A Cross Sectional Study of Trichinella in Pigs in CDR, Nepal Using Pepsin Digestion and ELISA Serology

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Introduction
Roundworms of the genus Trichinella are spread worldwide and are responsible for one of the most serious helminthic zoonoses. It is recognized that in many parts of the world none of the existing methods of control for Trichinella infection are applied because of the economic problems, erosions of veterinary infrastructures, failure of educational systems, ineffective abattoir control measures and non-awareness of the disease by medicals. These factors are responsible for Trichinella infection still in the food chain in large parts of the world (1). In Nepal there is a serological evidence of trichinellosis in pigs (2). In pigs high antibody titers against Trichinella reveal a recent infection because several months after the infection the antibody level begins to decreases. Therefore doubtful titers are not predictive enough in older animals. So a verification of the realistic prevalence is required using the Pepsin digestion. Objectives of this current study were; to investigate the Trichinella larval status in meat samples of slaughtered pigs by the Pepsin digestion method; to compare the results of the Pepsin digestion with corresponding serum samples by indirect antibody ELISA and finally to characterize the species of Trichinella larvae.

Materials and Methods
This study was carried out at the pig butcheries and located in the five districts (Kathmandu, Kavre, Dhading, Chitwan and Rauthat) of Central Development Region (CDR), Nepal. From each of the slaughtered pigs 25-30 g of diaphragmatic crus muscle together with 10 ml blood by heart puncture was taken. The demographic and husbandry criteria of the pigs were recorded. The butcheries were selected by convenient sampling, whereas the slaughter pigs were selected by simple random sampling. Batches of 10 pooled muscle samples were digested using the Pepsin digestion and serum samples were investigated by antibody ELISA using ES-Trichinella larval antigen, according to the SOP of the National Reference Laboratory for Trichinellosis (BfR), Germany. The positive and the questionable samples were examined using the endpoint titration for the confirmation according to Nöckler et al. (1995). The confirmatory diagnosis in the serum was done by the western blot (OE Mikrobiologie, Ref. LA163-1/BfR). The single meat probe sample was investigated by Trichinoscopy with the compressorium to identify the infected animal. The questionnaire was surveyed among 40 pig farm owners. These farms were located in the surveyed districts and their selection was based on convenient sampling.

Results and Discussion
In this study 26% of the pigs were from commercial, 17.2% from semi-commercial, 37.2% from scavenging and 19.6% from household raised system. The pigs were reared either indoor (44.4%), outdoor (37.8%) and mixed (17.7%). The breed characteristics of the sampled pigs were local 56.9% (Hurra, Bampudake, Wild), exotic 26.6% (Landrace, Yorkshire, Hampshire) and cross 16.5% (Dharane kalo). The collected samples were belonged to pigs below 1 year (37.8%), 1-2 years (56.1%) and more than 2 years of age (6.1%). The meat samples from a total of 551 pigs were analyzed through the Pepsin digestion and compressorium, but Trichinella larvae were not found in any sample. A total of 344 randomly selected sera were tested by AB-ELISA for antibodies against Trichinella spiralis. The trend of the serum samples showed that if antibody ELISA OD value of serum sample was more than 0.23 then the ELISA-index was arithmetically more than 12% which means either it was doubtful or positive. It was found that 14 samples were doubtful (12% ≤ ELISA-index <18%) and 2 samples were positive (≥ 18% ELISA-index) from antibody ELISA (Fig. 1). These doubtful and positive samples were confirmed by endpoint titer single dilution ELISA and it was found all these samples had the ELISA index less than 70% and border of titre less than 1.80. Based on the test evaluation criteria of endpoint ELISA all these tested serum samples failed to show antibodies against T. spiralis. These same 16 samples were further confirmed by western blot performed by BfR Berlin, Germany (Fig. 2). The ladder used for comparison had well-recognized molecular weight patterns for specific bands and based on the evaluation criteria, it had showed that none of the sample had shown two types of specific bands on its ladder. This means all the tested sera samples were negative for Trichinella genotype through western blot. However the status of pig farms with hygienic measures showed that 12 (30%) farms had not existed rodent control program,
29 (72.5%) had no architectural barrier for wildlife, 33 (82.5%) garbage dumped in vicinity and 17 (42.5%) had bird access from dumping area to the farms. The 26 (65%) of the farmers are providing feed containing leftovers and none of the farmers cook the offal before providing to pig. This study design was designed to determine the prevalence in pigs in the region, because the sampling strategies and collection were sufficient enough at 1% error to an estimate at 95% confidence interval. The Pepsin digestion test has a detection limit of 1-3 larvae/g meat according to directive 77/96/EEC using 1 g of muscle tissue is sufficient to detect where the aim is to prevent clinical trichinellosis (3). In this study 25-30 g muscle was taken and no sample was smaller than 5-10 g for Pepsin digestion, so it is very likely that the digestion method would have detected a positive result, if there had been one. Also in study the ES antigen was used which had more specificity than somatic antigens. Only 67% sera were tested randomly and the selected sera were of good quality, which is also a reason for increased sensitivity and specificity of ELISA (4). However, 37.8% of the samples were from pigs less than one year of age, where Trichinella was less reported, but since mode age was 1-2 years, so it was unlikely that the result of the serological testing of such animals were influenced by false negative results due to declining antibody titer. The low prevalence of Trichinella in Nepal and its comparison to the high prevalence in the China could be related to the differences in ecosystem, natural mountainous barrier and also because of no import export of food items between these geographically close nations. The current study indicates that the risk for humans contracting Trichinella infection from eating pork or pork products originated from such region was exceedingly low. It was necessary to search for the possible species as a reservoir for Trichinella in Nepal in the context that there is a wide range of suitable hosts. Since Nepal is landlocked but has open borders it is not possible to form efficient barriers to prevent the introduction and establishment of Trichinella in reservoir animals in a habitat. It is recommended that intensive national surveillance is essential for trichinellosis control in domestic pigs in Nepal. A herd’s status using a statistically based sample of finishing animals is to be monitored. Any suspicion of disease is followed at the field level by trace back, quarantine and laboratory testing. Monitoring and surveillance are followed through the official system, which means to organize surveys, to collect and collate the data and to operate quality control of routine laboratories. In case Trichinella won’t detected in the next 5 years country can apply for the region free certification.

References

Fig. 1 Results of ELISA index of Trichinella antibodies of randomly selected 343 serum samples including 14 doubtful and 2 positive

Fig. 2 Result of Western blot of 16 sera samples of pigs against Trichinella PC: positive control, NC: negative control