5. DISCUSSIONS AND CONCLUSION

5.1 Discussions

This study was the first study comparing the methods for the detection of *Trichinella* larvae in diaphragm muscles and specific antibodies in blood sera of pigs in the central development region of Nepal. The pig farm's management practices in the central development region were investigated with particular relevance to the transmission of trichinellosis. The Pepsin digestion method had failed to detect *Trichinella* larvae from meat samples although 5-10 g muscle per pig was used. However firstly through antibody ELISA of randomly selected 344 sera, 2 samples were positive and 14 were doubtful. The confirmation of these samples through end-point titration single dilution ELISA at central laboratory, Faculty of Veterinary Medicine (CMU) had failed to detect antibodies against *Trichinella spiralis*. Further confirmation of these 16 sera at the Bundesinstitut für Risikobewertung, Berlin through end-point single dilution ELISA and the western blot had also revealed that all tested serum samples were true negative for *Trichinella* genotype. This could be due to the testing and sampling process or it may be indicative of a very low prevalence of *Trichinella* spp. in surveyed areas of Nepal.

The total pork production in the central development region in 2005/2006 was 3428 metric tons. This amount was supplied 6% from the mountain districts, 31% from the hill districts and 63% from the plain districts (ABPSD, 2006). From the districts where more than 10 000 pigs were raised, the necessary amount of pigs was selected through probability proportional sampling. Through these approach different geographical areas which was the major pig producing areas of the central development region were included in the study. The sampling strategies and the collection were sufficient enough at 1% allowable error to an estimate percentage at 95% confidence interval. Therefore this study design was reliable to represent the prevalence of slaughtered pigs in the CDR.

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The Pepsin digestion test has a detection limit of 1-3 larvae/g according to the directive 77/96/EEC. A minimum of 1 g of muscle tissue is sufficient to detect *Trichinella* where the aim is to prevent clinical trichinellosis (Gamble *et al.,* 2000). In practice this is true for high larval densities but in case of low infection rates the larval are not distributed, homogenously. Using 1 g muscle samples from pigs, experimentally infected with *Trichinella spiralis*, the sensitivity of the pooled sample method of artificial digestion was between 3-5 lpg whereas a 5 g sample had an increased sensitivity of this method to approx 1 lpg (Nöckler *et al,* 2000). Since there was no significant difference between the 3 and 5 g samples in detecting the infection in pork containing 1.0-1.9 lpg, 3 g samples might be considered to be the minimum size (Forbes *et al*., 1998; Forbes and Gajadhar, 1999). Given that the average amount of diaphragmatic crus muscle collected in this study was around 25-30 g and no sample was smaller than 5-10 g it is very likely that the Pepsin digestion would have detected a positive result, if there had been one. However, this test did not have a direct impact on the reduction of the disease in pigs. The human error and accidents would always allow for some probability of infection from tested meat (van Knapen, 2000; Gajadhar and Forbes, 2002).

Many authors had reported on a successful use of *Trichinella* excretory-secretory antigens in the indirect ELISA for the detection of specific antibodies in various animal species (Murrel *et al.,* 1986; Smith, 1987; Smith and Snowdon, 1989; Nöckler *et al.,* 1995; Gamble *et al.,* 1996). The test antigen was considered to be an important factor for the identification of specific antibodies which is essential for the specificity of the ELISA result (Nöckler *et al.,* 2000). In this study the ES antigen was used which had more specificity than somatic antigens. Here only 67% (344/512) sera were tested randomly but with the specific consideration to select sera that were not hemolysed and of good quality. It also increased the sensitivity and specificity of ELISA (Dedek, 1992). However, 37.8% of the slaughtered pigs were less than one year of age where *Trichinella* investigation was less reported. But in this study the slaughtered pig had a mode value of 1-2 years of age. 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. It is also well known that a serum titer is detectable up

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to 130 weeks (Nöckler *et al.*, 2000). Therefore if the infection would have persisted in the observed population it might have been detected.

The sera tested by western blot had also shown negative finding. It showed that none of the tested serum had two specific bands in its ladder that is essential according to BfR evaluating procedure to be concluded as positive. So it is concluded that tested sera were true negative for *Trichinella* spp.

