SEROPREVALENCE OF *TRICHINELLA* IN SLAUGHTER PIGS IN KATHMANDU VALLEY, NEPAL

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Abstract. The aim of the present study was to determine the *Trichinella* seroprevalence in slaughter pigs in Kathmandu Valley, Nepal. Serum samples were obtained from 400 pigs at 4 major slaughterhouses and tested for *Trichinella* antibodies by ELISA using larval excretory-secretory (E/S) antigen. Four were positive and one was equivocal, giving a *Trichinella* seroprevalence of 1% (95% CI: 0.27 - 2.54). On titration, all positive and equivocal samples had titers greater than 1:80. Upon re-examination the equivocal sample failed to give a positive ELISA result. The pigs were from four major areas of Nepal, Kathmandu Valley, eastern Nepal, Terai and adjoining areas of the valley. Positive results were found from only Kathmandu Valley and adjoining areas. There was no significant difference in the prevalence between areas ($p = 0.43$). All four positive samples were from indoor managed pigs. The *Trichinella* seroprevalence determined in this study deserves a direct demonstration of the parasites for proof of the presence of *Trichinella* in Nepal and to discover the species and infection sources.

INTRODUCTION

Trichinella, a genus of nematode parasites belonging to the family *Trichuridae,* occurrs worldwide and infects all vertebrates including humans (Straw *et al,* 1999). Seven species of *Trichinella* are recognized (OIE, 2000). These are: 1. *Trichinella spiralis* (T-1), 2. *Trichinella nativa* (T-2), 3. *Trichinella britovi* (T-3), 4. *Trichinella pseudospiralis* (T-4), 5. *Trichinella murrelli* (T-5), 6. *Trichinella nelsoni* (T-7), and 7. *Trichinella papuae* (T-10). A new *Trichinella* species that infects both reptiles and mammals, called *Trichinella zimbabwensis,* was discovered in Zimbabwe (Pozio *et al,* 2002).

Trichinella spiralis, the most important species, is commonly associated with domestic pigs, therefore belonging to the domestic cycle. This species is distributed in temperate regions worldwide and is highly infective in pigs, mice and rats (Kapel, 2000). Infection in pigs is perpetuated by swill feeding, eating infected rodent carcasses, tail-biting, infestation by feces from freshly infected animals or feeding on non-sterilized human food residuals (Urquhart *et al,* 1996). Farm management practices play an important role in the outbreak of trichinellosis in pig farms (Gamble and Bush, 1999; Gamble *et al,* 1999).

The increasing annual growth rate of 4.55% in pigs is higher than in other food animals, such as 1.93% in buffaloes, 0.82% in sheep, and 2.03% in goats (CLDP, 2003). Therefore, investigation regarding prevalence of zoonotic agents like *Trichinella* is very important. Nepal is in the stage of implementing meat legislation and the identification of

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zoonosis related problems in the country which should be brought to the attention of the veterinary and public health authorities.

The objective of the current study was to determine the seroprevalence of *Trichinella* in slaughtered pigs in the Kathmandu Valley.

MATERIALS AND METHODS

The study area was the Kathmandu Valley, where the capital of Nepal is located. There are three districts in the valley: Kathmandu, Lalitpur/Patan and Bhaktapur. Pigs are slaughtered at 4 slaughterhouses in the valley. House 1 is located in the Patan District. Houses 2 and 3 are located in the Kathmandu District, and House 4 in Bhaktapur. The pigs come from different parts of the Kathmandu Valley, adjoining districts, Terai and eastern Nepal. Terai is a plain area, located in southern Nepal. Morang and Sunsari are main districts in the East from where black pigs are supplied. Pigs were raised indoors, mixed and/or outdoors. The breeds are indigenous (Bampudke, Chwanche and Hurra) (Joshi and Shaha, 2003) or improved breeds of pigs.

The study was a cross-sectional design in which the individual pig was the sampling unit. Sample size was calculated on the basis of an expected conservative prevalence of 50% (95% CI, 5% error) (Win Episcope 2.0) assuming a total of 0.9 million pigs in the country (CBS, 2002). The sampling methods were random sampling of slaughtered pigs at the time of slaughter. The sampling weeks were chosen randomly. Four hundred samples were examined for *Trichinella* antibodies with an equal number of samples from each slaughter house (100) since the overall number of pigs slaughtered at each house was similar. Sample collection was done 4 days a week from November 2004 to April 2005.

