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Profile of major pork associated zoonotic parasites from northern India: A detailed seminal study

RAVI PRATAP CHAUHAN[1], HIRA RAM[1]✉, R PANDA[1], A K NEHRA[1], T S VINAY[1], RAJAT GARG[1], P S BANERJEE[2], M KARIKALAN[1], A K SHARMA[1] and R K SINGH[1]

ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

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ABSTRACT

The present study was undertaken to document the status of three major pork associated zoonotic parasites, viz. *Taenia solium cysticercus, Sarcocystis suihominis* and *Trichinella* spp., in two north Indian states (Uttar Pradesh and Uttarakhand). Masseter, diaphragm, tongue and oesophageal muscles were collected from 450 pigs (domestic: 445, wild: 05) and examined during November 2014 to October 2017. Muscle tissue samples were initially screened by visual inspection followed by the muscle compression technique for visualization of the parasites. Tissue samples were also subjected to acid-pepsin digestion for the recovery of *Trichinella* spp. larvae. Out of the 450 pigs examined, *T. solium* cysticercus was detected in 41 (9.11%) samples, whereas sarcocysts were detected in 315 (70%) samples. Nurse cell bearing *Trichinella* spp. larvae were recorded in the muscle tissues of a single (0.22%) wild boar and none of the muscle tissue samples from domestic pigs were positive for *Trichinella* spp. infection. However, the exact species of *Trichinella* infecting wild boar could not be established morphologically. The findings of the study are of substantial importance for understanding the maintenance and distribution of zoonotic parasites transmitted through pork consumption in the country.

Keywords: *Cysticercus*, India, Pork, *Sarcocystis*, *Trichinella*

India has about 9.06 million pigs, out of which 80% are of indigenous/ non-descript breeds that are mostly reared under low input driven production systems. The remaining 20% pigs are exotic breeds/ crossbreds that are mostly managed at the semi-commercial/ commercial pig farms (20th Livestock Census). In low input driven pig production system, the animals are mostly housed near the human dwellings and are allowed to scavenge on garbage dumps and their feed is supplemented with locally available grains, vegetables, agricultural by-products and kitchen waste (Prakash *et al*. 2007). This makes them susceptible to various bacterial, viral and parasitic diseases, many of which are of zoonotic importance.

The major pork associated parasites of zoonotic importance include, protozoa like *Sarcocystis suihominis*, *Toxoplasma gondii*, and the helminths like *Taenia solium* and *Trichinella spiralis* (Djurkovic-Djakovic *et al*. 2013, Sharma *et al*. 2020). These parasitic infections are common in socio-economically weaker sections of the society in developing countries including India, where cultural, social and hygiene related factors are responsible for their maintenance (Kulkarni and Kumar 2014, Singh *et al*. 2018). Amongst the major zoonotic parasites of pork origin, *T. solium* cysticercosis is endemic in pigs and has been reported from several parts of India (Varma and Ahluwalia 1989, D'Souza and Hafeez 1999, Prasad *et al*. 2002, Hafeez *et al*. 2004, Sharma *et al*. 2004, Borkataki *et al*. 2012, Mohan *et al*. 2013, Saravanan *et al*. 2014, Singh *et al*. 2018). *Sarcocystis* spp. infection has also been reported in pigs from different parts of the country (Saleque and Bhatia 1991, Solanki *et al*. 1991, Prasanth, 1995, Avapal *et al*. 2004, Kaur and Bal 2016). Prevalence of *Trichinella* spp. infection in pigs is poorly explored with only two reports in domestic pigs from India (Niphadkar *et al*. 1979, Kumar *et al*. 2015). Most of the above studies have targeted individual parasite, and there is a scarcity of literature on the composite profiling of pork associated zoonotic parasites. Therefore, the present study aimed to record the status of pork associated zoonotic parasites in pigs of two North Indian states namely, Uttar Pradesh and Uttarakhand.

