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Scenario of porcine cysticercosis and human taeniasis in Maharashtra State, India

Vilas VAIDYA^{1,*}⁽¹⁾, Ashish PATURKAR¹, Ravindra ZENDE¹, Mukulesh GATNE², Dhananjay DIGHE³, Rupesh WAGHMARE¹, Shilpa MOON⁴, Sujata BHAVE¹, Priyanka JADHAV¹, Nilam WAVHAL¹ ¹Department of Veterinary Public Health, Bombay Veterinary College, Maharashtra Animal and Fishery Sciences University, Mumbai, India ²Department of Veterinary Parasitology, Bombay Veterinary College, Maharashtra Animal and Fishery Sciences University, Mumbai, India ³Department of Veterinary Preventive Medicine, Bombay Veterinary College, Maharashtra Animal and Fishery Sciences University, Mumbai, India ⁴Department of Veterinary Public Health, Nagpur Veterinary College, Maharashtra Animal and Fishery Sciences University, Nagpur, India

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Abstract: Cysticercosis/taeniasis is one of the neglected parasitic zoonoses in developing countries like India. A study was conducted for a period of 7 years from 2010 to 2017 during which 13,596 pig and 1238 human samples were screened to learn the exact scenario of cysticercosis/taeniasis in the different regions of Maharashtra State. Prevalence of cysticercosis/taeniasis in pigs and humans was recorded using various diagnostic methods such as meat inspection and serological methods involving ELISA. The overall prevalence of cysticercosis in pigs was found to be 0.88% by postmortem examination and 0.9% by PCR assay, whereas prevalence of taeniasis in humans was 3.15% by ELISA and 2.04% by PCR. As compared to the previous reports from the state, the findings of the present study revealed a lowered prevalence of the disease in both the human and the pig population. It was concluded that the declining trend of cysticercosis in Maharashtra State was the outcome of the implementation of appropriate and sustainable public health interventions achieved through demonstrations and the distribution of booklets in the vernacular language.

Key words: Cysticercosis, pigs, humans, postmortem, PCR assay, ELISA

1. Introduction

Cysticercosis, a ubiquitous disease of high economic and public health significance, is caused by Cysticercus cellulosae, the larval form of Taenia solium. It mainly affects the health and livelihood of subsistence farming communities in developing countries in Africa, Asia, and Latin America, where pigs are allowed to roam freely with free access to night soil (1). The disease is transmitted to humans by consumption of infective cysticerci in raw or undercooked pork or food and water contaminated with their eggs. The parasite commonly localizes in the central nervous system, causing neurocysticercosis, an emerging disease and one of the principal causes of epilepsy (2,3). The existence of scavenging or stray pigs and lack of sewage disposal make it a serious zoonosis in India, and the illegal and uninspected slaughtering of pigs perpetuates the transmission cycle of the parasite (4).

The inspection of pork carcasses for presence of C. cellulosae cysts is the key for prevention and control of

* Correspondence: vilasmvaidya@gmail.com

the disease. The infected carcasses have to be condemned completely or partially depending on the intensity of cysts, leading to great economic losses. Long-term measures include proper cooking of pork and awareness about consequences of eating uncooked or partially cooked pork (5). Coordinated efforts are required by veterinary and medical fraternities along with the local governing authorities to minimize the prevalence of livestock cysticercosis and human taeniasis, and this can be achieved by effectively using the application of proper diagnostic methods like meat inspection, PCR assays, and serological tests within well-designed, strategic, and feasible control programs.

In view of the above facts, the present research work was conducted in different slaughterhouses, retail shops, and pig farms of Maharashtra to identify the current scenario of porcine cysticercosis by strict postmortem inspection and of human taeniasis by testing different samples using proper methods as well as making demonstrations and distributing booklets to people about hygienic practices to minimize the incidence of the disease.

2. Materials and methods

This study was conducted between April 2010 and March 2017 by examining 13,596 pigs from different slaughterhouses/retail shops and pig farms and 1238 high-risk humans from different areas of Maharashtra by strict postmortem inspection, testing of samples using PCR assays with sequencing of PCR products, and serological methods, i.e. enzyme-linked immunosorbent assay (ELISA), flow-through assay (FTA), and western blot (WB), for the detection of cysticercosis and taeniasis. Simultaneously, efforts were made to conduct various demonstrations for the slaughterhouses/retail shops and farm workers and to distribute booklets in vernacular languages about hygienic practices.

2.1. Postmortem examination, collection, and processing of pig samples

In the slaughterhouses and retail shops, pig carcasses and visceral organs were examined by scientific postmortem inspection. Deep incisions were made at selected sites such as the shoulder muscle, thigh muscle, masseter muscle, neck, diaphragm, liver, and heart to detect cysticerci. The muscles/organs infested with cysticerci were collected, placed in an insulated box containing ice, and brought to the laboratory. The cysts were first separated from adherent host tissues and then collected in cold PBS. Further, cysts were gently washed with cold PBS three times and were stored separately in absolute ethanol at -20 °C for further use.

