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SEROPREVALENCE OF *TRICHINELLA SPP.* IN PIGS AND KNOWLEDGE, ATTITUDE AND PRACTICES OF PIG FARMERS OF EASTERN AND MIDWESTERN REGIONS OF NEPAL

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Abstract

A cross-sectional study was conducted on four major pig raising districts of eastern and mid-western region of Nepal from February to May 2014 to find out the seroprevalence of *Trichinella spp.* A total of 184 serum samples of pigs were collected and antibodies against *trichinella* were detected using ID screen *trichinella* indirect multi-species ELISA kit. The study revealed prevalence rate of 3.8% and difference in prevalence rate according to age, sex, breed, rearing system, ecozone, region & district were statistically insignificant ($P > 0.05$) as analysed by Chi-square test using PHStat version 2.5 and Fisher's exact test. This study confirms that antibodies of *Trichinella spp.* are circulating in pigs of Nepal. Further, the knowledge, attitude and practices survey of meat borne helminthic zoonoses was conducted among 50 pig raisers and pork consumers by face to face interview using a semi-structure questionnaire. This survey concludes that although there were significant portion of the respondents aware of meat borne helminthic zoonoses but there were still a noticeable proportion of respondents who didn't have a proper knowledge that upsurge public health risks. Moreover, present-day situation of their pig raising practices & pork consumption system possess them to a menace of public health zoonoses.

Keywords: *Trichinella*; ELISA; Eastern & Midwestern Nepal; Zoonoses

Introduction

Trichinellosis is a meat borne parasitic zoonoses caused by nematode *Trichinella spp.* and transmitted by eating raw or undercooked pork meat containing viable larva of the parasite. *Trichinella spp.* are intracellular parasites of vertebrates with an entire life cycle confined to the host. It remains a serious public threat in both developed and developing countries (Murrell and Pozio, 2000; Liu and Boireau, 2002). There are eight species and three morphologically indistinguishable additional genotypes in the genus (Murrell *et al.* 2000; Pozio *et al.*, 2000). *Trichinella spiralis* (genotype-T1) is the best known species, with high infectivity to swine and rats, with cosmopolitan distribution because of passive introduction with domestic pigs and synanthropic rats. Most infections in humans, domestic pigs, and synanthropic rats are related to this pathogen. *Trichinella spiralis* is an intracellular parasite specific for mammalian skeletal muscle. The infection occurs through the consumption of raw or undercooked meat from a wide variety of wild and domestic pigs (Dupouy-Camet, 2000).

In Nepal, majority of the pig population in Nepal are raised under free ranging condition under unhygienic and improper husbandry conditions. Free ranging "back yard" pigs and the practice of feeding offal is a very common management practice which potentially allows the transmission of trichinellosis. The certain ethnic groups in Nepal (Rai, Limbu, Magar, Gurung, Tamang, Tharu, Dalit) are mainly consuming raw pork in their regular dishes. The practice of consuming barbecued items such as momo, sukuti, sausage, salami and bacon is popular among Nepalese pork consumers.

Till now, in Nepal studies have been carried out in over 13 districts representing Eastern, Central and Western development regions. Either serum samples or meat samples are being tested. The techniques used include antibody ELISA screening and pepsin digestion methods. Positive & doubtful sera were further confirmed by endpoint single dilution ELISA & western blot methods. Out of 2074 serum samples tested by ELISA in different studies 36 (1.7%) turned out to be positive and 175 (8.4%) were doubtful but none of 1546 meat samples tested

positive. Out of 52 sera tested by western blot, only 2 revealed positive whereas 39 sera tested by endpoint ELISA revealed all negative results. However, this zoonosis has never been reported from this region Joshi *et al.* (2001). Further, Sporadic suspicions of trichinellosis in humans have been reported by different medical hospitals in Nepal but there is no confirmation yet. Therefore, to understand pig farmer's knowledge, attitude and their common practices of pig raising and pork consumption system, the knowledge, attitude and practices survey of meat borne helminthic zoonoses were prerequisite for designing the prevention & control strategies for possible zoonoses and public health impact.

