

Comparison of carcass and lingual examination for the diagnosis of porcine cysticercosis in Nepal

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Abstract: With the increase in pig production and consumption by smallholder, there have been problems with zoonotic parasitic diseases especially porcine cysticercosis in Nepal.

Materials and Methods: This study was conducted among 320 pigs from four different slaughter slabs of Chitwan and Kathmandu valley during June to November 2006. The objectives of this study were to determine prevalence by lingual examination, carcass examination and to identify predilection sites of *T. solium* cysticercosis in pigs as well as to compare the lingual examination, carcass examination.

Result: The prevalence rate of cysticercosis for lingual and carcass examination was 0.63%, 0.94% respectively. The incidence of cysticercosis was not significantly different ($P>0.05$) between the Chitwan and Kathmandu valley and lingual ($P>0.05$) and carcass examination ($P>0.05$). Similarly the incidence of the disease was not significantly ($P>0.05$) different among the slaughter slabs by lingual ($P>0.05$) and carcass examination ($P>0.05$). The common predilection sites of the cysts were skeletal muscle, diaphragm, heart, oesophagus and tongue. The sensitivity, specificity, positive and negative predictive values of Lingual examination, taking carcass examination, as gold standard was 50, 100, 100, 98.99 and 100, 82.65 respectively.

Conclusion: Among the animal factor under study breed was found significantly ($P<0.05$) associated with incidence of cysticercosis while the age and sex were not significant.

Keywords: Lingual, porcine cysticercosis, carcass, neuroparasitic

Introduction

Cysticercosis is one of the most important parasitic zoonoses of worldwide occurrence. The fully developed Cyst appears as oval and translucent in pig muscles measures 10-20 mm in diameter. Adult *T. solium*, first detected by Linnaeus, (1758) is dorsoventrally flattened, segmented, 3 to 5 m in length and found in the small intestine of man. It can survive for up to 25 years. Human cysticercosis is a major public health problem in many developing countries. Human Cysticercosis develops with accidental ingestion of eggs in contaminated food or by faeco-oral route.

Worldwide, more than four million people harbor the porcine tapeworm (Schantz, 1989) and 50 million individuals are infected with cystic stage (Schantz *et al.*, 1993). An estimated 50,000 people die from cysticercosis each year because of CNS or cardiac complications (Ghadishah & Burn, 2006). Neurocysticercosis is the

commonest neuroparasitic infection in humans (Arasu *et al.*, 2005) and the statistic figure shows NCC is the commonest cause of seizure in Nepal.

Pig is the intermediate host of the *T. solium* while man acts as both the intermediate as well as final host (Soulsby, 1982). Sheep, deer, dogs and cats are less frequently infected (Kumar and Gaur, 1994). The intermediate host is infected by ingestion of eggs or gravid segments excreted in the human faeces and the eggs transform into cysticerci in the muscles of the host within a period of three weeks to two months (White, 1997). The life cycle is completed when man ingests infected "measly" pork. Human Cysticercosis develops with accidental ingestion of eggs in contaminated food or by faeco-oral route (Soulsby, 1982). Pig farming is increasing in Nepal day by day with the annual population growth rate of 4.55%. The distribution of pig population in different ecological belt of country is 9.47% in mountain, 55.58% in hill and 34.95% in Tarai (CBS, 2006b). Per capita

meat consumption pattern in Nepal is 8.7 kg/per person/year (TLDP, 2003). Pork contributes about 7.32% of total share in Nepal and other as buff 64.68%, Chevon 19.41% and poultry 7.18% (CBS, 2006a) where as pork consumption is highest (38%) in world scenario followed by chicken (30%) and beef (25%) (Bhattarai, 2005). Porcine cysticercosis appears as asymptomatic but affects the quality of carcass resulting heavy economic losses in meat industry (Acevedo, 1982; Flisser, 1988). The highest proportions of cysts are found in the carcass of hind and forelimbs, while lower proportion are found in the tongue, heart, triceps brachii and diaphragm (Boa *et al.*, 2002). In heavily infected cases, cysts are also found in the liver, lungs and intercostal muscles (Kumar *et al.* 1991). Joshi *et al.* (2004a) reported 14.28% of porcine cysticercosis by meat inspection in Kathmandu in 1997. The prevalence rate of porcine cysticercosis in Chitwan valley has been found as 6.66% (Rana and Dhakal, 2005).

Objectives of the present study were:

- I. To determine prevalence of *T.solium* cysticercosis in pigs by ante and post mortem examination.
- II. To identify the predilection site of *T. solium* cyst in the pigs by carcass examination.
- III. To compare the result of lingual examination and carcass examination.

Material and Methods

This study was conducted at Chitwan and Kathmandu valley. Chitwan valley is extended from 27° 21' to 27° 46' north latitude & 83° 55' to 84° 48' east longitude with a total land area of 2218 square kilometer, located at an altitude of 305 to 1945m above the sea level. The Kathmandu valley consists of three districts as Kathmandu, Lalitpur and Bhaktapur. In these valleys there is no pig slaughterhouse so pigs are slaughtered in slaughter slabs. The four different slaughter slabs at Chitwan and Kathmandu valley were visited. The slabs were numbered as Slab 1, Slab 2, Slab 3, and Slab 4. The slab 1 is located in Chitwan Valley where as Slab 2, Slab 3 and Slab 4 are located in Kathmandu Valley. A total of 50 pigs are slaughtered every day in Kathmandu Valley (TLDP, 2002).