It was well documented that the management factors which are necessary for *Trichinella* control in farms were not practiced in Nepal. The pig farming was not commercialized and the bio-security measures were not implemented (Dhaubhadel, 1992). However, this study revealed no positive results through Pepsin digestion and ELISA serology. It was interesting to see that under similar farm conditions with similar study designs in different parts of world similar results were found. In the studies carried out in pigs or some sylvatic animals from Brazil, Colombia, Venezuela, Paraguay and Bolivia were also found no positive results through Pepsin digestion (Acha and Szyfres, 1986). Several studies were carried out in Colombia and Guatemala (Schenone, 1984), but there was no evidence of *Trichinella* found.

However, all randomly selected serum samples were negative but, initially, when the sera were tested through AB-ELISA, two samples were positive and 14 samples were doubtful. This may be due to the ES antigen, since it will increase the sensitivity and specificity of the test. Gamble *et al.* (1983) and van Knapen *et al.* (1984) had suggested the use of ES antigens allowing for the detection of natural infections even those with low parasitic burdens. Similarly, a survey conducted in Ecuador (Chavez-Larrea *et al.*, 2005) in 2331 pigs at slaughterhouse and 646 pigs of free roaming demonstrated the presence of specific antibodies in 44 samples but failed to confirm the presence of parasites. Since in that study ES antigen was used like in our study the results may not comment, unambiguously.

Since the risk factors were abundant for *Trichinella* its initial prevalence was low. Epidemiological calculation was done to estimate the maximum probable

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prevalence of *Trichinella* in CDR which was 0.52%. The similar prevalence rates of 0.47% and 1% were estimated, respectively, by Joshi *et al.* (2005) and Sapkota *et al.* (2006). However, in their studies only sera were tested so the reliability of the true prevalence is questioned.

Feeding of offal and kitchen wastes were a very common practice in pig farming in Nepal (Joshi *et al.,* 2005). Questionnaire surveys showed that 65% of the farm pigs were allowed to scavenge for feed or they were being tethered during the day along roadsides and were fed with household refuse. These husbandry conditions result in a high mortality and might lead to cannibalism amongst pigs (Yepez-Mulia *et al.,* 1994). 82.5% of the farm owners of pig farms which had responded to the interview had their farm access to garbage. It was a well-documented source of *Trichinella* outbreak (Acha and Szyfres, 2003). This may indicated that either there was no reservoir in Nepal or rats that were get assess to the dead carcass did not reach up to the pig farms. This issue, however, went beyond the scope of this study.

The low prevalence of *Trichinella* in the pig population of the central development region of Nepal in comparison to the high prevalence in the pig population of China could be related to the differences in the ecosystems. It may be due to a natural mountainous barrier of two neighboring countries and also due to the fact that there is no import export of food items between these geographically close nations. In China the prevalence of the *Trichinella* infection in pig was 5% (n = 40 000) and in similar epidemiological studies (n = 2000) in the northern, southern and coastal region a 7.5% positive rate was indicated (Chan and Ko, 1992). However, there were no pig isolates from Tibet (Xizang province of west China) that is the bordering province to Nepal. Also it was expected that the occurring species would be most probably *Trichinella britovi* and *Trichinella pseudospiralis*, because of the temperate climate in that area (Wang *et al.*, 2007). But it was well known that the first outbreak of human trichinellosis in China came from Tibet and in-between 1964-1998 there were 5 outbreaks with a 1/117 mortality rate (Takahashi *et al.,* 2000). From 2000-2003 there were 3 outbreaks with a mortality rate of 4/50 (Wang *et al.,* 2006). Sampling from 2000-2003 in the adjacent province to Tibet and Qinghai indicated a

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3.2% prevalence of infected pork. Concerning this aspect the emergence of *Trichinella* in Nepal in near future cannot be ignored.

However, this preliminary survey to estimate the prevalence of *Trichinella* in Nepal did not reflected that the central development region is free from *Trichinella*. The recent finding that *Trichinella* infection was documented in animals of 3 islands (Ireland, Croatia, Sardinia) previously considered to be *Trichinella* free, strongly supports the concept that there is neither a region nor an area nor a country that could be considered to be *Trichinella* free, totally (EFSA, 2005; Pozio and Zarlenga, 2005). This was well explained through the outbreak in Sardinia (the Mediterranean island) that was supposed to be *Trichinella* (Pozio *et al.*, 2006) free after negative results from an examination of 4427 sera from domestic pigs, 668 wild boars and 8 red foxes and meat from 2036 wild boar and 32 red foxes (Pintore *et al.*, 1996). But the outbreak in 2005 (11 persons were infected) raised the questions of the validity of the concept of a *Trichinella* free area.

