



Seroprevalence of porcine cysticercosis in Maharashtra

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ABSTRACT

Porcine cysticercosis is caused by *Cysticercus cellulosae*, a larval stage of parasite *Taenia solium*. Humans get infected by consuming eggs of *T. solium* through contamination of food and water. Owing to economic and zoonotic importance of porcine cysticercosis in India, a research plan was designed to study seroprevalence of cysticercosis in Maharashtra. Blood samples (172) were collected from different regions. The seroprevalence of cysticercosis by ELISA, FTA and western blot was 8.5%, 7.5% and 6.98%, respectively. Cohen's kappa coefficient was used to analyse for the percentage of agreement with meat inspection test. The sensitivity and specificity between ELISA and FTA ($k=0.958$) showed no significant difference. The study indicates that ELISA and FTA were more sensitive than western blot. Western blot of whole cyst antigen revealed immunoreactivity at 42 to 250 kDa bands. The seroprevalence of porcine cysticercosis in Maharashtra had increased significantly. FTA is easier to perform and faster than ELISA test. This simple test appears to be suitable for practical use at field level, especially for large-scale ante-mortem screening of pigs against cysticercosis.

Key words: Cysticercosis, ELISA, FTA, Porcine, Western blot

Porcine cysticercosis is an emerging and re-emerging parasitic disease for both developed and developing countries (Craig and Pawlowski 2002) which are closely associated with social and environmental conditions such as poor hygiene and free roaming of pigs. Considering the rearing of pigs in India, they are susceptible to contract different types of parasitic infections, some of which can have economic and zoonotic significance.

Porcine cysticercosis is caused by *Cysticercus cellulosae*, a larval stage of parasite *Taenia solium*. Humans get infection by consuming eggs of *T. solium* through contamination of food (unclean green vegetables) and water.

Approximately 50 million people in the world are infected with the parasites and approximately 50,000 die of cysticercosis annually (Sarti *et al.* 2000, Willingham and Schantz 2004). Although population based epidemiological data on taeniasis in humans and cysticercosis in pigs is

lacking in India, large number of neurocysticercosis patients managed in the hospitals in all states indicate that it is a major public health concern.

By knowing the public health significance and economic impact of the disease, there is need to know burden of cysticercosis in pig population by using rapid, reliable and cost-effective serological method, so that effective preventive and control measures along with suitable diagnostic method can be suggested to minimize the incidence of disease. FTA being useful in field was compared with ELISA & Western blot.

MATERIALS AND METHODS

The survey was conducted to inspect slaughtered pigs during February 2016 to June 2016 for identification of disease burden. Pig carcasses (815) were examined for mealy pork and 172 pig blood samples collected from different regions in Maharashtra.

Post mortem examination, collection of cysticerci cysts and processing: Pig carcasses and visceral organs were examined by a scientific post mortem inspection in the slaughter houses/retail shops. Deep incisions were given at predilection sites such as shoulder muscle, thigh muscle, masseter muscle, neck, diaphragm, liver and heart to detect cysticerci. The infested muscle/organ with cysticerci were collected and brought to the laboratory in chill condition. The cysts were first removed from adherent host tissues and then collected in cold Phosphate Buffer Solution (PBS). Further, cysts were gently washed with cold PBS three times

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and were stored separately in absolute ethanol at -20°C for further use.

Testing of pig sera by ELISA, flow through assay and Western blotting: Aseptically collected blood samples from slaughtered and farmed pigs, and separated serum samples were placed at -20°C until use. The commercially available ELISA kit (CUSABIO Pig cysticercosis (CYT) antibody (IgG) ELISA kit, China) was standardized as per the manufacturer's instructions and used.

The FTA test was performed as per Sreedevi *et al.* (2011) with modification. The cellulose acetate membranes of 0.45 micron were used. The lowest concentration of antigen that showed clear pink dot was 360 ng/ul and was chosen as optimum sensitizing dose. Appearance of two pink dots indicated a positive reaction; only one dot appeared on control side, the test was considered negative.