2.2. Molecular identification and sequence analysis

For molecular identification of *T. solium* (*C. cellulosae*), DNA was extracted from cysts using the DNAsure Tissue Mini Kit (Genetix Biotech Asia Pvt. Ltd., New Delhi, India) as per the manufacturer's guidelines. The quantified DNA was subjected to PCR analysis targeting the *Cox1* and *TBR* genes as per the method described by Sreedevi et al. (6) and Yamasaki et al. (7), respectively. The amplified products were analyzed and confirmed by agarose gel electrophoresis. The obtained PCR products of 7 positive cyst samples of pigs were sent for sequencing to Bio Innovations (Mumbai, India).

2.3. Testing of pig sera by ELISA, FTA, and WB

Collected serum samples from slaughtered and farmed pigs from different areas of Maharashtra were tested using standardized commercially available ELISA kits as per the manufacturer's instruction, standardized FTA as per Sreedevi et al. (8), and standardized WB as per the procedures of Towbin et al. (9), Agudelo-Flórez et al. (10), and Fernando et al. (11) to detect the seroprevalence of the disease.

2.4. Collection of human samples and testing by ELISA and PCR assay

The approval of the institutional human ethical research committee was received before the collection of human samples (i.e. blood and stools). Fresh blood (2 mL) of high-risk humans, i.e. workers in pig slaughterhouses/ retail shops and pig farms, pork consumers, and suspected hospitalized patients, was collected in a sterile vial. The serum was then separated and tested by a commercially available ELISA kit (M/s. Genxbio Health Sciences Pvt. Ltd., Delhi, India) to analyze the seroprevalence.

Stool samples from suspected patients admitted to hospitals were collected aseptically in sterile vials and processed for the isolation of DNA using the Power Fecal DNA isolation kit. The isolated DNA from the stool was further subjected to standardization of the PCR assay using a Gradient Cycler PCR machine (Eppendorf) as per the method of Yamasaki et al. (7) using primers that amplified a target repeated sequence of the cytochrome c oxidase subunit 1 (*Cox1*) gene. The assay was optimized and amplified products were analyzed and confirmed by agarose gel electrophoresis. The PCR products of positive stool samples were sent for sequencing to Bio Innovations.

2.5. Demonstrations for the high-risk workers

A number of demonstrations were organized yearly for workers of Maharashtra State working in various pig slaughterhouses/retail shops and pig farms about practices of personal hygiene, proper disposal of condemned meat/carcasses, and maintenance of good environmental sanitation. Booklets in vernacular languages were distributed for awareness of the disease where the sampling was done. Moreover, demonstrations were organized to teach avoidance of the free range system of rearing and provide information about the healthiness of consuming cooked pork and pork products. Veterinarians were educated about strictly following meat inspection procedures and meat hygiene practices at the abattoirs. The importance of personal hygiene, avoidance of open-air defecation, and use of latrines was also demonstrated.

2.6. Data analysis

All the data obtained from pigs and humans were recorded as shown in the Table and analyzed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). The prevalence (%) of cysticercosis was calculated with the formula described by Thrusfield (12). Pearson's chi-square (χ^2) statistical test was applied to determine the year-wise linear trend in percent prevalence of cysticercosis in pigs by postmortem examination and taeniasis in human by ELISA as per the EpiTools Epidemiological Calculator available at http://epitools.ausvet.com.

	Pigs	Humans					
Year	% Prevalence by postmortem examination	% Prevalence by PCR assay	% Prevalence by ELISA	% Prevalence by FTA	% Prevalence by Western Blot	% Prevalence by ELISA	% Prevalence by PCR assay
2010-11	1.09 (44/4042)	ND	ND	ND	ND	8.75 (7/80)	ND
2011-12	1.02 (1/98)	ND	ND	ND	ND	6.52 (6/92)	ND
2012-13	1.00 (13/1291)	1.00 (13/1291)	ND	ND	ND	3.87 (7/181)	ND
2013-14	0.98 (18/1820)	ND	ND	ND	ND	2.51 (13/517)	2.04 (2/98)
2014-15	0.82 (12/1460)	0.75 (7/937)	7.97 (11/138)	ND	ND	2.13 (4/188)	ND
2015-16	0.74 (15/2036)	ND	6.25 (10/160)	ND	ND	1.98 (2/101)	ND
2016-17	0.60 (17/2849)	ND	5.75 (13/226)	5.31 (12/226)	5.31 (12/226)	0 (0/79)	ND
Total	0.88 (120/13596)	0.9 (20/2228)	6.49 (34/524)	5.31 (12/226)	5.31 (12/226)	3.15 (39/1238)	2.04 (2/98)

Table. Prevalence of cysticercosis in pig and humans.

