Studies on Prevalence of Cysticercosis and its Economic Impact on Cattle, Buffalo and Pig Meat Production

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ABSTRACT

The present study was carried out to determine the prevalence of cysticercosis in cattle (male), buffalo and pigs. A total of 753 cattle (male), 640 buffaloes and 4042 pigs slaughtered at Deonar abattoir, Mumbai were inspected for presence of cysticercosis. Percent prevalence of cysticercosis in cattle and buffalo was noted as 0%, whereas *Cysticercus cellulosae* infection in 23 pigs examined had a prevalence of 0.57%. Analysis of whole cyst antigen, scolex antigen and host tissue antigen with SDS-PAGE showed a total of 15, 7 and 11 protein fractions, respectively, with molecular weights ranging from 25.1 to 64.9 kDa, 26.6 to 66.8 kDa and 23.7 to 64.9 kDa, respectively. 13 cysticercosis infected pig carcasses were totally condemned, whereas 10 were partially condemned for human consumption. A total of 396 kg pork and 15 hearts were condemned accounting to the loss of Rs. 39600 (0.392%) and Rs. 105 (0.37%), respectively, thus amounting to a total economic loss due to *C. cellulosae* in pig meat production was Rs. 39705 (0.392%).

Keywords: Cysticercosis, economic impact, prevalence, SDS-PAGE.

Introduction

Taeniasis is one of the important meat borne parasitic zoonotic disease, caused by adult stages of taeniid tapeworms *viz.*, *Taenia saginata* and *Taenia solium* in cattle, buffaloes and pigs, respectively. In animals, cysticercosis is caused by the immature stages (metacestode or cysticerci) called as *Cysticercus bovis* in cattle (*T. saginata*) and *Cysticercus cellulosae* in pigs (*T. solium*). These immature stages enter into the animal body through contaminated fodder, silage or hay in the form of gravid proglottids and/or eggs followed by hatching in intestine and subsequently via blood circulation enter into vital organs and musculature. The disease in animals is clinically manifested by emaciation, weakness and decreased production and work capacity (Soulsby, 1982). Man acquires infection by ingesting the eggs from fecal contaminated food or water or larvae from eating undercooked measly pork or measly beef that contains viable cysticerci.

Immunodiagnostic methods are shown to be very effective in detecting cysticercosis in humans and animals (Husain et al., 2001). These immunodiagnostic methods can also be helpful in detecting cysticercosis in animals before slaughtering, which will help in decreasing the economic losses. Therefore, there is a need to investigate more systematically the prevalence, economic impact and using more rapid, reliable and cost effective method for diagnosis of cysticercosis. Thus, characterization of antigenic protein of cysticercus cyst is essential for development of immunodiagnostic techniques. Keeping these points in mind, the present study was undertaken to assess the prevalence of cysticercosis in cattle, buffalo and pigs, characterization of cysticercus proteins and its economic impact on meat production.

Materials and Methods

Sample collection

A total of 753 cattle (male), 640 buffaloes and 4042 pigs slaughtered at Deonar abattoir, Mumbai were inspected for presence of cysticerci infection during the period of March, 2010 to June, 2010. A detailed scientific post-mortem inspection was carried out by taking deep incisions at common predilection areas/sites such as shoulder muscle, thigh muscle, masseter muscle, neck, diaphragm and heart. In positive cases, the intensity of

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infection was recorded by counting the number of cysts per unit area of muscle sample (7.5x2.5 cm). The intensity of infection was graded into three categories *viz.* low (1 cyst), moderate (2-3 cysts) and heavy (more than 3 cysts) as described by Kulkarni (1984). All the samples were kept on ice and brought to laboratory, Department of Veterinary Public Health, and kept at -20°C till further processing. The muscle tissues were washed with cold (4°C) phosphate buffer saline (PBS) prior to processing of tissue and cyst for preparation of antigen. Cysts were first separated from adherent host tissues and then collected in cold PBS. Further cysts were gently washed with cold PBS three times.

**Preparation of antigens**

For preparation of whole cyst antigen, the whole cysts collected from pig were triturated with mortar and pestle adding glass powder and cold PBS (4°C) and then homogenized using glass tissue homogeniser. The homogenate was further disrupted by sonication four times (1 min. sonication followed by 30 sec cooling time) at 20 kHz, 1 mA for a total of 4 min on ice and then centrifuged at 9000 rpm for 60 min in refrigerated centrifuge to separate the tissue debris and coarse particulate matter. The supernatant was used as whole cyst antigen (Dhanalakshmi *et al.*, 2006). For the preparation of scolex antigen (SA), approximately 10 g of the scoleces were taken for preparing scolex antigen. The procedure used for preparation of SA was similar as described for preparation of WCA. The host tissue antigen (HA) was prepared as per the procedure described by Dhanalakshmi *et al.* (2005). In brief, the muscle tissues collected from uninfected pigs’ predilection sites were homogenised in cold PBS (4°C), centrifuged at 9000 rpm for 15 min in refrigerated centrifuge and supernatant was collected using pasture pipette and used as host tissue antigen. The prepared antigens were immediately transferred to sterile plastic tubes and kept in deep freezer at -18°C till further characterization.

