CROSS-SECTIONAL STUDY OF *TRICHINELLA* SPP IN PIGS IN CDR, NEPAL USING PEPSIN DIGESTION AND ELISA SEROLOGY

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Abstract. This epidemiological cross-sectional study was conducted in five major pig-producing districts of CDR, Nepal from November 2006 to April 2007. A total of 576 slaughtered pigs were randomly selected and diaphragmatic crus muscles (*n*=551), corresponding sera (*n*=487) and sera only (*n*=25) were collected from them. Meat samples were examined by pepsin digestion to evaluate for larvae of *Trichinella* spp. The sera were investigated using ELISA to evaluate for antibodies against *T. spiralis*. The doubtful and positive sera from the ELISA test were investigated by end-point ELISA, and the Western blot was used for confirmatory diagnosis. Pepsin digestion did not detect the larvae of *Trichinella* spp. AB-ELISA showed 2 positive and 14 doubtful results, out of 344 sera analyzed. End-point ELISA and the Western blot had revealed that all 16 samples were truly negative. A questionnaire revealed no rodent control (70%), left over feeding practices (65%), dumping of farm waste (82.5%) and uncooked meat was being used as feed (100%). This study reveals that *Trichinella* spp has a low prevalence.

INTRODUCTION

Roundworms of the genus *Trichinella* are found worldwide and are responsible for one of the most serious helminthic zoonoses. The epidemiological data on *Trichinella* spp infection in pigs show that this infection is usually confined to small farms with traditional pig rearing practices or grazing in wild areas (Pozio, 2000). In many parts of the world no methods of control of *Trichinella* spp infection are applied due to economic problems, erosion of the veterinary infrastructure, failure of the educational system, ineffective abattoir control measures and lack of awareness of the disease by medical workers. These factors are responsible for *Trichinella* spp infection still in the food chain in large parts of the world (van Knapen, 2000). In Nepal there is serological evidence of trichinellosis in pigs (Joshi *et al,* 2005). In pigs high antibody titers against *Trichinella* spp reveal a recent infection since several months after infection the antibody level begins to decrease. Therefore, equivocal titers are not predictive enough in older animals. Verification of realistic prevalence is performed using pepsin digestion. In this study, evaluation of *Trichinella* spp infection in slaughtered pigs was carried out.

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MATERIALS AND METHODS

Study design and study population

This was a cross-sectional observational study carried out at pig butcheries located in five districts (Kathmandu, Kavre, Dhading, Chitwan and Rauthat) of CDR, Nepal. Muscle samples and/or serum samples were collected for *Trichinella* spp investigation. The pigs were reared commercially, semi-commercially, by scavenging or the household way.

Definition of samples and sampling strategies

From each slaughtered pig 25-30 g of diaphragmatic crus muscle along with 10 ml of blood obtained by heart puncture were obtained. Meat materials were stored in cooling boxes and blood samples were centrifuged after standing over night. The demographic and husbandry criteria regarding the pigs were recorded. The butcheries were selected by convenient sampling and the slaughtered pigs through simple random sampling.

Laboratory analysis

Batches of 10 pooled muscle samples (5 g each) were digested using pepsin digestion and serum samples were investigated by ELISA using ES-*Trichinella spiralis* larval antigen, according to the National Reference Laboratory for Trichinellosis, Germany. Positive and doubtful sera were examined using end-point titration for confirmation according to Nöckler *et al* (1995). Confirmatory diagnosis of the serum was done using Western blot (OE Mikrobiologie, Ref No. LA163-1/BfR).

Questionnaire survey

A questionnaire was filled out by 40 pig farm owners. The farms were located in the surveyed districts and their selection was based on convenience sampling. The questionnaire was administered by direct interview to gather information regarding farm management practices used to prevent trichinella infection.