*ND: Not done.

3. Results

3.1. Prevalence of cysticercosis in pigs

Every year postmortem examinations of pig carcasses/ visceral organs at different slaughterhouses/retail shops of Maharashtra State were carried out. Details of the carcasses examined and the positive cyst samples obtained are given in parentheses in the Table. Overall prevalence during the study period was found to be 0.88% in slaughtered pigs by postmortem inspection. The extracted DNA from cysts was subjected to PCR analysis with two sets of oligonucleotide primers based on amplification of the DNA for the identification of *T. solium* from the infected pigs. PCR products with molecular sizes of 286 and 984 bp were amplified, which target large subunit rRNA (*TBR*) and cytochrome c oxidase subunit 1 (*Cox1*) genes, respectively (Figure 1). Year-wise percent positivity of the cysticerci by postmortem examination and the percent prevalence of cyst samples by PCR are depicted in the Table and it was noted that year-wise prevalence of the disease was decreasing in Maharashtra. The results indicated that PCR assay is a valuable diagnostic tool for confirmation of cysticerci in pigs detected during postmortem inspection. Seven PCR-positive samples were confirmed by sequencing at species level as *T. solium* and *T. asiatica*. Sequences were matched using NCBI BLAST software and aligned and compared with previously published sequences of *T. solium*. Isolation of *T. asiatica* in a pig cyst sample seems to be the first such report in India. Resulting nucleotide data were submitted

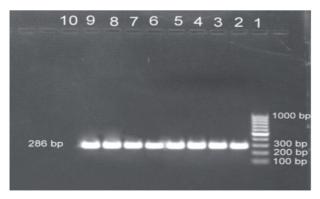


Figure 1. PCR assay with TBR primers to detect *C. cellulosae* in pig meat. Lane 1: 1000-bp DNA ladder, lanes 2–9: positive test samples extracted from cysticercosis-positive pig meat, lane 10: negative control.

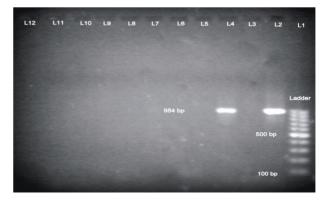


Figure 2. PCR assay with Cox1 primers to detect *T. solium* from humans. Lane L1: 100-bp DNA ladder, lanes L2 and L4: positive stool samples from seropositive humans, lanes L3 and L5–L11: negative stool samples from seropositive humans, lane L12: negative control.

to GenBank with an accession number from NCBI for one pig cyst sample of KR701908.

Sera samples of pigs were tested by standardized ELISA, FTA, and WB. The seroprevalence study of cysticercosis by these methods is depicted in the Table. Among the three serological tests used in 2016 and 2017, it was found that commercially available ELISA was more sensitive than FTA and WB.

3.2. Prevalence of taeniasis in high-risk humans

Aseptically processed serum samples were examined by ELISA and the overall seropositivity in high-risk groups was found to be 3.15%, although there was a year-wise decrease in the prevalence as shown in the Table. None of the sera samples for the year 2016–17 were found to be positive. Stool sample analysis by PCR assay revealed 2.04% prevalence of taeniasis in patients from hospitals (Figure 2). Both of these two PCR-positive samples were confirmed by sequencing at species level, i.e. *T. solium*, using NCBI BLAST software.

3.3. Awareness campaign for high-risk humans

Awareness was created among different workers involved in slaughtering and farming of pigs by conducting various demonstrations/campaigns in vernacular languages in every visit to the different areas of Maharashtra State where the sampling was done. In some of the endemic areas initially more demonstrations were given for improvement of the health status of workers as well as animals and for sanitary practices. It was observed that day by day people became more aware of the disease and have started applying hygienic practices in pig farming as well as in slaughtering procedures. At the end of the study, satisfactory observations with regards to the improved health status of workers and sanitary conditions in pig farms and slaughterhouses/retail shops were noted.

3.4. Data analysis

Pearson's chi-square (χ^2) statistical test was used to calculate the year-wise linear trend in percent prevalence of cysticercosis in pigs and taeniasis in humans with P-values of 0.4619 and 0.0122, respectively, which showed a nonsignificant difference at the 5% level of significance.

4. Discussion

The life cycle of the parasite is sustained in pigs because of the coexistence of poor sanitary conditions, free range management of pigs, and inadequate meat inspection. Considering the public health significance of the disease, a periodic study was carried out for detection of cysticercosis in pigs as well as taeniasis in high-risk humans in different parts of the state using different diagnostic methods to detect the exact epidemiological status of the disease in a particular area, so that effective prevention and control measures could be undertaken to eradicate the disease.

