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Abstract

Cysticercosis caused by *Taenia solium* metacestode, is one of the major public health disease. Infected pigs play an important role in human taeniosis and cysticercosis. Recently much progress has been made towards immunodiagnosis of *T. solium* metacestode infection in pigs that have improved diagnostic sensitivity and specificity at field level. DNA approaches are now being used for accurate species specific identification of *Taenia* spp. Recent advances in diagnosis of porcine cysticercosis both antemortem and postmortem diagnostic techniques are reviewed in this article.

Key Words: *Taenia Solium* Metacestode; Serodiagnosis; Molecular Diagnosis; Pigs

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Introduction

Cysticercosis, caused by metacestode of *Taenia solium* is primarily an infection of pigs that acts as an intermediate host of *T. solium* and is a neglected disease [1] and an under rated zoonotic disease classified in list B by the OIE. Pigs become infected by the ingestion of eggs of *T. solium* during scavenging that are present in the stools of human beings who are the definitive host of *T. solium*. Earlier the larval stage or metacestode of *T. solium* was known as *Cysticercus cellulosae*, now the name is nowhere in use and is referred as metacestode or larval stage of *T. solium*. The larval stages (metacestodes) of *T. solium* form distinctive pearly-white cysts (cysticerci) and infection in man and pig is called cysticercosis. It is the leading cause of epilepsy in human population of the developing countries due to neurocysticercosis [2]. Now the disease is considered as emerging and reemerging parasitic disease [3]. Pigs can become infected at any age. Piglets become infected around the age of 2- 4 weeks and predominately during dry season. The metacestodes were found in the liver of piglets of 2 months of age and in older animals aged 4 to 6 months, the larvae were also found in the muscles. In a 6-month-old pig, larvae were found in the muscle and brain [4]. Breeders have a significantly higher prevalence

than weaners. Similarly fatteners had a significantly higher prevalence than weaned pigs [5]. Contrarily some studies show no relation between age and prevalence of infection [6-8].

Cysticercosis affects food security in pigs threatening human health, as pig will always be one of the major sources of food for mankind but is rarely associated with high mortality in intended host. Swine infected with *T. solium* metacestodes play a fundamental role in the transmission and maintenance of human taeniosis and cysticercosis with the consequent need for effective services of animal health and inspection of products of animal origin. Approximately 2.5 million people worldwide carry *T. solium* tapeworm and not less than 20 million people are infected with *T. solium* metacestode and 50,000 die of neurocysticercosis annually [9].

Porcine cysticercosis has even emerged as an important constraint for the nutritional and economic well being of smallholder farming communities due to down grading or total condemnation of affected meat in endemic area. About 68 per cent of hogs were condemned due to cysticercosis in Central America from 1959 to 1961 [10]. The estimate in Mexico for the cost of losses of pig production in 1980 was estimated as US \$ 43 million [11]. The annual losses in 10 West and Central African countries were 25 million Euros [12] and US \$ 121 million in China [13]. In India, the economic loss due to total carcass condemnation was reported as Rs. 64,600/- from Aligarh, Uttar Pradesh [6] and Rs. 2, 61,661 from Andhra Pradesh, which constituted 4.22 per cent of the overall cost of the pigs [14]. Protection of human health against zoonoses is mandatory by effective control of the disease for which the epidemiological data should be collected for improved monitoring arrangement. The identification of high prevalence zones, reliable sensitive and specific field applicable inexpensive procedures for its early and accurate detection is the need of the hour. Different techniques being followed for detection of *T. solium* metacestode infection in pigs including recent approaches are discussed in the following sections.

Diagnosis

In the recent past much progress has been made in research on diagnosis, treatment and prevention of human taeniosis and porcine cysticercosis, although more operational research is still

needed. In spite of this, global eradication of *T. solium* infection is still unlikely in the near future [15]. The lack of hygiene in the rural communities, free roaming of pigs, lack of education of pig owners, lack of control in the trade of pigs and their meat and lack of conscientious meat inspection has a direct bearing on the presence of adult worm in human [7,16-21]. Diagnosis of porcine cysticercosis is primarily of two types; one is antemortem inspection by tongue examination, clinical signs and serological studies, the second being the post mortem inspection.