MATERIALS AND METHODS

Study area: The study area included two north Indian states [Uttar Pradesh (UP) and Uttarakhand (UK)] that are well known for the vast biodiversity in terms of flora and fauna. There are a total of 7 National Parks (UP-1, UK-6) and 32 Wildlife Sanctuaries (UP-25, UK-7) in these two states with a demarcated area of 13923.85 square kilometers (http://www.natureconservation.in). Domestic pig rearing

Present address: [1]ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh. [2]Eastern Regional Station, ICAR-Indian Veterinary Research Institute, Kolkata, West Bengal. ✉Corresponding author email: hiraram.35@gmail.com

in these states is based mainly on traditional system with focus on low input driven farming. Besides this, a fair population of wild boars (*Sus scrofa*) is also spread in both states, especially inside the reserve forests demarcated for conservation of a variety of wild animals.

Collection of tissue samples: During November, 2014 to October, 2017, muscle tissue samples (oesophagus, diaphragm, tongue, heart and skeletal muscle) were randomly collected from 445 adult domestic pigs (non-descript) slaughtered at local abattoirs located in both the states (UP-375, UK-70) and from five adult wild boars that were received at the Wildlife Centre of the institute from the forest department of UP for parasitological investigations. The collected samples (~40 g of each muscle from each pig) were brought to the Parasitology Division of the institute on ice in sterile plastic bags and stored at 4°C till further laboratory investigations were conducted. This study has ethical permission from the Institute Animal Ethics Committee for conducting research work in the Project No. IVRI/PARA/14-17-004.

Laboratory investigations: The muscle tissue samples were incised to detect *Taenia solium* cysticerci. Thereafter, small pieces (3-5 mm) of individual muscles were cut, pressed between two glass slides and examined under low magnification of a stereo-microscope for the detection of sarcocysts and nurse cells or larvae of *Trichinella* spp. (Luzon *et al*. 2015). Trypsin-EDTA digestion method was employed to recover tissue cysts (sarcocysts) of *Sarcocystis* spp. (Banerjee 1998), whereas, artificial muscle digestion technique (acid-pepsin digestion) as suggested by ICT (http://www.trichinellosis.org/)wasperformedtorecoverthe *Trichinella* larvae from individual tissue sample (20 g each). Following digestion, sarcocysts or larvae were collected by repeated sedimentation and washing. The recovered larvae of *Trichinella* spp. were counted in the sediment and larval load in terms of larvae per gram of muscle tissue (lpg) was calculated. Recovered Sarcocysts and *Trichinella* larvae were examined microscopically for their morphological characteristics for identification of the species.

Statistical analysis: The results obtained on the incidence of pork associated zoonotic parasites in Uttar Pradesh and Uttarakhand were compared by Chi-square test (Snedecor and Cochran 1994).

RESULTS AND DISCUSSION

The results of laboratory investigations carried out on muscle tissues collected from the pigs of two states are given in Table 1. Gross examination of muscle tissues revealed presence of *T. solium* cysticercus in 9.11% (41 out of 450) of domestic pigs screened. Cysticerci were recovered from tongue, oesophageal and skeletal muscles only. All the cysticerci recovered from muscles were fully viable. None of the wild pig muscle tissues examined were harbouring *T. solium* cysticerci. Incidence of cysticerci in pig was significantly higher ($p<0.05$) in Uttar Pradesh (10.4%) than in Uttarakhand (2.85%).

Microscopic examination of muscle tissues (masseter, diaphragm, tongue, oesophagus) of each animal by compression technique revealed presence of sarcocysts in 53.78% pigs (240 domestic and 2 wild). Trypsin-EDTA digestion method revealed presence of sarcocysts in 70% pigs (312 domestic and 3 wild). Incidence of sarcocysts in pig was significantly higher ($p<0.01$) in Uttar Pradesh (72.53%) than in Uttarakhand (57.14%). Morphological identification revealed that the recovered microsarcocysts belonged to *S. suihominis* and *S. miescheriana* (Fig. 1). Single species infection with *S. mischeriana* was recorded in 17.78% (56 out of 315), *S. suihominis* in 35.24% (111 out of 315), while a mixed infection of both species was recorded in 46.98% (148 out of 315) pigs that were positive for sarcocysts.