The role of the rodents as a reservoir in domestic habitat is a topic of debate (Schad *et al.*, 1987). A survey carried out in Croatia had suggested that the brown rat acts as vector or a victim rather than as a reservoir (Stojcevic *et al.*, 2004). However, 70% of the responded farmers of Nepal had no rodent control program in his/her farm. Since the rodent is as confirmed source it might be the major route of disease transmission from farm to farm in Nepal. It was reported that infected rats represent an offshoot of the domestic cycle, being recipient of infection from that cycle (Campbell, 1983).

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The finding of this study may postulate there was only a very low prevalence of *Trichinella* in the CDR of Nepal. But the questionnaire survey showed that the farming management system and the epidemiological factors supported the transmission of parasites. It should raise the attention of scientific authorities to search, precisely, with high sample sizes and with high sensitive techniques so that the quality assurance regarding the *Trichinella* status of the region could be developed. It was assumed that in economically less developed conditions pigs

presented at slaughterhouses were those which were raised under improved hygiene standards in opposition to those pigs roaming around in villages, slaughtered in rudimentary conditions and destined in local consumption. In that case the presence and zoonotic importance of *Trichinella* should be confirmed. The decision whether this should bear practical consequences for the routine meat inspection must be evaluated in respect of Nepal.

While the surveillance of domestic pigs, wild animals and games could be improved (Zimmermann, 1983) the current study indicates that the risk for humans contracting *Trichinella* infection from eating pork or pork products from pigs of the surveyed districts of the CDR was exceedingly low in comparison to other food borne disease problems existing in that region. 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. It was beyond the scope of this study but it was thought that before documenting a possible reservoir it is necessary to know at least two criteria: the area where the host species is detected should be similar in size or larger than the area where *Trichinella* is present; and the host species should maintain the infection for years, independently, of the presence of *Trichinella* in other animals living in the same area (Pozio, 2005).

However, a complicated issue for *Trichinella* is the presence of wildlife reservoir both in terrestrial and marine mammals (EFSA, 2005). 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.

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5.2 Conclusion and recommendations

The negative results through Pepsin digestion, end-point titer single dilution antibody ELISA and western blot during the period of this study conclude that pigs of the central development region of Nepal were not infected or had a very low infection with trichinellosis at the slaughterhouse level. However, negative finding do not guarantee that the CDR is free from *Trichinella* since comprehensive and complete

knowledge on the epidemiological situation of the parasite in domestic pigs and wildlife within country is unknown. Freedom from *Trichinella* infection within a given area is difficult to document. Despite a negative result from hundreds of millions of swine carcasses it is assumed that the infection exists there at a low prevalence (EFSA, 2005). This study is not applicable for species characterization. Based on the findings of this study the following recommendations are made for the detection of *Trichinella* parasites in order to reduce the risk of trichinellosis in the Nepalese population.

1. Surveillance and monitoring for trichinellosis in pigs

It is recommended that intensive national surveillance is essential for trichinellosis control in domestic pigs in Nepal based on the OIE guideline. To achieve this goal in an efficient manner the production practices that prevent trichinae infection have to be documented. 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. Surveillance and monitoring 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 that *Trichinella* (after all this effort) will not be investigated within the next 5 years the country can apply for the region free certification.

2. Implementation of the meat inspection act

The meat inspection act should be implemented as soon as possible to insure safe meat control during slaughtering, transportation and distribution. In addition to that safe and controlled elimination of all offal and condemned materials is also recommended. The official meat inspection act of the country should require regulations that implement and support a quality assurance program, proper training in both identification methods for everyone involved in the chain of trichinellosis control. For the development of proficiency programs it is necessary to evaluate the quality of the examiners and the examination process. The establishment of cost

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effective laboratory facilities for the diagnosis of trichinellosis should be brought to the attention of the veterinary and public health authorities of Nepal.

3. Awareness regarding trichinellosis

Awareness generating programs should be launched focusing on safe meat consumption habits. The suggested target groups for those programs are butchers, food shop vendors and ethnic communities. In addition the authorities must, systematically, carry out veterinary and sanitary educational programs for both swine breeders and consumers to maximize prevention and control measures. If properly implemented erroneous results in the examination of meat for *Trichinella* can be substantially reduced or eliminated. To achieve these goals, veterinary and human medicines should co-operate in trying to solve any problems of zoonotic origin.

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