The available literature in India showed that there is a regional variation in the prevalence of porcine cysticercosis, which was also observed in our study. Year-wise prevalence of cysticercosis recorded in the current study by postmortem examination is considered to be distinctly low as compared to Deka et al. (13), who reported 20.8% from Guwahati, Assam. Pathak et al. (14) reported 17% while Pathak and Gaur (15) reported 9.33% prevalence of cysticercosis in Uttar Pradesh. D'Souza and Hafeez (16) reported 4.33% to 8.66% prevalence in pigs slaughtered in different districts of Andhra Pradesh. Sharma et al. (17) reported 6.35% prevalence of cysticercosis in pigs slaughtered in Ludhiana, Punjab. Hafeez et al. (18) recorded 3.52%, 5.50%, 5.73%, and 5.38% positivity in the states of Andhra Pradesh, Tamil Nadu, Karnataka, and Kerala, respectively. Moreover, in Maharashtra, Kulkarni (19), Shinde (20), and Munde (21) reported a prevalence of 2.85%, 6.02%, and 1.78% of C. cellulosae, respectively, in pigs slaughtered at the Deonar abattoir in Mumbai.

The prevalence of cysticercosis in pigs by PCR assay was recorded to be 1% and 0.75% for the years 2012–13 and 2014–15, respectively, as shown in the Table. Application of PCR assay for validating meat inspection results was also done by Sreedevi et al. (6), who stated that PCR assay proved to be a successful tool for an objective confirmation of postmortem examination of *T. solium* in slaughtered pigs.

Seroprevalence of cysticercosis in pigs by ELISA kit was done for a consecutive 3 years and in 2016-17 the positive sera were again confirmed with FTA and WB. Results of the present study correlate with those of Pondja et al. (22), who reported variation from 5.6% to 66.7% in Mozambique, whereas Porphyre et al. (23) noted 2.3% to 2.6% prevalence in Antananarivo abattoirs. In India, our findings also correlate with the work of Pathak et al. (24) for ELISA, Sreedevi et al. (8) for FTA, and Palampalle (25) for WB, who reported similar observations with these methods for the diagnosis of cysticercosis in pigs. Hafeez et al. (18) noted 6.50%, 6.22%, 6.40%, and 6.50% of sera samples to be positive in Andhra Pradesh, Tamil Nadu, Karnataka, and Kerala, respectively, by ELISA, and these results are in agreement with our findings. The use of various serological tests in our study indicated that these assays are suitable for the serosurveillance of large numbers of samples in endemic areas.

Prevalence in high-risk humans in the present study was found to be less than that reported by Vora (26), who recorded 22.4% prevalence in humans of rural Goa. In another study, Mahajan et al. (27) reported a prevalence of taeniasis ranging from 0.5% to 2% in hospitalized patients in northern India and 12% to 15% in labor colonies where pigs were raised. This PCR study noted positivity in two stool samples obtained from suspected patients, which showed seropositivity with the ELISA kit. The observations noted by Davaasuren et al. (28) using multiplex-PCR in fecal samples from taeniasis patients in Mongolia are higher than those of the present study.

The emergence or reemergence of Taenia spp. infection in pigs is influenced by several risk factors, mainly poor sanitation, a free-roaming pig rearing system, and their slaughter within the household under unsuitable conditions (29). Main causes for the persistence of the disease in endemic areas include lack of hygiene in rural communities, inadequacy of education, and insufficient authority for controlling the pork market. In our study, various risk factors including personal hygiene, improper disposal of animal waste, environmental sanitation, free range rearing of pigs, and inappropriate meat inspection practices were identified. By considering these risk factors, consistent efforts were made by our research team for successful creation of awareness in high-risk humans for the betterment of animal and human health. Also, because of the initiative of the Government of India since 2014 with the 'Clean India' campaign and provision of financial assistance to the poor to build toilets to avoid openair defecation, environmental sanitation has improved.

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Therefore, this study found that the combination of the various above-mentioned efforts could help to decrease the prevalence of disease in human and pig populations.

In conclusion, cysticercosis/taeniasis is a neglected zoonotic disease that is an important animal and public health concern. The present study showed a decreasing trend of the prevalence of the disease in both human and pig populations using various diagnostic methods, i.e. postmortem findings and molecular and serological methods, over a span of 7 years. Reduction in prevalence was achieved by successfully creating awareness among high-risk humans about hygienic measures and through the 'Clean India' campaign initiated by the Government of India. Continuous efforts are required for monitoring the disease and proper implementation of veterinary and public health interventions to minimize the incidence in endemic regions.

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