Microscopic examination of muscle tissues of each pig by compression technique and acid-pepsin digestion revealed presence of nurse cells/ larvae of *Trichinella* spp. in one wild boar (0.22%) only. The concentration of *Trichinella* spp. larvae was maximum in masseter (8 larvae/g muscle), followed by diaphragm (5 larvae/g muscle), tongue (4 larvae/g muscle) and intercostal muscles (3 larvae/g muscle). Morphological variations in the shape of larvae (thick and thin) were noted, but the species of *Trichinella* infecting pigs in the areas under study could not be established morphologically. None of the domestic pig muscle tissues examined was harbouring nurse cells/ larvae of *Trichinella* spp.

Consumption of raw or undercooked pork poses a high risk to humans as it may lead to transmission of zoonotic parasites like *Taenia solium* cysticercus, *Sarcocystis suihominis* and *Trichinella* spp. Higher prevalence of these zoonotic infections in developing countries is associated with poverty, lack of personal hygiene, open defecation practices, scarcity of potable water, abundance of stray animals, high population density and certain culinary

Table 1. Screening of pig muscles for detection of zoonotic parasites

Origin of samples	Type of pig	Number of pigs screened	*T. solium cysticerci* +ve	*Sarcocystis* sp. +ve		*Trichinella* sp. +ve	
			(Visual inspection)	(Compression technique)	(Trypsin-EDTA digestion method)	Compression technique	Acid-pepsin digestion
Uttar Pradesh	Domestic	375	39[a] (10.4%)	214[a] (57.07%)	272[x] (72.53%)	-	-
	Wild	5	-	2 (40%)	3 (60%)	1 (20%)	1 (20%)
Uttarakhand	Domestic	70	2[b] (2.85%)	26[b] (37.14%)	40[y] (57.14%)	-	-
Total		450	41 (9.11%)	242 (53.78%)	315 (70%)	1 (0.22%)	1 (0.22%)

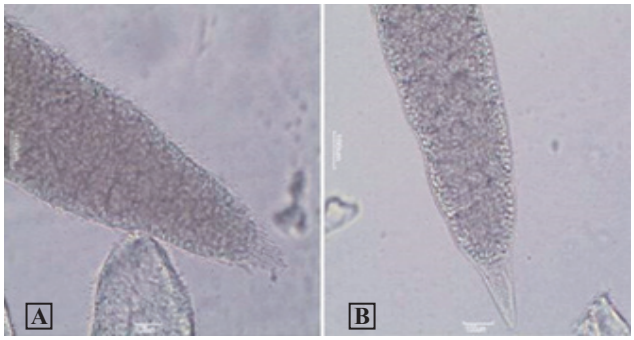

Fig. 1. Sarcocyst of *Sarcocystis suihominis* (A) and *S. miescheriana* (B).

