

Research Article**PREVALENCE OF PORCINE CYSTICERCOSIS AND ITS ASSOCIATED FACTORS IN KATHMANDU VALLEY, NEPAL****R. Chaulagain^{1*}, B. Sharma¹, S. P. Shrestha² and S. Acharya³**¹ Himalayan College of Agricultural Sciences and Technology, Kathmandu² Nepal Agriculture Research Council, Nepal, Khumaltar, Lalitpur, Nepal,³ Utah State University,**ABSTRACT**

A cross sectional study on prevalence of Porcine Cysticercosis and its associated factors was conducted among 384 pigs by using serum samples in the year 2014 at Kathmandu Valley, Nepal by Ag-ELISA test. The statistical analyses was done by using R 3.0.3 software (R Core Team, 2014) for possible associations between positive cases and each factor of interest. Among 384 pigs tested, 33 animals were found positive against Cysticercosis infection (apparent seroprevalence was 8.59%; 95% C.I. 6.18-11.82%; and the true seroprevalence was 7.9%; 95% C.I. 5.3-11.1%). In bivariate analysis, seroprevalence was found to be significantly associated with breed (improved and local breed were 10.4%; 95% C.I. 7.5-14.5% and 2.3%; 95% C.I. 0.6-7.8% respectively); abortion history in females (18.2%; 95% C.I. 9.5-31.9%) and housing system (indoor housing and free-range grazing were 3.4%; 95% C.I. 1.9-5.9% and 36.7%; 95% C.I. 25.6-49.3% respectively) of pig. In the multivariate analysis, the odds of improved breed being seropositive was 5.1 (95% C.I. 1.2-21.8) times greater that of local; whereas free-grazing pigs showed 16.5 (95% C.I. 7.4-35.6) times higher rate of sero-prevalence than that of indoor raised ones; and both the variables remained significant ($p < 0.01$). The study indicated Kathmandu valley of Nepal as an area of enzootic stability for Cysticercosis infection.

Key words: pigs, Cysticercosis seroprevalence, Ag-ELISA**INTRODUCTION**

Cysticercosis is prevalent worldwide and endemic in humans who eat raw or inadequately cooked pork. This disease in humans and pigs is entrenched in developing countries and emerging as a major health problem (Sciutto et al., 2000). *Taenia solium* is a neglected zoonotic parasite endemic to most underdeveloped countries and regions where pig raising and pork consumption are not restricted (Dorny et al., 2009). Its life cycle involves humans as definitive and pigs as intermediate hosts, but its clinical importance is mainly related to the accidental intake of tapeworm eggs by humans and their development into cysticerci in the host's central nervous system - a condition called neurocysticercosis (Devleeschauwer et al., 2012). Neurocysticercosis (NCC) is the most common parasitic disease of the central nervous system and is a major cause of epilepsy and neurological morbidity in humans in endemic areas of the world. International travel and immigration are bringing neuro-cysticercosis to areas where it is not endemic. The incidence of NCC is also

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increasing in the developed countries (Agarawal and Khanal, 2007). The presence of this parasite in Nepal was first reported more than 30 years ago (Joshi *et al.*, 2004); yet further research is limited. As a result, very little is known about the local risk factors and the public health importance of this zoonosis. To combat this economically ravaging zoonoses reliable diagnosis and understanding of its epidemiology has been vital. Keeping in mind the magnitude and seriousness of the disease due to its public health concern the present investigation was proposed and conducted. The present study was conducted with an objective to study the seroprevalence of Taeniasis /Cysticercosis in pigs of Kathmandu valley by Ag-ELISA test and determine associated factors.

MATERIALS AND METHODS

Before blood samples were collected, permission was obtained from farm owners or slaughterhouse managers. Trained veterinarians collected the sample with no or minimal pain to animal and communicated test results among the farm owners

Study Site

The study was done in pig farms and slaughter slabs of Kathmandu valley consisting of three districts: Kathmandu, Bhaktapur and Lalitpur. The valley lies in central region of Nepal. Kathmandu is the capital city and Lalitpur is also a major city; both are major market for pork and all three districts are major supplier of pork.