RESULTS

Twenty-six percent of the pigs selected for this study were from commercial farms, 17.2% were from semi-commercial farms, 37.2% were from scavenging and 19.6% were raised by households. The pigs were reared indoors in 44.4%, outdoors in 37.8% and mixed in 17.7%. The breeds of the sampled pigs were local in 56.9%, exotic in 26.6% and cross breed in 16.5%. The samples were taken from pigs less than 1 year old in 37.8%, 1-2 years old in 56.1% and greater than 2 years old in 6.1%.

Meat sample investigation by pepsin digestion

Meat samples from 551 pigs were analyzed by pepsin digestion, but *Trichinella* spp larvae were not found in any samples.

Serum sample investigation through indirect ELISA

Three hundred forty-four randomly selected sera were tested by AB-ELISA for antibodies against *T. spiralis*. The ELISA-OD indices for these sera are shown in Fig 1. The results show that if the antibody ELISA-OD value of the serum sample was greater than 0.23 then the ELISA-index was arithmatically greater than 12%, which means it was either equivocal or positive. Fourteen samples were equivocal (12-18% ELISA-index) and 2 were positive (≥18% ELISA-index). These equivocal and positive samples were re-evaluated by end-point titer ELISA. All had an ELISA-index less than 70% and a titer < 1:80. Based on this criteria, all tested serum samples failed to show antibodies against *T. spiralis*.

These same 16 samples were further evaluated by Western blot performed by BfR Berlin, Germany (Fig 2). The results revealed well recognized bands of 43.0 (42.7-43.2), 46.9 (46.7-47.2), 62.0 (61.5-62.5), 66.1 (65.4-66.9) and 102.0 (101.5-102.4) kDa. None of the samples were positive for *Trichinella* on Western blot.

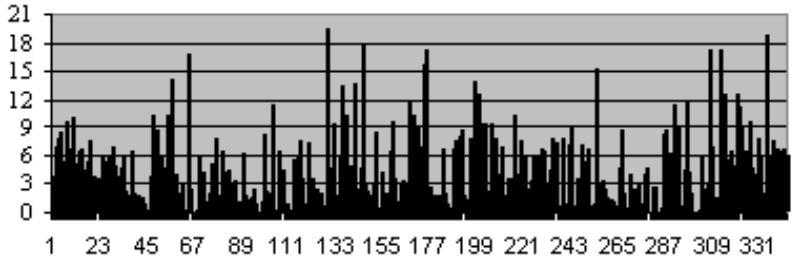

Fig 1–ELISA index for *T. spiralis* antibodies randomly selected from 343 serum samples, including 14 equivocal and 2 positive results.

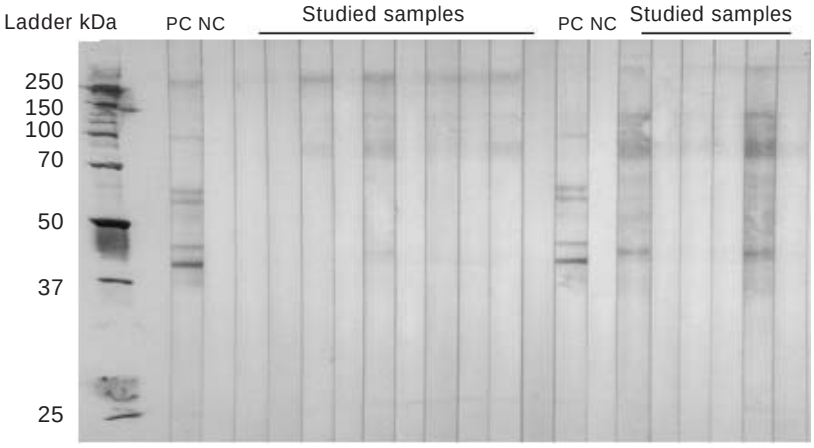

Fig 2–Result of Western blot on 16 serum samples positive on ELISA for *Trichinella* spp.

Pig producer questionnaire survey

Twelve farms (30%) had no rodent control program, 29 (72.5%) had no barrier against external wildlife, 33 (82.5%) a garbage dump vicinity and 17 (42.5%) had bird access to the garbage dump area, Twenty-six farmers (65%) provided feed containing leftovers and none of the farmers cooked the offal before feeding it to the pigs.