Antemortem diagnosis

The antemortem diagnosis, based on clinical signs is usually not possible because clinical symptoms are not well defined in pigs. Clinical manifestation is noticeable only when the cysticerci get lodged in the eye or brain of pig when nervous symptoms are exhibited [22]. The cysticerci in brain are the main cause of late-onset epilepsy in human in tropical countries [2]. In contrast to their much studied effects upon human health, the impact of intracranial cysticerci upon pig health has not been fully explored and the information on clinical signs in infected pigs is scanty. Excessive salivation, blinking and lachrymation and in some cases sub-conjunctival nodules were the only reported signs [22,23]. Pigs that had many larvae in the brain appeared quieter and lay down for longer period than the other [20], some pigs however exhibited no apparent signs [24,25]. Gross and histopathological studies of brains of pigs revealed wide variation (3% to 60%) in NCC prevalence [22,26]. Proton magnetic resonance spectroscopy using creatin as a marker is valuable in prediction of viability of porcine neurocysticercosis [27].

The current method for detecting infected pigs in the field is tongue inspection. In tongue inspection, the pigs are considered positive for infection if cyst-like nodules are either seen or felt on palpation [28]. In vivo examination of tongue shows high specificity although its sensitivity is low, which is not always desirable [20,28,29], but several studies used palpable lingual cysts as indicator to estimate the prevalence of porcine cysticercosis and to study the potential risk factors associated with porcine cysticercosis [30].

Immuno diagnosis

Reliable antemortem serological test based on detection of specific antibody and antigen proves very useful in confirmatory diagnosis.

Immune responses

In infected animals the level of the serum antigen and antibody varies with the intensity of the infection. In heavily infected pigs, both antigen and antibodies can be detected at least 29 days and up to 200 days post infection (pi), while in lightly infected pigs antigen and antibodies are first observed between 61-97 days pi [31]. Immunity due to primary infection lasts at least 5 months. At 2 months of infection in piglets experimentally infected with eggs of *T. solium*, antigens of 24 and 39-42 kDa are most frequently recognized. In pigs with only a few caseous cysts in muscles and/or vesicular cysts in brains no antibodies could be detected [32]. The animals with viable cysts at necropsy have high antigen levels, whereas animals with no cysts or only degenerated cysts have low or no antigen levels and the trend is that the number of viable cysts decreases with the age at which the animals are infected [33]. An intense humoral response is observed in piglets experimen-

tally infected with *T. solium* eggs, from 10-30 days pi, and persisted up to 90-140 days [34-35] but cellular responses occur (increase in CD4 + T cells) at 60 days pi [36]. Interpretation of seropositive cases in piglets might be complicated by the maternal antibodies transferred from colostrum from a positive sow to its piglets which persists for 7 months, which has to be considered while studying the prevalence of cysticercosis in pigs [37].

T. solium metacestode antigens

Type of antigen used is mainly responsible for the sensitivity and specificity of cysticercosis diagnosis. So far crude and purified whole cyst antigen (WCA), cyst fluid antigen (CF), scolex and its fractionated antigens (SA), membrane antigens (MA), antigen B, excretory and secretory products of metacestode (E/S) of *T. solium*, were used with different sensitivity and specificity in different diagnostic methods. CFA are more sensitive than other components of *T. solium* metacestode as they are enriched with sensitive diagnostic glycoproteins [38-39] where as excretory-secretory antigens (E/S) are more specific than sensitive [40-41]. Use of Antigen B is limited in differential diagnosis of *T. solium* and *T. hydatigena* as it is also found in adult and larva of *T. hydatigena* [42].

