Original Research

Studies on Occurrence of *Trichinella* spp. in Pork Meat Sold in Goa and Assam States of India

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Abstract

Trichinellosis is rare but an important zoonotic disease around the world which occurs due to consumption of raw or under cooked pork infected with parasitic larvae of the genus Trichinella. The occurrence of Trichinellosis in domestic animal populations is mainly due to poor management practices which allow them to feed on meat infected with Trichinella. Certain parts of the India such as Goa and Assam are reported to have high demand for pork and pork products which is met through good piggery farming system. However, no significant study has been carried out on occurrence of trichinellosis in these states. Considering the above facts, the present study was carried out to know the exact burden or existence of Trichinellosis in pigs of Goa and Assam states of India. A total of 421 samples (diaphragm and tongue/masseter muscle) were collected from different retail pork shops from Goa (n=346) and Assam (n=75). The samples so obtained were subjected to Acid-pepsin digestion assay for the detection of Trichinella larvae. None of the samples were found to be positive for Trichinella spp. indicating very low or zero occurrence in Goa and Assam. Further studies should direct towards continuous monitoring and surveillance of trichinellosis in pigs, to declare pig industry in these regions free from Trichinella.

Key words: Pork, Acid-pepsin digestion assay, Trichinellosis, Occurrence

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Introduction

Trichinellosis is an important meat borne parasitic disease of zoonotic importance which occurs due to the consumption of raw or under cooked pork (OIE 2008; Gottstein *et al.*, 2009) infected with the nematode larva called namely *Trichinella*. The genus *Trichinella* has been placed under the Phylum; Nemathelminthes, Class; Nematoda, Order; Enoplida, Superfamily; Trichuroidea and Family;

Trichinellidae (Soulsby, 1982). Though pork consumption is negligible in overall India, there is an increase demand in certain parts of the country such as Karnataka, Andhra Pradesh, Tamil Nadu, Kerala, Bangalore, Assam and Goa (Suri, 2012). The total contribution of pork production in India is 5.23% of the total world production, of which free scavenging pigs contribute to the major portions. Due to free rearing of pigs, these animals have access to raw (meat-containing) waste products or access to wildlife, wildlife carcasses, or rats (Gottstein *et al*., 2009) infected with *Trichinella* larvae and could pose as an important risk factor for the occurrence of trichinellosis in humans (Singh *et al*., 2013).

In animals, the parasite causes no clinical symptoms whereas in humans, the symptoms are fever, muscle soreness and pain, gastrointestinal symptoms, facial oedema and retinal haemorrhages (Gottstein *et al*., 2009). Studies are being carried out all over the world regarding the occurrence of trichinellosis in pigs and humans. However, the disease is poorly studied in India. Report on occurrence of *Trichinella* larvae in domestic pigs in India was reported for the first time by Niphadkar *et al*. (1979). Several studies across the country is carried out on occurrence of trichinellosis in humans (Mohan *et al*., 2002 and Sharma *et al*., 2014). Recent outbreaks of human trichinellosis in Uttarakhand state has resulted in the death of 11 people (Sethi *et al*., 2013) that has alarmed the countries public health department and pork industry.

Although infection with *Trichinella* is globally distributed (FAO/WHO/OIE, 2007), its prevalence in India is poorly understood. Hence considering the above facts, the present study was undertaken with an objective to know the occurrence of the disease in Goa and Assam states

Materials and Methods

The survey was conducted for a period of six months i.e. from March-2017 till August 2017. Collectively 346 diaphragm and tongue samples were collected from four different pork retail shops from Goa. In Assam a total of 75 diaphragm and tongue samples were collected from local markets placed at two different districts. The samples were collected at an interval of every two months with an average of 100 samples from Goa, whereas in Assam state samples were collected only once. The data regarding details of the samples collected is shown in Table 1. The present study was carried out at Department of Veterinary Public Health, Bombay Veterinary College, Mumbai. A total of 421 pig samples (diaphragm and tongue/masseter muscle) were collected aseptically from different retail pork shops (n=46) of local markets from Goa (n=346) and Assam (n=75). History regarding source of pigs was taken from butchers. The samples collected aseptically were packed in polyethylene bags and transported to laboratory under cold conditions and stored at -18^{0}C till further processing. The samples were thawed to a temperature of 4-8^{0}C and then subjected to processing for the detection of *Trichinella* spp. larvae.