habits of people (Singh *et al*. 2010). In the present study, *Taenia solium* cysticercus was recorded in 9.11% pigs, with a significantly ($p<0.05$) higher incidence of infection in Uttar Pradesh (10.13%) as compared to Uttarakhand (2.85%). Similarly, a significantly higher ($p<0.05$) incidence of *Sarcocystis* spp. infection was recorded in Uttar Pradesh (72.37%) as compared to Uttarakhand (57.17%). The microsarcocysts recovered from muscle tissues were morphologically identified as *S. suihominis* and *S. miescheriana*, out of which the former is of zoonotic potential with man acting as a definitive host. Humans can also act as intermediate host for *S. suihominis* and acquire the infection by ingesting food or water contaminated with oocysts excreted in the faeces of infected animals. Pigs may acquire these infections by scavenging on human excreta or feed and water infected with *T. solium* eggs or *Sarcocystis* spp. oocysts. Lower incidence of these infections in Uttarakhand may be due to the hilly terrain of the state that makes it less conducive for scavenging of free-range pigs. Also, the human population density (censusindia.gov.in) and pig population (http://dahd.nic.in) in Uttarakhand is much lower (189 persons/km^2, .002 million pigs) than Uttar Pradesh (828 persons/km^2, 1.33 million pigs), which may affect the transmission dynamics of the infection. High prevalence of porcine cysticercosis (1.7% to 20.8%) and sarcocystosis (more than 65%) have been reported from north Indian states (Gupta and Gautam 1984, Pathak *et al*. 1984, Deka *et al*. 1985, Agnihotri *et al.* 1987, Varma and Ahluwalia 1989, Saleque and Bhatia 1991, Prasanth 1995, Prasad *et al*. 2002, Avapal *et al*. 2003, 2004, Sharma *et al*. 2004, Singh *et al*. 2018). Besides this, porcine neurocysticercosis has been reported in 3% free range pigs slaughtered for human consumption at a local abattoir of Uttar Pradesh (Prakash *et al*. 2007). Prasanth (1995) also reported prevalence of *S. suihominis* (48.9%) and *S. miescheriana* (40.2%) from pigs of Western Uttar Pradesh.

In the present study, *Trichinella* spp. larvae were recovered from the muscle tissues of a wild boar that was submitted by the Forest Department of Pilibhit district of Uttar Pradesh. This forest reserve holds a very good population of wild boars and other wild animals including the tigers (*Panthera tigris*) and leopards (*Panthera pardus*). Since, these big cats frequently predate on wild boars in the forest reserve, it is speculated that *Trichinella* spp. infection may also be present in other carnivores of the forest. Sporadic reports on *Trichinella* spp. infection in different animal species like cats (Maplestone and Bhaduri 1942, Kalapesi and Rao 1954), civet cats (Schad and Chowdhury 1967, Parmeter *et al*. 1968), rodents (Niphadkar 1973), domestic pigs (Niphadkar *et al*. 1979) and humans (Alipuria *et al*. 1996, Sethi *et al*. 2010) from India are on records. A 0.69% prevalence of *Trichinella* spp. was also recorded in domestic pigs slaughtered in the Maharashtra state of India (Kumar *et al*. 2015).

The species of *Trichinella* infecting the wild boar could not be established because it is not possible to differentiate the larvae of *Trichinella* spp. on the basis of morphology. However, the recovered larvae were encapsulated. Based upon their capability of encapsulation in the muscles of the host, the larvae recovered belonged to the encapsulated clade (*T. britovi*, *T. murrelli*, *T. nativa*, *T. nelsoni*, *T. patagoniensis, T. spiralis* and 3 genotypes T6, T8 and T9).

The concentration of *Trichinella* spp. larvae recorded in the study ranged from 3 larvae/ g of intercostal muscle to 8 larvae/g of masseter muscle of wild boar. It has been estimated that ingestion of 100 *Trichinella* larvae can cause a clinical disease in humans (FAO/WHO 2013) and an intake of more than 500 larvae is fatal (Murrell 1985, Battelli *et al*. 1994, Oivanen 2005). If we extrapolate the larval concentration in muscles recorded in present study (average 5 larvae/gram of muscle tissue), then about 100 g of infected muscle, if consumed as raw or semi-cooked, will be sufficient to cause a clinical disease in humans in the study area. Multiple outbreaks of trichinellosis in humans in the remote areas of Uttarakhand, resulting in moratlity of 11 humans, have been reported following consumption of wild boar meat (Sethi *et al*. 2013).

Thorough meat inspection should be done during slaughter of food animals in order to prevent pork-borne parasitic infections in humans. Further, more extensive and systematic epidemiological survey needs to be undertaken in order to address the distribution pattern of these parasites in pigs (including wild boars) as well as humans.

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