Sampling Framework

A total of 384 blood samples were collected randomly from pigs of both small and large farms brought at slaughter slabs and from different pig farms in Kathmandu valley as the total pig population in Kathmandu is 10,000. Preliminary survey was done to know the pig farms and abattoirs which showed approximately 50 commercial pig farms with the average herd of 15-50 pigs and 20 abattoirs with the average slaughter per day of 5-15 pigs in Kathmandu valley (Field Survey, 2014). An average of 6 samples per farm was collected from 50 pig farms and an average of 5 samples per abattoir was collected from 20 abattoirs.

The calculation of sample size is as per Daniel (1999): $n = t^2 \times p(1 - p) / m^2$ where, n = required sample size; t = confidence level at 95% (which equals to 1.96); p = estimated prevalence (0.5); and, m = margin of error at 5% (Standard error of 0.05). With 50% prevalence and 5% error and 95% confidence limit, the sample size is 384.

Sample Analysis

About 10 ml. of blood samples was collected aseptically in a sterile blood collecting vial directly from the jugular vein and from the heart at the places where stabbing or hammering was done; and was immediately transported in ice-cooled box to the Laboratory of Animal Health Research Division (AHRD), NARC, Lalitpur, Nepal. Then the blood samples were centrifuged for collecting the serum. Serum samples were tested by ELISA kit (apDia diagnostic company, Belgium) as per manufacturer's instruction.

Data Analysis

For statistical analysis, the raw data were managed on Microsoft® Excel sheet then exported to R 3.0.3 software (R Core Team, 2014) for further analysis. Apparent prevalence of cysticercosis was calculated by dividing the number of positive cases by total cases. True prevalence was calculated from apparent prevalence and specificity and sensitivity of test method provided by manufacturer using EpiTools ([http://epitools.ausvet.com.au/content.php?page=True Prevalence](http://epitools.ausvet.com.au/content.php?page=True%20Prevalence)).

Summary statistics were generated using R software. Bivariate association between the outcome and individual explanatory variables were assessed using the Pearson's Chi-square test or Fisher Exact test. Liberal cut-off of 20% was used for variable selection to be included in the multivariable analysis. Multivariable analysis was performed by logistic regression model using glm (generalized linear model) function. Final multivariable model was selected using the backward variable selection approach. P-values less than 0.05 were considered significant.

RESULTS

Among the total sampled pigs, 260 were female and 124 were males and the mean and median age of sampled animals was 11.62 and 10.00 respectively.

Among 384 pigs tested, 33 were found positive by ELISA. The apparent seroprevalence, at the individual animal level, was (8.59%, 95% C.I. 6.18-11.82%). The true seroprevalence, after accounting for sensitivity and specificity of the test, was 7.9% (95% C.I. 5.3-11.1%).

Cysticercosis seroprevalence was evaluated based on several variables, as depicted in the bivariate analysis presented in Table 1, and found to be significantly associated with breed, abortion history (in females) and housing system of pig. The seroprevalence in improved and local breed were 10.4% (95% C.I. 7.5-14.5%) and 2.3% (95% C.I. 0.6-7.8%) respectively. The seroprevalence in indoor housing and free-range grazing were 3.4% (95% C.I. 1.9-5.9%) and 36.7% (95% C.I. 25.6-49.3%) respectively. The seroprevalence in sows with history of abortion was found to be 18.2% (95% C.I. 9.5-31.9%).

Table 1. Bivariate analysis for association between Cysticercosis sero-positive pigs and the individual explanatory variables.

Variable and category	Positive animals Number (%)	Odds ratio (95% C.I. ¹)	P-value
Age group			
Upto 5 years	4 (5.1)	1	0.067
6 to 10 years	7 (5.7)	1.13 (0.32 – 3.99)	
Above 10 years	22 (12.1)	2.57 (0.86 – 7.75)	
Sex			
Female	26 (10.0)	1	0.155
Male	7 (5.7)	0.54 (0.23 – 1.28)	
Breed			
Local	2 (2.3)	1	0.018 *
Improved	31 (10.4)	4.88 (1.14 – 20.80)	