**Protein estimation of host tissue and cysticercus cyst antigen**

The host tissue and cysticercus cyst antigens obtained from infected cases were subjected to protein estimation using Lowry’s method modified by Hartree (1972) with the help of readymade protein estimation kit (Bangalore-Geneti™) and UV-visible spectrophotometer (Model: Pharma Spec UV-1700, Shimadzu Corporation, Japan). The antigen samples (host and cysticercus cyst) showing concentration above 150 mg% only subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

The molecular weights of the electrophoretic protein fractions of host tissue and cysticercus whole cyst and scolex antigens from pigs were studied by polyacrylamide slab gel electrophoresis method as described by Lammeli (1970) and Sreenivasamurthy *et al.* (1999), with slight modifications. Chemicals and reagents used during PAGE were of analytical grade and procured from Sisco Research Laboratory, Mumbai. The protein marker used in the present study was procured from Bangalore-Geneti™, Bangalore, India.

**Procedure of SDS-PAGE**

Sample buffer prepared was mixed in prepared host tissue and cysticercus cyst antigens (WCA and SA) at 2:1 ratio in eppendorf tubes and protein was denatured by boiling at 100°C for 5 min. About 20-25 µl of the sample was loaded in each well and one well with standard protein marker (PMWB by Bangalore-Geneti: range 3.5 kDa to 205 kDa) using micropipette. Electrophoresis was carried out at constant voltage of 100 V till marker dye (bromophenol blue) reached the bottom of the gel (2 to 3 h.). At the end of the run, the gel slab was removed from the electrophoresis plates and placed in a glass dish with distilled water for rinsing. Then the gel was subjected to silver nitrate staining method for staining of protein fractions. The molecular weights of unknown protein fractions were determined as per the method described by Shapiro *et al.* (1967) by plotting a graph between the molecular weights of protein markers and their relative mobility (Rf) on semilogarithmic graph paper. The Rf value was calculated as follows:

\[
R_f = \frac{\text{Distance travelled by protein markers}}{\text{Distance travelled by tracking dye}}
\]

**Economic loss determination**

Economic losses due to cysticerci infection to the pig meat production were estimated by recording the weight of condemned carcasses and their present prevalent market price as per the following formula used by Munde (1999).

\[
\text{Total Economic loss } = \text{Total weight of carcasses condemned} \times \text{X Price per kg of meat}
\]

**Results and Discussion**

All the cattle (male) and buffalo carcasses examined in the study were found to be negative for *Cysticercus bovis* infection. Amongst the 4042 pig carcasses examined, *Cysticercus cellulosae* infection was recorded in 23 pigs with prevalence of 0.57% (Table 1). These findings are in full agreement with Munde (1999), who also reported non occurrence of cysticercosis in cattle and buffaloes slaughtered at Deonar abattoir, Mumbai. Ratnam and Khanna (1988) also reported zero prevalence of cysticercosis in buffaloes slaughtered at Calcutta;
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Out of 23 positive carcasses, 21 (91.30%) showed cysts in the shoulder muscle, 15 (65.21%) in heart muscles, 14 (60.86%) in neck muscles and 13 (56.52%) each were found in thigh muscles and masseter muscles (Fig.1). The intensity of *C. cellulosae* infection in pigs was also estimated by observing the number of cysts per unit area (7.5 x 2.5 cm). Amongst 23 positive carcasses for cysticercosis, 12 (52.17%) showed low infection (1 cyst per unit area), 4 (17.39%) had moderate infection (2-3 cysts/unit area) and remaining 7 (30.43%) showed heavy or severe infection (more than 3 cysts per unit area). In the present study, highest prevalence of cysticerci was noted in shoulder region (91.3%), which is in agreement with the observations of Kulkarni (1984), Shinde (1991) and Munde (1999) who reported 53.27%, 100% and 92% prevalence in shoulder muscle, respectively. The next preferred site noted was heart (65.21%), which is also in accordance with Munde (1999) who reported 84% prevalence but not with Pramanik *et al.* (1985) and Shinde (1991) who reported 30% and 8% respective prevalence in heart. The prevalence of cysticerci noted in thigh region (56.52%) also in accordance with Shinde (1991) who reported 60% prevalence in thigh muscles but not with Kulkarni (1984) who reported 6.52% prevalence in thigh muscles.