Materials and Methods

Study Area and Design

A cross sectional study of four major pig producing areas of Eastern and Midwestern Nepal, viz, Sunsari, Dhankuta, Banke, Surkhet consisting of two different ecozones i.e. terai and hill was done from February to May 2014. A total of 184 serum samples were collected, consisting of 92 samples from each ecozone, similarly 46 samples from each district, of pigs raised either intensively, semi intensively in commercial pig farms or free ranging pigs raised in household way. Further, the knowledge, attitude and practices survey of meat borne helminthic zoonoses, viz, cysticercosis & trichinellosis was conducted among 50 pig raisers and pork consumers by face to face interview using a semi-structure questionnaire.

Sampling Technique

The study area was purposively selected with purposive & convenience sampling of free ranging pigs whereas purposive & convenience sampling based on age, sex, breed constituting 10 % of the herd for semi intensive and intensive population.

Serological Test

Serological test was done using *Trichinella* Indirect multi-species ELISA kit (Sensitivity = 90.47%, n= 42, Specificity = 100%, CI_{95%}: 98.95 – 100%, n=362), based on the use of excretory/ secretory (E/S) antigen that detects anti-*Trichinella* antibodies directed against *T. spiralis*, *pseudospiralis*, *britovi* and *nativa* in serum, plasma or meat juice from pigs, wild boar and horses. ELISA was done as per the guidelines provided in ID Screen *Trichinella* Indirect Multi- species protocol.

Statistical Analysis

Data entry, management and analysis were done using program Microsoft Office Excel 2010, Chi-square (χ^2) analysis using commercial software PHStat version 2.5 with significance level defined at the $p < 0.05$ and fisher's exact tests using R statistical package.

Results and Discussion

The study reveals 7 serum samples (3.8%) to be positive whereas 7 serum samples (3.8%) were considered as doubtful, later they are assumed as positive and remaining 170 serum samples (92.4%) were negative (Fig. 1). The Fig. 2 to 7 indicate seroprevalence of *trichinella* in pigs according to different parameters in eastern and Midwestern region of Nepal.

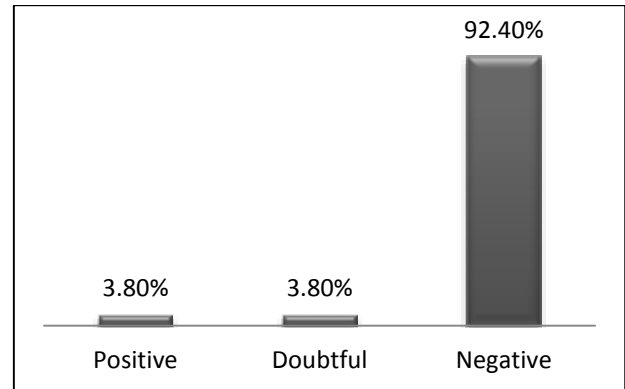


Fig. 1: Seroprevalence of *Trichinella*

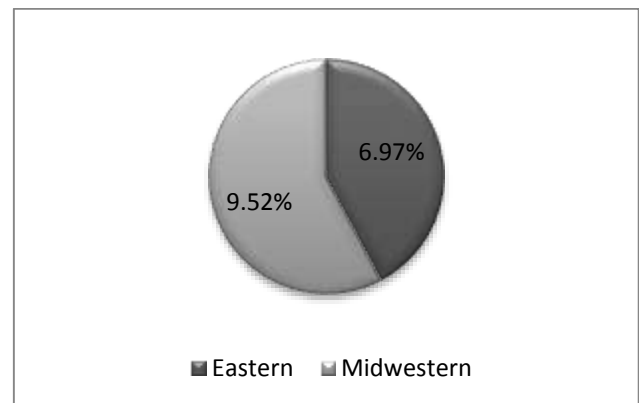


Fig. 2: Prevalence by ecozone (region)