Western blot was performed using WCA as per the method of Towbin *et al.* (1979), Agudelo-Flórez *et al.* (2009) and Fernando *et al.* (2011) with few modifications.

Evaluation of efficacy of ELISA, flow through assay and Western blotting: In pigs, the results of post-mortem and enumeration of metacestodes are considered as gold standard and used as a tool for validation of immunodiagnostic tests (Dorny *et al.* 2004). Hence, with the use of Cohen's kappa coefficient, the results were analysed for the percentage of agreement with meat inspection test. It is a statistical measure of inter-rater agreement for qualitative (categorical) items, i.e. between two tests, especially in the absence of a standard and is defined as kappa or κ (Fleiss *et al.* 2004). The kappa value was calculated by Graph Pad Software @ 2016.

RESULTS AND DISCUSSION

The prevalence of porcine cysticercosis by post mortem was 0.49% (4/815). The available literature showed region-wise variation in prevalence. The results of current study are distinctly low as compared to Deka *et al.* (1985) from Guwahati who had reported 20.8% prevalence. Pathak and Gaur (1989) reported 8.33% prevalence of cysticercosis in Uttar Pradesh; whereas, Mandakhalikar *et al.* (2009), Palampalle (2012) and Jadhav (2014) reported prevalence of *C. cellulosae* as 0.89%, 1.26% and 0.9%, respectively in pigs, in Mumbai, Maharashtra.

The seroprevalence of cysticercosis by ELISA, FTA and western blot was 8.1% (14/172), 7.5% (13/172) and 6.98% (12/172), respectively. The results of the present study were not in agreement with Pouedet *et al.* (2002) and Gomes *et al.* (2007) as they reported seroprevalence of 21.8% and 57% in Cameroon and Brazil respectively; while the results were higher than Kagira *et al.* (2010) who reported 4% prevalence in Busia district, Kenya. Pondja *et al.* (2015) reported variation from 5.6% to 66.7% in Mozambique whereas Porphyre *et al.* (2016) found out 2.3% to 2.6% prevalence in Antananarivo abattoirs. In India, Hafeez *et al.* (2004) observed seropositivity of 6.50%, 6.22%, 6.40% and 6.50% in Andhra Pradesh, Tamil Nadu, Karnataka and Kerala respectively; the results were in agreement with

present study. Mohan *et al.* (2012) reported very high seroprevalence (59.8%) while Jadhav (2014) reported only 2.89% seropositivity.

In the present study, WCA showed 19 differentiated bands with molecular weight ranging from 10 kDa to 250 kDa in silver staining whereas Palampalle (2012) noted bands with molecular weight ranging from 12 to 103 kDa. The band pattern of present study principally coincided with the pattern described by Tsang *et al.* (1991), Ko and Ng (1998), Sreenivasamurthy *et al.* (1999), Dhanalakshmi (2005) and Sreedevi (2010) particularly in relation to molecular weight range of fractionated proteins.

The results of Western blot revealed that number of polypeptides of molecular weights between 50 to 98 kDa reacted positively with hyper immune sera (control) at 30 sec exposure and 42 to 250 kDa at 1–2 min exposure, respectively. In the present study, known positive serum samples showed positive reactivity to 55 kDa and band between 17 kDa–36 kDa. However, Palampalle (2012) reported that similar molecular weight polypeptides reacted positively with hyper immune sera in peak II of WCA. This type of trend, i.e. positive sera showing positive reaction against number of high as well as low molecular weight proteins of *C. cellulosae* fractionated on SDS-PAGE was also noted by Gottstein *et al.* (1986), Cho *et al.* (1987) and Tsang *et al.* (1991). However, proteins of high molecular weight cross reacting with sera from other heterologous parasitic cases such as human hydatidosis (Gottstein *et al.* 1986), porcine hydatidosis (Ko and Ng 1998) and other helminthic infections had been reported. In contrast to this, amongst different polypeptides of *C. cellulosae* showing positive reaction to low molecular weight proteins had been reported to be more specific than the other components (Lu *et al.* 1991, Xu and Liu 1992, Pathak *et al.* 1994, Kaur *et al.* 1995 and Dhanalakshmi *et al.* 2005).