The blood from each pig was collected from the heart during the evisceration and was immediately transported to the Central Veterinary Laboratory (CVL), Tripureshwor, Kathmandu and centrifuged to separate the serum. The serum samples were stored at -25ºC at the same laboratory until examination. The ELISA test was conducted at the Regional Center for Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Thailand. The ELISA using *T. spiralis* larval E/S antigen was conducted according to Nöckler *et al* (1995). Serum samples were tested for specific antibody at a dilution of 1:100. The microtiter plate was measured when the positive control serum reached an optical density (OD) of 1.3 to 1.4 after 10 to 20 minutes. ELISA results were evaluated as sample/positive control (S/P) ratio (%) by comparing the mean OD value of the duplicate sample to the mean OD value of the quadruplicate positive control serum (each previously corrected for the mean OD values of the blank plate). S/P ratio of 8% and 14% were considered as negative/inconclusive and inconclusive/positive thresholds, respectively. Positive and equivocal serum samples were re-examined using an endpoint titration test (from 1:10 to 1:1,280) for confirmation.

RESULTS

Out of 400 serum samples tested by ELISA at a screening dilution of 1:100, four were positive and one was equivocal giving a *Trichinella* seroprevalence of 1% (95% CI: 0.27 - 2.54). Ontitration, all positive and equivocal samples had titer greater than 1:80. Upon re-examination, the equivocal sample failed to give a positive ELISA result.

Table 1 shows the distribution of serum samples found positive for *Trichinella* antibodies by farming system and area of pig origin. Two hundred eighty-five serum samples (72%) belonged to indoor managed pigs and 115 (28%) were from mixed or outdoor farmed pigs. All 4 positive sera were from indoor managed pigs giving a *Trichinella* prevalence of 1.4%.

Table 1
Distribution of samples found positive for *Trichinella* antibodies by farming system and area of pig origin, Kathmandu Valley, Nepal, November 2004 to May 2005.

	Total serum samples	% Positive	95% CI
Farming systems			
Indoor	285	1.4 (4)	0.38-3.55
Mixed	113	0 (1) [a]	
Outdoor	2	0	
Areas			
KTM Valley	193	1.55 (3)	0.32-4.47
Eastern Nepal	104	0 (1) [a]	
Terai	54	0	
Adjoin districts	49	2 (1)	0.05-10.85

( )[a]= equivocal sample; ( ) = positive samples

There was no significant difference in the seroprevalences among the farming systems (Kruskal Wallis, H = 1.62, df = 2, p = 0.44).

About 48.2% (193/400) of the pigs sampled were from Kathmandu Valley, 26% (104/400) were from eastern Nepal, 13.5% (54/400) were from Terai and 12.2% (49/400) were from adjoining areas of Kathmandu Valley. *Trichinella* positive samples were found in Kathmandu Valley and adjoining areas only giving seroprevalences of 1.55% (3/193) and 2% (1/49) in those areas, respectively. There was no significant difference in the seroprevalence of trichinellosis among these areas (Kruskal Wallis, H = 2.72, df = 3, p = 0.43).

DISCUSSION

Pig farming and marketing have increased dramatically in the country in recent years due to a growing demand for pork (Joshi *et al*, 2003). Hygiene and sanitation in the country are very poor. This is one of the major causes of parasitic diseases in both humans and livestock. The common practice of feeding offal and kitchen waste, free ranging and back yard piggeries, potentially contributes to the transmission of *Trichinella* (Joshi *et al*, 2004).

Of the 400 samples tested, 4 were positive for *Trichinella* antibodies, giving a seroprevalence of 1% in slaughtered pigs in Kathmandu Valley. This finding shows that pigs in Kathmandu Valley and adjoining areas may be infected with this nematode.

Joshi *et al* (2004) reported a *Trichinella* seroprevalence of 0.47% by EITB (2 out of 425) in slaughter pigs in Kathmandu Valley. However, using synthetic beta-tyvelose antigen in ELISA they obtained an unexpectedly high number of positive cases. According to Gamble *et al* (1983), the use of E/S antigens increases the sensitivity and specificity of the diagnosis of *T. spiralis* in pigs, thus increasing the detection of natural infections, even those with very low parasitic densities. Recently, it was reported that the synthetic glycan antigen beta-tyvelose appears to be less sensitive than the E/S antigens and beta-tyvelose antigen may not be suitable to screen for trichinellosis in pig herds (Moller *et al,* 2005). The findings of this study can be compared with the findings in some parts of China as the seroprevalence reported in pigs in China varies from 0.09% to 29.63% (Wang and Cui, 2001).

In this study all the positive samples were from pigs under the age of 9 months. Ten per-

cent (40/400) of the serum samples were from the pigs of over 1.5 years. It is worthy noting that ELISA fails to detect infected pigs during both early and very late stages of infection (OIE, 2000). Thus, it is likely that positive cases may have been missed and the prevalence could be even higher than that reported here. It has been reported that the time to seroconversion is correlated to infection dose and there are no known cross-reactions using E/S antigens in ELISA (Nöckler *et al*, 2000). The equivocal result obtained in this study may be due to old infection or low infection dose. This indicates the importance of conducting the research to demonstrate the presence of parasites by pepsin digestion (gold standard).