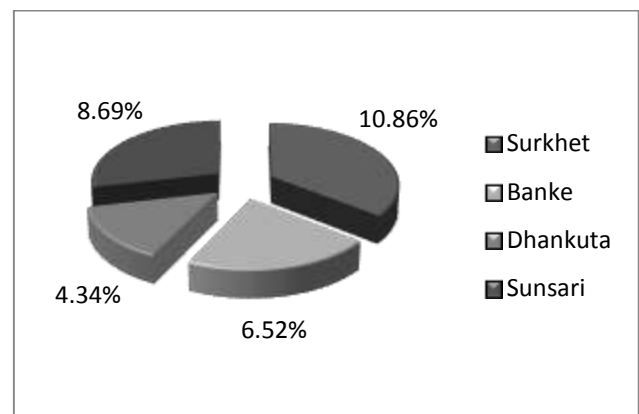


Fig. 3: Prevalence by district

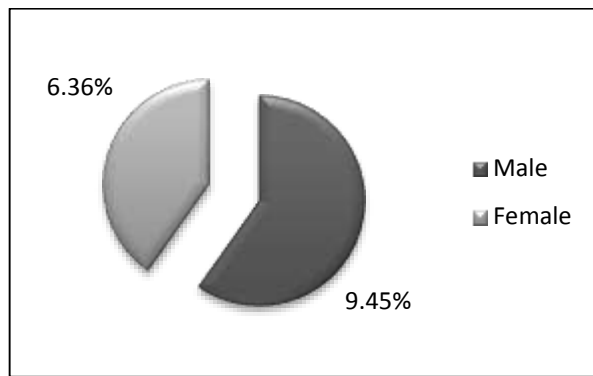


Fig. 4: Prevalence by sex

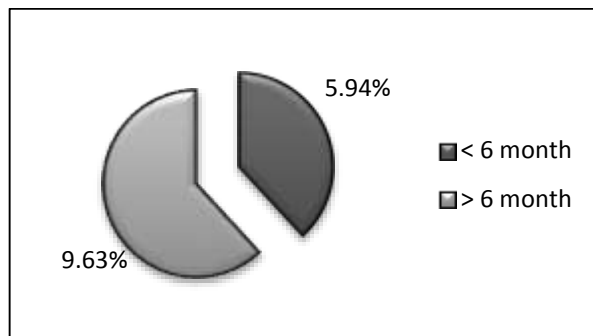


Fig. 5: Prevalence by age

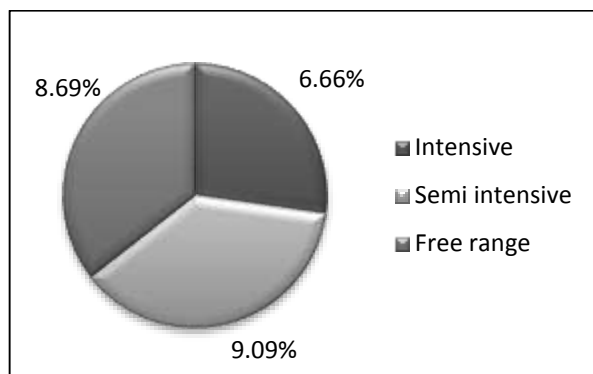


Fig. 6: Prevalence by Rearing System

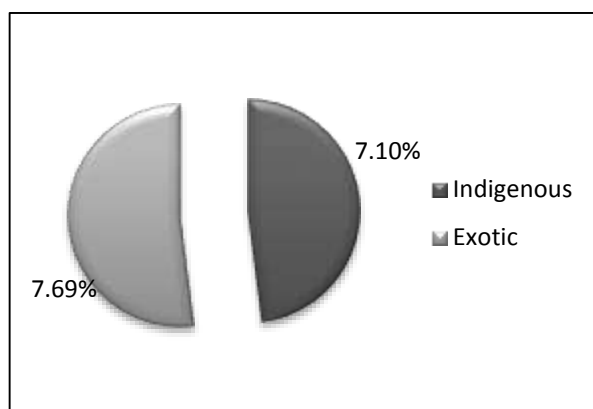


Fig. 7: Prevalence by breed

In hilly region, 7 serum samples (7.6%) were found positive which is similar in findings of Terai region, where 7 serum samples were positive (7.6%). In eastern region of Nepal, 6

serum samples (6.9%) were found positive whereas in contrast in Midwestern region, 8 serum samples were found positive (9.5%). Pigs of less than 6 months of age, reveals 6 serum samples (5.9%) positive whereas in pigs greater than 6 months age, 8 serum samples were positive (9.6%). 7 female pig serum samples (6.3%) were found positive, similarly in male, 7 serum samples were positive (9.45%). Of intensively raised pigs 7 serum samples (6.6%) were found positive whereas in semi intensively raised pigs 3 serum samples were positive (8.6%) and 4 serum samples (9%) were positive of free ranging pigs. In exotic breed of pigs 12 serum samples (7.6%) were found positive whereas in indigenous pigs 2 serum samples were positive (7.1%). However, The difference in prevalence rate according to age, sex, breed, rearing system, eco zone, region & district was found statically non-significant ($p > 0.05$).