The sensitivity and specificity of ELISA was somewhat contrary to that reported by Pathak *et al.* (1994) (sensitivity and specificity of 90 and 100% respectively) (Table 1).

The present study was in agreement with Sreedevi *et al.* (2011) who reported 92% sensitivity and 100% specificity in porcine cysticercosis by FTA. However, Gan *et al.* (2006) reported higher sensitivity (98.3%) in detection of human cysticercosis using crude cyst fluid antigen. On the contrary, Hernandez-Cruz *et al.* (2009) recorded lower sensitivity of 84.6% in immunobinding dot-blot assay for serodiagnosis of human cysticercosis using whole cyst antigen.

Verastegui *et al.* (1992) reported sensitivity varying from 58–65% and specificity was 100% in diagnosis of hydatid disease in humans by Western blot. In contrast, Pathak *et al.* (1994) reported the sensitivity and specificity as 90% and 100%, respectively. However, Agudelo-Florez *et al.* (2009) found that western blot was 86.4% sensitive and 93.2% specific for porcine cysticercosis. It is stated that the method of preparation of antigen and the type of antigen had influence on the sensitivity and specificity of the test (Dottorini *et al.* 1981).

The study indicates that Western blot is less sensitive

Table 1. Comparative diagnostic test evaluation measures of ELISA, FTA and Western blot

Parameter	ELISA	FTA	Western blot
Sensitivity	100	100	75
Specificity	94.04	94.64	94.64
Accuracy of the test	94.18	94.76	94.19

than ELISA and FTA. Pathak *et al.* (1994) reported that ELISA was less sensitive (70%) and specific (73%) while Western blot was 90% sensitive and 100% specific. Accuracy of all the tests, i.e. ELISA, FTA and Western blot was similar.

The present study revealed that, there is no significant difference in sensitivity and specificity between ELISA and FTA as strength of agreement between these two tests was very good (Table 2). Similar results were noted by Sreedevi *et al.* (2011). Few reports noted results that agree to those detected by routine ELISA during diagnosis of hydatidosis and toxocarosis by comparing FTA with ELISA (Eliades *et al.* 1998, Dubinsky *et al.* 2000). The strength of agreement between ELISA and Western blot was moderate. Similar findings were noted in between FTA and Western blot.

The seroprevalence of porcine cysticercosis in Maharashtra had increased significantly. This demands a proper consideration to the unnoticed spread of the parasitic disease. The proper awareness for preventive measure practices have to be set up along with quality screening and diagnostic strategy to reduce the impact of disease. The FTA is easier to perform and faster than ELISA test. The results can be interpreted visually for the detection of antibodies against porcine cysticercosis. This simple test appears to be suitable for practical use at field level, especially for large-scale ante-mortem screening of pigs against cysticercosis. However, purification of antigen might improve the accuracy of diagnosis.

The seroprevalence of cysticercosis by ELISA, FTA and Western blot was 8.5%, 7.5% and 6.98%, respectively. The sensitivity and specificity between ELISA and FTA ($\kappa = 0.958$) showed no significant difference. Western blot of whole cyst antigen revealed immunoreactivity of 42 to 250 kDa. The study indicates that ELISA and FTA were more sensitive than Western blot. The FTA is easier to perform and can be used at field level for screening of disease. The seroprevalence of cysticercosis in

Table 2. Summary of K value of ELISA, FTA and Western Blot calculated by Graph Pad Software

	ELISA and FTA	ELISA and Western Blot	FTA and Western Blot
K value	0.96	0.424	0.45
95% confidence interval	0.881–1.000	0.159–0.743	0.140–0.707
Strength of agreement	Very good	Moderate	Moderate

Maharashtra had increased significantly. This necessitates the proper preventive measures to combat the disease.

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