Variable and category	Positive animals Number (%)	Odds ratio (95% C.I. ¹)	P-value
Housing			
Indoor	11 (3.4)	1	3.00e-17 **
Free-range	22 (36.7)	16.47 (7.42 – 36.59)	
Abortion history			
No	18 (8.3)	1	0.047 *
Yes	8 (18.2)	2.44 (0.99 – 6.05)	
Growth rate			
Good	17 (6.9)	1	0.125
Poor	16 (11.5)	1.75 (0.85 – 3.57)	
Partition type			
No partition	8 (5.7)	1	0.172 (FET) ²
Semi-solid	23 (10.3)	1.94 (0.85 – 4.48)	
Solid	2 (12.5)	2.43 (0.47 – 12.57)	
Pigs per pen			
Upto two pigs	29 (10.2)	1	0.056
More than two	4 (4.0)	0.37 (0.13 – 1.07)	
Location			
Bhaktapur	16 (8.9)	1	0.968
Kathmandu	11 (8.3)	0.91 (0.41 – 2.04)	
Lalitpur	6 (8.2)	0.91 (0.34 – 2.42)	

¹C.I.: Confidence Interval; ²FET: Fisher's Exact Test; * and ** = significance levels at P<0.05 and P<0.01 respectively

Multivariate analysis (Table 2) showed the significant (p<0.01) association of breed (p<0.01) and housing system. The model containing breed and housing system was selected as the final model. Based on final model, the odds of improved breed being seropositive was 5.1 (95% C.I. 1.2-21.8) times greater than that of local breed being seropositive. The odds of free-grazing pigs being seropositive were 16.5 (95% C.I. 7.4-35.6) times higher than that of indoor raised pigs.

Table 2. Multivariate generalized linear model of cysticercosis in pig.

Variable and category	Odds Ratio (95% C.I. ¹)	P-value
Breed		
Local	1	0.016 *
Improved	5.1 (1.2 – 21.8)	
Housing system		
Indoor	1	2.439e-12 **
Free range	16.5 (7.4 – 36.6)	

¹C.I.: Confidence Interval, * and ** = significance levels at P<0.05 and P<0.01 respectively

DISCUSSION

This paper describes a cross-sectional study on porcine cysticercosis in Kathmandu valley. There have been some studies on porcine, canine and human cysticercosis; however this is the first study estimating seroprevalence of porcine cysticercosis in Kathmandu valley using an adequate sample size and showing associated factors for seropositivity. Our main findings were seropositivity in 8% of pigs tested. This finding is lower than that reported by Joshi et al. (2004) where they detected 14.29% in 2004. Reduction in seropositivity might be due to the awareness of farmers in pig rearing and management. Recently, farmers are rearing pigs in cleaner pens and regularly deworm the animals compared to the past.

Pigs raised on free range were 16 times more likely to be positive compared to rear indoors. Improved (Cross and Exotic) breeds were 5 times more likely to be positive compared to indigenous breeds. In present study, the seropositivity of pigs was 7.9%. Devleesschauwer et al. (2012) conducted epidemiological study of *Taenia* spp on various ecoregions and socially varied household of Nepal and reported that backyard pig raising farmer had more seropositive cysticercosis similar to our finding. Similar results coincides with the study done in Africa by Pondja et al. (2010) and reported free-range pig husbandry system (OR=3.81, 95% CI = 2.08-7.06) as important risk factor. Free ranging has also been associated with porcine cysticercosis in Africa (Sikasunge et al., 2007 and Bimi et al., 2012), as it allows pigs easy contact to feces.

This results coincides the fact with the study done by Devleesschauwer et al. (2012); that the transmission of the zoonotic pork tapeworms *Taenia solium* depends on a combination of specific risk factors, such as open defecation, backyard pig raising and the consumption of raw or undercooked pork and viscera (Devleesschauwer et al., 2012). This difference between the prevalence rate of crossbred exotic breed and indigenous breeds is might be due to that cross bred cattle population were more predisposed than indigenous cattle because of natural resistance and endemic stability between host-parasite relationships (Radostits et. al. 2000 cited by Jyotisree et. al. 2013).

As vaccines against cysticercosis are not available in Nepal, antigens detected in pigs indicated natural exposure to *Taenia* infection. Cysticercosis positive animals have been found in neet. al.ighbouring country India. Ratnam *et al.* (1983) reported a 7% prevalence of cysticercosis in pig carcasses examined at slaughterhouses in Calcutta (east India). In a study performed in the north Indian state of Uttar Pradesh, between 1980 and 1985, 3,550 pig carcasses were screened for cysticercosis and 9.3% were found to be positive for infection (Pathak and Gaur, 1989). Prasad et al. (2002) examined 50 pigs that were slaughtered in 3 villages in the north Indian state of Uttar Pradesh and found that 13 (26%) were infected with cysticercosis. Nepal shares open borders with India and livestock movement between two countries is frequent with very less quarantine check-posts leading to passage of infected animals through borders.