Crude somatic antigens from *T. solium* metacestode revealed a protein band pattern ranging between 8 kDa and 200 kDa. Four poly peptides 8, 11, 16 and 23 kDa were specially recognized by pigs with confirmed cysticercosis [43]. Later, purification of glycoproteins and production of recombinant antigens was achieved to improve the performance of serology. 26kDa and /or 8 kDa antigens in crude saline extract of *T. solium* metacestodes were compared by immunoblot with Gp 13-50 antigens in a lentil-lectin semipurified glycoprotein extract of *T. solium* for antibody recognition. The seroprevalence represented a non significant difference with both antigens [16]. HP10 epitope-bearing antigens have been demonstrated in *T. solium* and *T. crassiceps* cyst fluid antigen and excretion/secretions for detection of antigens and antibodies in infected pigs [31]. The fractionated first peak of fluid antigen showed highest sensitivity and specificity followed by scolex and membrane antigens of *T. solium* in ELISA in naturally infected pigs [38]. Glycoproteins (GPs) purified by a single step iso-electric focusing electrophoresis (IEFE) [44-45] and recombinant chimeric antigen (RecTs) of *T. solium* [45] are good candidates for antibody detection in porcine cysticercosis. Analysis of *T. solium* metacestode cyst fluid by high performance liquid chromatography (HPLC) revealed 14 kDa fraction (F3) which showed high performance in Ab-ELISA with serum samples of pigs experimentally infected with *T. solium* eggs but when applied on field samples the performances of the F3-ELISA were lower than those of a crude cyst fluid antigen [46]. Both GPs and RecTs (African American, or Asian) are suitable for serological monitoring in infected pigs worldwide as they showed a correlation higher than 92% in serological tests. Comparison of native GPs with RecTs by ELISA demonstrated no statistical difference in sensitivity [47]. The use of TS-14 recombinant antigen in ELISA test (Ab-ELISA) can be useful for the diagnosis of cysticercosis in pigs with low infection [48].

Crude antigens of *T. solium* metacestode serologically cross react with other helminth parasites of pig [39,43,46,49]. Immunoperoxidase and indirect immunofluorescence studies showed distribution of cross reacting antigens mainly on the tegument of *T. solium* metacestode [50]. No cross reactions were observed with serum samples from pigs infected with other parasites using the HPLC purified fraction (F3) of cyst fluid [46]. Though purifica-

tion of antigen improves the test performance in terms of sensitivity and specificity, it requires large quantity of antigen and use of laborious and expensive procedures for specific antigen purification. Use of multiple antigens which are isolated and purified from closely related species (*Taenia crassiceps*) show cross reaction with *T. solium*. Hence, a crude metacestode antigen of *T. crassiceps* was used for detection of antibodies in cysticercosis infected pigs [51,29]. Sensitivity was high with cyst fluid and crude antigens of *T. crassiceps* compared to that of *T. solium* metacestode in diagnosis of porcine cysticercosis [52,53].

Antibody detection methods

Several immunoglobulin classes are produced as specific antibodies against the parasite i.e. IgG, IgM and little of IgA. The most frequent and persistent is IgG.

Conglutinating complement absorption test (CCAT): This is superior to CFT, IHA and BFT. Titer of antibody 1:80 and above is considered as positive in naturally infected pigs [54].

Indirect fluorescent antibody test (IFAT): Sensitivity is high with eggs of *T. solium* antigen and specificity was high with first peak of scolex antigen without any cross reaction with *C. tenuicollis* infected animal [55]. Practical utility is limited due to the requirement of special equipment.

Intradermal test (ID): It is simple, practical and sensitive method. Increase in thickness > 1.75 cm after 30 min is considered as positive [56]. Reaction completely disappears 48 hrs after inoculation. False negative and false positive reactions are observed at inoculation site [56-57].

Double immunodiffusion test (DID): Circulating antibodies could be detected in experimentally and naturally cysticercosis infected pigs [58]. DD test is time consuming and lack sensitivity [57].