DISCUSSION

In these districts more than 10,000 pigs are raised. The pigs were selected through probability proportional sampling. The sampling strategies were within a 1% allowable error to give an estimated 95% CI. Therefore, this study design reliably represents the prevalence of trichinella infection in slaughtered pigs in the region. Pepsin digestion has a detection limit of 1-3 larvae/g of meat according to the directive 77/96/EEC. A minimum of 1 g of muscle tissue is sufficient to detect *Trichinella* spp where the aim is to prevent clinical trichinellosis (Gamble *et al,* 2000). Given that the average amount of diaphragmatic muscle collected in this study was 25-30 g and no sample was smaller than 5-10 g it is likely the pepsin digestion method would have detected a positive sample, if there had been one. Human error and accidents could allow for some probability of infection in the tested meat (van Knapen, 2000). In this study, the ES *Trichinella* antigen was used, which had greater specificity than somatic antigens. Sixty-seven percent of sera were tested randomly and were of good quality, which is also a reason for the increased Se and Sp of ELISA (Dedek, 1992). However, 37.8% of the samples were from pigs less than a year old, when *Trichinella* spp infection is less common. Since the mode age was 1-2 years, it is unlikely the results are influenced by false negative results due to declining antibody titers.

The risk factors were abundant for *Trichinella* spp, but its prevalence was low.

Epidemiological calculations carried out using Episcope 2.0 (EPIDECON) estimated a maximum probable prevalence of 0.52%. The low prevalence of *Trichinella* spp in the pig population in Nepal in comparison to the high prevalence in China may be related to differences in the ecosystems. This may be due to the natural mountainous barrier between the two neighboring countries and to the fact that there are no imports or exports of food items between these geographically close nations. In China, the prevalence of trichinella infection in pigs is 5% (*n*=40,000) and in similar epidemiological studies (*n*=2,000) in the northern, southern and coastal region a 7.5% positivity rate has been found (Chan and Ko, 1992). However, there were no pig isolates from Xizang Province, China, which is the province bordering Nepal. The first outbreak of human trichinellosis in China came from Xizang, and during 2000-2003 there were 3 outbreaks with a mortality rate of 4/50 (Wang *et al,* 2006). Given this data the emergence of *Trichinella* spp infection in the near future in Napal cannot be ignored.

However, our survey to estimate the prevalence of *Trichinella* spp in Nepal does not confirm the region is infection free. *Trichinella* spp infection was documented in animals of 3 locations (Ireland, Croatia, Sardinia) previously considered to be free of infection. This strongly supports the concept that there is neither a region nor a country that may be considered totally free. This is well illustrated through the outbreak in Sardinia which was supposed to be free of infection after negative results were obtained after examining 4,427 sera of domestic pigs and 668 sera of wild boars (Pozio *et al,* 2006).

While the surveillance of domestic pigs may be improved, the current study indicates that the risk for humans contracting *Trichinella* spp infection from eating pork or pork products originating from this region is exceedingly low. It is necessary to search for species which may act as a reservoir for *Trichinella* spp in Nepal. Since Nepal is landlocked and has open borders it is not possible to form efficient barriers to prevent the introduction and establishment of *Trichinella* spp in reservoir animals. A negative finding does not guarantee the region is free from *Trichinella* spp infection, since a complete knowledge of the epidemiological situation of the parasite in domestic pigs and wildlife within country is unknown. Intensive national surveillance is essential for trichinellosis control in domestic pigs in Nepal based on OIE guidelines. To achieve this goal the production practices that prevent trichinella infection have to be documented. A herd's status needs be monitored. All suspected cases need to be traced back, quarantined and tested. Monitoring and surveillance should be followed through the official system, which means organizing surveys to collect and collate data and to evaluate quality control of laboratories. After this, if *Trichinella* spp is not detected over the next 5 years, the country can apply for region free certification.

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