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Table 1: Details of pig samples collected from pork retail shops of Goa and Assam

S. No.	Source of Sample		Total no. of slaughtered pigs inspected	Number of shops	No. of samples collected			
					Diaphragm		Tongue	
					M	F	M	F
1	Goa	South Goa	213	20	137	76	13	10
		North Goa	98	8	58	40	12	0
2	Assam	Kamrup	46	12	34	12	5	3
		Marigaon	21	6	13	8	0	0
	Grand Total		**378**	**46**	**242**	**136**	**30**	**13**

The samples were subjected to Acid-pepsin digestion assay as per the protocol of OIE (2012) for identification of *Trichinella* larvae. From each sample, approximately 5 g of the tissue was weighed and triturated using pastel and mortal for which 250 ml of 0.55% Acid (Conc. HCl) and 0.5 g Pepsin (1:10000) was added. The resultant fluid/Digests was transferred into beaker and subjected for muscle disintegration on a magnetic stir plate at 45° C for 30 min for separation of larvae from muscle. At the end of 30 min, the digest was allowed to settle and the supernatant was decanted. The sediment was poured through a sieve into separatory funnel and allowed to settle for 30 min. Around 10 ml of the sediment fluid was transferred on to petri dish and examined under trichinoscope at 4X magnification (Fig. 1, 2 and 3).

Fig.1: Diaphragm sample

Fig.2: Homogenization of sample

Fig.3: Trichinoscope

Fig. 1, 2 and 3: Images showing procedure of Acid pepsin digestion assay

Results and Discussions

Trichinellosis is an important meat borne anthropo-zoonotic parasitic disease caused by consumption of raw or under cooked meat of domestic and wild animals infected with larvae belonging to the genus *Trichinella*. Trichinellosis in domestic animals, wild animals and humans has been reported across the globe and it is considered as an emerging or re-emerging food-borne disease of zoonotic importance. India has a rich livestock resource with 13.5 million pig population (Agricultural Research Data Book, 2007). Each year 84% pigs are slaughtered compared to 6% cattle, 11% buffaloes, 33% sheep and 38% goats. The major

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proportion of pork production in India comes from unorganized sector comprising of free ranging and backyard pig farming, but in Goa and Assam pig rearing farming system is well established. These free ranging and back yard pig production is at particular risk of acquiring the *Trichinella* infection as they have a more probability of getting exposed to source of infection like infected meat scraps and garbage. Estimate of loss to Indian pig production industry due to trichinellosis cannot be made, since no systematic data on prevalence or occurrence of trichinellosis at national level are available.

Hence, the present study was carried out to know the probable level of trichinellosis in two Indian states namely Goa and Assam as they are largest pork consumers. A total of 421 samples (diaphragm and tongue/masseter muscle) were analyzed from different retail pork shops from Goa (n=346) and Assam (n=75). History of the sourcing of pigs indicate that, pigs were procured from the exclusively stall fed pigs farms, pigs are rarely sourced from the traders selling free range pigs. The samples so obtained were brought to Department of Veterinary Public Health, Bombay Veterinary College in chilled condition and subjected to Acid-pepsin digestion assay as per the protocol of OIE (2012) for the detection of *Trichinella* larvae.