Indirect haemagglutination test (IHA): False positive reactions and cross reactions with the serum of pigs infected with other helminth parasites occurs. This test is highly sensitive but less specific [57-58]. Though HA test lack repeatability and accuracy is still extensively used because of its high sensitivity and ease of application.

Counter immunoelectrophoresis (CIEP): Test is more rapid, simple and less expensive compare to other tests [59] and is more sensitive and specific than DID [58]. Sensitivity and specificity was 84.5 to 86.6 and 88.5 to 94.2 per cent with scolex and its fractionated antigens in diagnosis of porcine cysticercosis and could be used as field test in antemortem diagnosis [58,60]. Highest cysticercosis positive cases were detected in infected pigs by CIEP than in ELISA [61].

Latex agglutination test (LAT): In LAT, antigens /antibodies are combined to latex as a particle vector and the reactions between antigen and their specific antibodies are visualized as agglutination. LAT was as efficient as ELISA when antigen B was used [62].

Enzyme linked immunosorbent assay (ELISA): Till today ELISA is universally and extensively accepted technique for detection of antibodies because of its high sensitivity and specificity,

depending on the type of antigens used [29,39,46,52,58,61,63-64]. Several methods based on ELISA are established, including Dot-ELISA, gel ELISA [65] and Protein-A ELISA [66] which are of higher sensitive and specific and more convenient than ELISA.

Enzyme linked immunotransfer blot (EITB): Over the past decade EITB has been widely used for diagnosis of cysticercosis in pig serum samples [67]. It is highly sensitive (90-97.5%) and specific (100%) test than ELISA for antibody detection in pigs [28,42,67-70]. Though, many workers reported higher sensitivity of EITB than ELISA in the diagnosis of porcine cysticercosis, in developing countries, ELISA is preferred because of its better availability, simplicity and lower cost compared with immunoblot [71]. However, the sensitivity and specificity of ELISA was as par to those of the immunoblot [13]. ELISA was more sensitive than immunoblot when evaluated with RecTs of *T. solium* and reliable for differentiation of pigs infected with larvae of *T. solium* and those either uninfected or infected with other *Taeniid* species [45].

Antigen detection methods

It is difficult to determine cysticercosis positive pigs only by antibody detection methods because antibodies might continue to be present even after cure. The specific antibodies can be detected only after 1 week of post infection and reach the peak after 6-7 weeks [34-35] where as circulating antigens exist very early and will disappear as soon as parasite is killed. So, infected animals can be detected at the early stage of infection from the level of larval circulating antigens. Identification of infected pigs with viable larvae is achieved through detection of their secretory and excretory products using a monoclonal antibody-based capture assay [7,31,72-73]. Sensitivity and specificity of Ag-ELISA (86.7% and 94.7%) is more than Ab-ELISA (35.8% and 91.7%) in estimating the prevalence of porcine cysticercosis [29]. B158/B60 Ag-ELISA and HP10 Ag-ELISA are used to detect antigen-positive pigs and to understand the level of disease transmission [64,74-75] which was more sensitive than EITB [74]. Use of MoAb-TS14 for the detection of circulating antigen (Ag-ELISA) was not appropriate for pigs with low infection but, the test was successful for naturally heavily infected pigs [48].

Serological methods are more sensitive than tongue palpation for the detection of porcine cysticercosis [28], but several tongue positive pigs could not be confirmed by EITB [76] and Ag-ELISA [7]. Whereas, few authors [77] were able to detect pigs harboring even single cyst using Ag-ELISA. Differences in the sensitivity of the test may be related to the permeability of the host capsule around the metacestode that influences the amount of excretory-secretory products released into the circulation [78].