However, they reported 2.62% prevalence of *C. bovis* in cattle. Kulkarni (1984); Shinde (1991) and Munde (1999) reported 2.85%, 6.02% and 1.78% prevalence of *C. cellulosae* in pigs slaughtered at Deonar abattoir, Mumbai. Recently, Mandakhalikar *et al.* (2009) screened pigs slaughtered at Deonar abattoir, Mumbai and reported 0.89% prevalence of *C. cellulosae*. From the analysis of data generated by these previous workers revealed that over the period of 10-15 years the prevalence of cysticercosis in pigs found to be declined gradually. Thus, the prevalence of cysticercosis observed in the present study is in agreement with the observations of previous research workers.
molecules. From the above data, inspecting shoulder muscle is the best possible site for detection of infection.

The whole cyst antigen showed a total of 15 protein fractions with molecular weights ranging from 25.1 to 64.9 kDa by SDS-PAGE. The molecular weights of these protein bands were 64.9, 56.2, 50.1, 42.2, 39.9, 37.6, 36.5, 35.5, 34.5, 33.5, 31.6, 29.8, 28.1, 27.4 and 25.1 with increasing mobility (Fig. 2). The scolex antigen showed 7 protein fractions with molecular weights ranging from 26.6 to 66.8 kDa (Fig. 3). Molecular weights of the detected scolex protein fractions were found to be 66.8, 50.1, 39.8, 37.6, 35.5, 29.8 and 26.6 kDa of which 50.1, 39.8, 37.6 and 29.9 kDa were common with the WCA. The host tissue antigen of pig showed 12 protein fractions ranging from 23.7 to 64.9 kDa (Fig. 3). The molecular weights of protein bands were 64.9, 59.6, 53.1, 44.6, 39.9, 36.5, 35.5, 28.2, 26.6, 25 and 23.7 kDa. The comparative analysis of band pattern of WCA, SA and HA indicated that protein fraction of molecular weight 35.5 kDa was common in all three antigens analyzed. Protein fractions of molecular weight 64.9 and 25.1 were found common in WCA and HA, whereas 26.6 kDa was found common in SA and HA.

Grogl et al. (1985) reported 51 protein fractions in the whole cyst antigen with molecular weights ranging from 13.5 kDa to 260 kDa after silver staining. While Sreenivasmurthy et al. (1999) reported 18 bands in WCA ranging from 14 to 97.4 kDa and major component of protein was reported in between 20 to 34 kDa. These findings are in accordance with the results found in the present study. Seung-Yull Cho et al. (1987) reported 34 bands in the saline extract of scolex of C. cellulosae of which 94, 64, 39, 34, 26, 24, 21, 15, 10 and 7 kDa were common with the cystic fluid antigen. Dhanalakshmi et al. (2005) reported a total of 18 and 15 bands in the WCA and SA, respectively ranging from 13 kDa to 97.4 kDa and major component of molecular weight 35.5 kDa was common in all three antigens analyzed. Protein fractions of molecular weight 64.9 and 25.1 were found common in WCA and HA, whereas 26.6 kDa was found common in SA and HA.

Out of 23 cysticercus infected pig carcasses, 13 (7 heavy, 4 moderate and 2 low infected) were totally condemned, whereas 10 low infected carcasses were partially condemned for human consumption after removing and rejecting the infected part of the carcass. Thus, a total of 396 kg pork and 15 hearts were condemned accounting to the loss of Rs. 39600 [@ Rs. 100/kg (0.392%)] and Rs. 105 [@ Rs. 7/heart (0.37%)], respectively. Therefore, total economic losses due to C. cellulosae cysticercosis to the pig meat production was Rs. 39705 i.e. 0.392%. India has 13.5 million pigs and the slaughter rate is about 84% as per Ranjhan (2003). When economic loss is considered in the total condemned meat produced all over the country and on total number of animals slaughtered, the magnitude of the problem can be appreciated. Therefore, to reduce this loss, detection of cysticercosis infection before slaughtering of the animal is necessary and could be done by using immunodiagnostic tests. Overall, the prevalence of cysticercosis in pigs examined during the present study is very low (0.57%) as compared to the previous research workers. Most of the research workers stated that the ingestion of raw meat or undercooked meat is main source of acquiring infection by the human beings. There should be strict implementation of quality management systems such as HACCP, ISO-9000 and ISO-22000 during production, processing, storage, distribution and sale of meat at retail outlets. Besides this, better sanitary facilities should be maintained (adequate number of latrines) at villages, awareness about this infections among the people, proper disposal of infected meat and thorough inspection of carcasses by the veterinarians should be strictly implemented to avoid threat to the public health and to promote animal health.

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References


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