It is observed that the Trichinoscopy examination of the digested sediment by Acid- Pepsin method usually reveals a comma shaped or C- shaped motile or non-motile larvae of *Trichinella* in the positive cases. But to our surprise none of the samples were found to be positive for *Trichinella* spp. indicating zero occurrence of the trichinellosis in both the states i.e. Goa and Assam. Similar results have been observed by Konwar *et al.* (2017) who could not able to isolate *Trichinella* from pigs of Assam region but observed seropositivity of 2.87 percent by ELISA. Whereas, there is no single study report on *Trichinella* from pigs of Goa. Similarly, a survey has been conducted before and after 1979 in other parts of the country. However, only few positive cases have been detected.

The studies conducted by Shastri (1966) in Bombay and Ramamurthi and Ranganathan (1968) in Madras, respectively could not isolate *Trichinella* from swine carcasses. In another study Gaurat (2005) who examined 501 pig carcasses for *Trichinella* infection could not get any positive case; Contrarily, Pethe (1991) could identify three *Trichinella* positive pork carcasses out of 500 samples screened. Recently Jundale, (2015) and Panchal, (2016) have reported one and three pig samples positive out of 372 (0.27%) and 348 (0.86%) carcasses, respectively screened in Maharashtra state. Several studies have reported the failure of Acid-pepsin digestion assay to detect *Trichinella* larvae this could be attributed to low sensitivity of the technique i.e. directive 77/96/EEC on Pepsin digestion test has a confirmed detection limit of 1-3 larvae/g (Karn, 2007; Konwar *et al.*,2017).

In our study, we employed only Acid-pepsin digestion assay to detect *Trichinella* larvae; several other techniques such as serological assay and molecular assay has proven their efficiency in detection of the parasite as in comparison to Acid pepsin assay (Faubert *et al.*,1985; Frey *et al.*, 2009; Richomme *et al.*, 2010). Further studies should direct towards detection of the parasite by the other methods whose sensitivity

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is more. Study conducted by Murrel *et al*. (2000) has shown positive co-relation between pigs and rodents with respect to the outbreak of trichinellosis. On the other hand, the study samples were collected from organised commercial pig farms which were fed on rice bear waste, rice bran and kitchen waste. These commercial stall-fed animals have less or no access to the reservoirs of *Trichinella* infection like rats or wild pigs. This could also be one of the reason for negative results. Further, seasonal variation in trichinellosis (for absence or presence) could not be derived since the studies were conducted for a short period of time. Negative results through Acid pepsin digestion assay during the period of this study do not assure that the surveyed regions are free from *Trichinella* spp. To prove that *Trichinella* is not prevalent in these states, it requires the regular monitoring and surveillance of large number of pig in population by various techniques like ELISA, Acid pepsin digestion assay and molecular diagnosis.

Despite of having generous information on biology, life cycle pattern and geographical distribution, trichinellosis is still a serious problem worldwide. Since the time of its discovery till date, the parasite has been isolated from many mammalian, avian and reptile species. This parasitic zoonosis is not only a public health hazard affecting human patients but also rises economic concern (Gottstein *et al.*, 2009). The epidemiology of the disease could be attributed to several factors like socio-economic background, cultural habits, political factors, geographic location and migration of humans and animals. Further good farm practices, strict post-mortem inspection of pork and game meat, avoidance of consumption of raw or semi-cooked pork/, thorough cooking of meat, effective freezing of pork (-15°C for not less than 3 weeks) and prompt field diagnosis using serological tests helps in proper monitoring and control of this disease.

Conclusion

Zoonotic trichinellosis remains largely underreported and under diagnosed in Indian scenario. Lack of strict meat inspection, consumption of undercooked meat, unawareness regarding the disease and poor epidemiological data has resulted in the recent outbreaks that have alarmed the countries public health administration. As none of the samples were found to be positive for *Trichinella* spp. indicating zero occurrence of the trichinellosis in both the states i.e. Goa and Assam. Keeping in view of the above facts, regular monitoring and surveillance of the parasite has to be carried in these states to achieve *Trichinella* free status.

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Conflict of Interest

There is no conflict of interest.

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