Immunogold techniques

The sensitivity of the currently available diagnostic techniques (Ag – ELISA, Ab – ELISA assays, EITB and tongue inspection) is low in pigs with low levels of cyst burdens [5,31,79] and requires access to laboratory with proper instrumentation and trained personnel. In 2008, ASARECA developed a pen-side diagnostic kit for *T. solium* cysticercosis in pigs under a project titled “Diagnostic and control tools and strategies for *T. solium* cysticercosis”. The principle of assay is similar to lateral flow assay, is used for detection of circulating antigen in which antibody labeled colloidal gold is an indicator for development of visible line on device. Recently, flow through assay or dot immunobinding assay is used at field level, in which colloidal gold is used as marker for detection

of antibodies especially for large scale screening of pigs against cysticercosis [39]. This test is user friendly, very simple, can be completed within 3 minutes without any equipment and is cost effective with visual results (colored dots in positive case).

Post Mortem Inspection

Meat inspection of pigs at slaughter is the only public health measure implemented to prevent *T. solium* transmission to humans, where inspected carcasses are detected at post mortem and subsequently downgraded or condemned [43,68]. In pigs, results of autopsy and enumeration of the cysts in the carcass considered as gold standard and provide a tool for validation of the immunodiagnostic tests [80]. Meat inspection is a major useful method when animals are heavily infected, but not when animals carry a few viable cysticerci [41,81]. Despite the efficacy of conventional inspection procedures, 40-50 per cent of the cases are not detected in mild infections [63]. The main drawback of routine meat inspection is its lack of sensitivity and objectivity as the procedure is restricted to certain predilection sites and is highly dependent on the expertise of the inspector as well as on the stage of cysts. Generalized infection of the carcass make it unfit for human consumption, however, lightly infected carcasses are not condemned but provided long term storage at low temperature at -10°C for 4 days greatly affect the value of the meat and hence the profit of owner.

Molecular techniques

Because of the lack of sensitivity and objectivity of meat inspection, an objective test to underpin the observation of meat inspector is needed. More over the degenerated metacestodes can be confounded with milk spots [82], hydatid cysts [83], *T. hydatigena* cysticerci, sarcocystis and piece of fat and left over of muscle fasciae. The identification of degenerated cysts also assumes importance for carcass judgment. Molecular diagnosis based on PCR test assumed significance due to its high specificity and sensitivity and can be used as simple presence / absence assay to detect the parasite of interest [84]. Consequently, PCR based techniques are being employed to study genetic variability, for species-specific identification of *Taeniid* spp. cysticerci and to validate meat inspection results in porcine cysticercosis [85-87], which is an appropriate postmortem test that could be applied on meat samples in suspected cases. Suspected lesion from the liver that resembled milk spot was also confirmed by PCR [87]. Milk spots in the liver of the pigs infected experimentally with *T. solium* eggs are confirmed by histology, ruling out *Ascaris* infection [82]. The performance of PCR and ELISA assays were compared for ante-mortem diagnosis of porcine cysticercosis, the ELISA assay showed high sensitivity and good specificity while the PCR assays showed high specificity but a low sensitivity [88]. Though, PCR based techniques are not difficult and have high sensitivity and specificity, demand expensive infrastructure and is not suitable as rapid on-site diagnostic test preventing the general use of this methods. Recently, proteomic analysis of *T. solium* metacestode excretion-secretion proteins was studied to improve the current diagnostic tools [89].

Conclusion

New and improved reliable diagnostic methods and strategies to identify infected pigs for the surveillance, prevention and control of this zoonotic disease are now available but cost and acces-

sibility remain drawback if these tests are to be used in endemic areas in developing countries. More attention should be given in improving antibody and antigen assays as user friendly by modifying them to be more practical and economical at field level e.g., dipsticks and lateral flow tests. Flow through assays like Malaria Kit in human, would greatly contribute to strengthen the control of metacestode of *T. solium* infection. The cysticercosis vaccines for pigs based on onchosphere antigens show great promise for blocking transmission to humans. Further research is required to ensure effective field application of the vaccines with regard to delivery and duration of protection.

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