INTRODUCTION

Trichinella, a genus of nematode parasites belonging to the family Trichuridae, occurs 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 -25°C 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). On titration, 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%.
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

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