The management practices of farms are associated with the occurrence of *Trichinella* (Gamble and Bush, 1999; Gamble *et al,* 1999). Rodents play an important role in the transmission of *Trichinella* in the domestic cycle. Kitchen waste, which is not heat treated properly is a risk factor for infection (Urquhart *et al,* 1996). In Nepal, pig farming is not yet fully commercialized and the bio-security measures have not been introduced to pig farms (Dhaubhadel, 1992). Feeding of offal and kitchen waste is very common in pig farming in Nepal (Joshi *et al,* 2004). In this study, the positive samples were from indoor farms, in which the pigs were confined in the same place until marketed. Therefore, cannibalism and the access of rodents to the indoor farms can not be excluded. This may explain why positive cases were recorded in the pigs from this type of farming system.

The serological evidence of *Trichinella* should be proved by the direct demonstration of the parasite using the digestion method, which is considered as the gold standard. Parasite species identification should as be performed. Therefore, establishment of cost-effective laboratory facilities should be brought to the attention of the veterinary and public health authorities in Nepal.

Kitchen waste and offal should be sterilized before feeding to pigs. *Trichinella* positive meat should not be delivered to the market unless it is well cooked. Similarly, intensive pig farming with adoption of proper biosecurity measures is advocated to prevent the transmission of *Trichinella*.

REFERENCES

CBS: Central Bureau of Statistics. Population census-2001, Nepal. *National report.* 2002.

CLDP: Project Appraisal for Community Livestock Development Project HMG. *Nepal/ADB,* 2003.

Dhaubhadel TS. The role of monogastric and small stock. In: Abington JB, ed. Sustainable livestock production in the mountain agro-ecosystem of Nepal, 1992. Rome: FAO animal production and health paper 105, Chapter 6. [Cited 2006 July 21]. Available from: URL: http://www.fao.org/DOCREP/004/T0706E/T0706E00.htm#TOC

Gamble HR, Anderson WR, Graham CE, Murrell KD. Diagnosis of swine trichinosis by enzyme-linked immunosorbent assay (ELISA) using an excretory-secretory antigen. *Vet Parasitol* 1983; 13: 349-61.

Gamble HR, Bush E. Seroprevalence of *Trichinella* infection in domestic swine based on the National Animal Health Monitoring System 1990 and 1995 swine surveys. *Vet Parasitol* 1999; 80: 303-10.

Gamble HR, Brady RC, Bulaga LL, *et al*. Prevalence and risk association for *Trichinella* infection in domestic pigs in the northeastern United States. *Vet Parasitol* 1999; 82: 59-69.

Joshi BR, Shaha BKP. Role of indigenous farm animal genetic resources in poverty reduction in Nepal. Kathmandu: 7th National Conference of Nepal Veterinary Association (VETCON'03). 5-7th November 2003: 34-42.

Joshi DD, Maharjan M, Johansen MV, Willingham AL, Sharma M. Improving meat inspection and control in resource-poor communities: the Nepal example. *Acta Trop* 2003 ; 87: 119-27.

Joshi DD, Moller LN, Maharjan M, Kapel CMO.

Serological evidence of trichinellosis in local pigs of Nepal. San Diego, California: 11th international conference on trichinellosis. 8-12th August, 2004: 82.

Kapel CM. Host diversity and biological characteristics of the *Trichinella* genotypes and their effect on transmission. *Vet Parasitol* 2000; 93: 263-78.

Moller LN, Petersen E, Gamble HR, Kapel CM. Comparison of two antigens for demonstration of *Trichinella* spp antibodies in blood and muscle fluid of foxes, pigs and wild boars. *Vet Parasitol* 2005 (in press).

Nöckler K, Voigt WP, Protz D, Miko A, Ziedler K. Diagnosis of trichinellosis in living pigs using indirect ELISA. *Berl Münch Tierarztl Wochenschr* 1995; 108: 167-74.

Nöckler K, Pozio E, Voigt WP, Heidrich J. Detection of *Trichinella* infection in food animals. *Vet Parasitol* 2000; 93: 335-50.

OIE. Manual of diagnostic tests and vaccines for terrestrial animals, trichinellosis. Paris: Part 2, Section 2.2, Chapter 2.2.9. 2000.

Pozio E, Foggin CM, Marucci G, *et al*. *Trichinella zimbabwensis n.sp.* (Nematoda), a new non-encapsulated species from crocodiles (*Crocodylus niloticus*) in Zimbabwe also infecting mammals. *Int J Parasitol* 2002; 32: 1787-99.

Straw BE, D'Allaire S, Mengeling WL, Taylor DJ. Disecises in swine. 8th ed. Oxford, UK: Blackwell Science, 1999.

Urquhart GM, Armour J, Duncan JL, Dunn AM, Jenings FW. Veterinary parasitology. 2nd ed. London: Blackwell Science, 1996.

Wang ZQ, Cui J. The epidemiology of human trichinellosis in China during 1964-1999. *Parasitology* 2001; 8: 63-6.