CONCLUSION

The findings of this study has suggested that there is cysticercosis prevalent in pigs supplying pork to Kathmandu valley. This also implies that the infected animals can serve as potential threat to other animals as well as human. Breeds and housing system were identified as potential factors associated with porcine cysticercosis. We recommend increasing the surveillance program for cysticercosis within Nepal and make farmers aware about management and control of disease.

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REFERENCES

- Agarawal, A. and Khanal, G. P. (2007). *Neurocysticercosis: A Review*. Nepal Journal of Neuroscience 3:80-84.
- Bimi, L., Laar A.K., and Anto, F. (2012). *Prevalence and Risk Factors of Taeniasis in the Bunkpurugu-Yunyoo District of Northern Ghana*. J Bacteriol Parasitol 3:129 doi: 10.4172/21559597.1000129.
- Devleesschauwer, B., Aryal A., Joshi D. D., Rijal S., Sherchand J. B., Praet N., Speybroeck N., Duchateau L., Vercauteren J. and Dorny P. (2012). *Epidemiology of Taenia solium in Nepal: is it influenced by the social characteristics of the population and the presence of Taenia asiatica?* Short Communication. Tropical Medicine and International Health. Volume 17 (8): 1019–1022. doi:10.1111/j.1365-3156.2012.03017.x.
- Dorny, P., Praet, N., Deckers, N. and Gabriel, S. (2009). *Emerging foodborne parasites*. Veterinary Parasitology 163, 196–206.
- Joshi, D. D., Maharjan M., Johnsen M. V., Willingham A. L., Gaihr Y. and Sharma M.. (2004). *Taeniasis / Cysticercosis situation in Nepal*. The Southeast Asian Journal of Tropical Medicine and Public Health 34(Suppl. 1): S252–S258.
- Jyothisree, C., Srinivas, N. and Samatha, Y. (2013). *A study on prevalence and clinico-therapeutic management of babesiosis in H.F cross bred cattle in Anantapur district of Andhra Pradesh*. International Journal of Food, Agriculture and Veterinary Sciences ISSN: 2277-209X (Online). Available at <http://www.cibtech.org/jfav.htm>. 2013 Vol. 3 (2) May-August. Pp. 88-91.
- Pathak, K.M., & Gaur, S.N. (1989). *Prevalence and economic implications of Taenia solium taeniasis and cysticercosis in Uttar Pradesh State of India*. Acta Leiden, 57:197-200.
- Pondja A., Neves L., Mlangwa J., Afonso S., Fafetine J., Lee Willingham III A., Thamsborg S. M., Johansen M. V. (2010). *Prevalence and Risk Factors of Porcine Cysticercosis in Angónia District, Mozambique*. Retrieved February 2, 2010 from <http://dx.doi.org/10.1371/journal.pntd.0000594>.
- Prasad, K.N., Chawla, S., & Jain, D. (2002). *Human and porcine Taenia solium infection in rural north India*. Trans. R. Soc. Trop. Med. Hyg. 96:515-6.
- R Core Team. (2014). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.

- Radostits, O. M., Gay, C. C., Blood, D. C. and Hinchcliff K. W. (2000). *Veterinary Medicine. A text book of the diseases of cattle, sheep, pigs, goats and horses*, 9th Ed., W.B. Saunders Co. Ltd., London, UK.
- Ratnam, S., Khanna, P.N., & Bandyopadhyay, A.K. (1983). *Incidence of taeniasis in man*. Indian J Public Health 27:70-4.
- Sciutto E., Fragoso G. and Fleury A. *Taenia solium* disease in humans and pigs. Microbes Infect. (2000). 2:1875-90.
- Sikasunge, C.S., Phiri, I. K., Phiri A.M., Dorny P., Siziya S. and Willingham A.L. (2007). *Risk factors associated with porcine cysticercosis in selected districts of Eastern and Southern provinces of Zambia*. Veterinary Parasitology. 143.1:59-66.