Further, the study of survey concludes, about 66% of the respondents were aware of meat borne helminthic zoonotic disease from pigs caused by consumption of undercooked pork meat whereas 34% of the respondents were unaware. Majority of the respondents (84%) think pigs as dirty animals whereas minority of the respondents (16%) thinks reverse of it. Majority of the respondents (88%) consume pork & they prefer mostly (72.2%) by frying method of cooking whereas minority (27.2%) of the respondents prefer by boiled method of cooking. Although, semi intensive & intensive pig farming is increasing day by day, farmers used to raise pigs mostly under free ranging condition (74%). Most of them (66%) dewormed their pigs whereas 34% of farmers were not aware of deworming their pigs. Farmers usually (79.2%) prefer to slaughter their pigs at butchers, where in context of Nepal meat inspection act is not regulated. Majority (62%) of pig raising farmer's don't have their latrines in house & their pigs are exposed (62%) to wild boars, rodents, dogs, cats & birds which play role in the transmission of *Trichinella* to their domestic pigs. The table indicates the retrospective review of trichinellosis in pigs in Nepal.

Since comprehensive and complete knowledge on the epidemiological situation of the parasite in domestic pig and wild life with in the country is unknown. Freedom from *Trichinella* infection with in a given area is difficult to document. However, its validity and accuracy could be challenged through increased sample sizes. Furthermore, continuous surveillance and monitoring have to be implemented before concluding that Nepal is free from infection. Using confirmatory techniques with sufficient sample size should be conducted to confirm their findings and assess the public health risk. These results therefore suggest for greater attention and more intensive surveillance need in Nepal. Enhanced surveillance will enable for additional burden estimates and understanding the impact of this parasitic disease in pig as well as in human beings

Table 1: Review of surveillance of trichinellosis in pigs in Nepal

S.N.	Author	Year	Samples	Site	Breed	Methods	Findings
1.	Joshi <i>et al.</i>	2005	425 serum	Kathmandu	Local pigs	Screening by ELISA	2 serum positive on western blot
			Muscles (10 pooled and 52 single)			HCL pepsin digestion	Negative
2.	Joshi <i>et al.</i>	2009	645 serum	Kathmandu, Bhaktapur, Lalitpur, Sunsari, Morang	Local indigenous and cross breed	Ab ELISA using Excretory/secretory antigens from L1 larvae	165 positive (25.58%) 19 (2.95%) strong positive 146 doubtful
			298 muscles			HCLpepsin digestion	Negative
3.	Sapkota <i>et al.</i>	2006	400 serum	Kathmandu valley, Eastern Nepal, Terai and adjoining areas of the valley slaughter house		Ab ELISA using Excretory/secretory antigens from L1 larvae	4 positive (1%) 1 equivocal(negative on reexamination)
4.	Karn <i>et al.</i>	2007	344 serum	Central region(Kathmandu, Kavre, Dhading, Chitwan and Rauthat)		Ab ELISA Positive and doubtful by end point ELISA and western blot for confirmation	2 positive 14 doubtful Negative on western blot and end point ELISA
			551 muscles			HCLpepsin digestion	Negative
5.	Upadhaya, G.R.	2012	260 serum	Western and central slaughter house (Kathmandu, bhaktapur, lalitpur) (rupandehi, chitwan, kaski, baglung and syangja)		Ab ELISA using Excretory/secretory antigens from L1 larvae	9(3.46%) positive 14 doubtful (5.38%) Negative on end point ELISA
			375 muscles			HCL pepsin digestion	Negative
6.	Sharma, P.	2012	210 muscles	Kathmandu valley		HCLpepsin digestion	Negative
7.	K.C. S.	–	50 muscles			HCLpepsin digestion	Negative

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