Slaughter Slab1: This slaughter slab is located in Chitwan valley near lions club Narayanghad. Mostly the Yorkshire, Landrace, Hampshire crossbreeds are slaughtered. About 4 pigs are slaughtered per day; **Slaughter slab2:** This slaughter slab is one of the oldest and located at Tilganga, Kathmandu. It is not registered. Here mostly the hurrah pigs brought from Terai region are slaughtered. They also slaughter the crossbreed pigs from Kathmandu valley when

there is scarcity of hurrah pigs due to strike. About 8-9 pigs are slaughtered per day; **Slaughter Slab3:** This slab is located in Talchhikhel, Lalitpur and Established 3 years ago. It is one of the modern pig slaughtered slab and established by the help of TLDP. A meat shop is attached with this slab. Slaughtering of mostly the male Dharane pigs is one of the peculiar features of this slab. Slaughter pigs in these slabs come from Dharan and its periphery. About 6-8 pigs are slaughtered per day; **Slaughter slab 4:** This is the oldest slaughter slab located at Talchhikhel, Lalitpur and commonly known as "Dharane Kalo Sungur Meat Center".

Sample Size Determination

Sanchez, (1989) mentioned the formula for sample size determination of problem dealing with the counts like descriptive study or one population problem in the book Research methods in health and medicine as follows: -

$$n = \frac{K^2pq}{d^2}$$

Where,

n = sample size

K = reliability coefficient based on the level of confidence

p = estimate of frequency event q = 1-p

d= maximum amount of deviation from true frequency

The value of K is constant = 1.96 or 95 percent confidence level

The estimate frequency of *Cysticercus cellulosae* in Chitwan valley = 6.66 % (Rana & Dhakal, 2005)

So, p = 0.0666 q = 1-0.0666 = 0.9334

At 5% level of error (allowable error), d = 0.05 So,

$n = \frac{K^2pq}{d^2} = \frac{1.96 \times 1.96 \times 0.0666 \times 0.9334}{0.05 \times 0.05} = 95.52$

So, the minimum sample size will be 96 at 5 % level of error.

Sampling schedule

Sample was collected during the period of July to December 2006. In Chitwan valley sample was collected in July and August where as in Kathmandu valley from September to December 2006. Total 320 pigs were examined for cysticercosis with an equal number sampling unit (80) in each slaughter slab.

Lingual Examination

The pig was controlled with the help of a rope and a wooden jack was kept between the jaws. The tongue was exposed

Comparison of carcass and lingual examination

and inspected visually and by palpation.

Carcass Examination

The head, carcass and viscera were thoroughly examined visually as per the OIE guideline. Meat inspection was done by visual inspection of the carcass and its cut surfaces for the detection of cyst. The muscles of diaphragm, heart, shoulder, thigh and abdomen were thoroughly examined visually; similarly the masseters and the pterygoid muscles were examined on incisions.

Collection and Scoring of Cyst

The positive tissue samples in lingual and carcass examination were examined in detail. The scoring of the cyst was done as follows.

No cyst = 0

Cyst 1-5 = 1

Cyst > 5 = 2

Statistical Analysis

The data were collected in the Dummy Tables during the study period were analyzed by using Microsoft Excel-2002 and SPSS-12. These data were entered on SPSS-12 and descriptive statistical information was calculated.

The prevalence of *T. solium* Cysticercus in slaughter pig (P_1):

$$P_1 = \frac{\text{Total number of cysticercosis cases observed}}{\text{Total number of pigs examined}} \times 100$$

Sensitivity & specificity are important measures of the diagnostic accuracy of a test but can't be used to estimate the probability of disease in an individual animal. Positive & negative predictive values provide estimates of probability of disease but both positive predictive value & negative predictive value vary according to disease prevalence (Akobeng, 2007).

Sensitivity is the proportion of true positives that are correctly identified by the test.

$$\text{Sensitivity} = TP / TP + FN$$

Specificity is the proportion of true negatives that are correctly identified by the test.

$$\text{Specificity} = TN / TN + FP$$

		Gold standard		
		Positive	Negative	
Test	Positive	True Positive (TP)	False Positive (FP)	TP + FP
	Negative	False Negative (FN)	True Negative (TN)	FN + TN
		TP + FN	FP + TN	

Positive Predictive value- is the proportions of the test-positive animals that are truly infected. Positive Predictive Value (PPV) = $TP / TP + FP$

Negative Predictive value- is the proportion of the test-negative animals that are truly not infected. Negative Predictive Value (NPV) = $TN / FN + TN$

Relative risk (RR) is the risk of an event (or of developing a disease) relative to exposure. Relative risk is a ratio of the probability of the event occurring in the exposed group versus the control (non-exposed) group.

		The Outcome	
		Disease developed	Disease not developed
The Exposure	Exposed	a	b
	Not exposed	a	b
Incidence ratio among exposed		a / (a+b)	
Relative Risk = Incidence ration among non-exposed		= c / (c+d)	

Results

Lingual Examination

Among the 320 pigs examined only 2 pigs were found positive during lingual examination. So the prevalence of Cysticercosis on lingual examination is as below

$$P_1 = \frac{\text{Total number of cysticercosis observed on lingual examination}}{\text{Total number of pigs examined lingually}} \times 100$$

$$= \frac{2}{320} \times 100 = 0.63\%$$

Table 1: The distribution of Cysticercosis positive pig during lingual examination by area of origin and slaughter slabs in Chitwan and Kathmandu valley

SN	Particulars		Total carcass	Percentage +ve	+ve	-ve	χ ² (P)
1	Area of examination	Chitwan	80	1.25	1	79	0.671 (P0.413)
		Kathmandu	240	0.42	1	239	
2	Slaughter Slabs	1	80	1.25	1	79	2.013 (P0.570)
		2	80	1.25	1	79	
		3	80	0	0	80	
		4	80	0	0	80	

The distribution of Cysticercosis lingual positive by area of examination and slaughter slabs is shown in the table 3. Out of total 320 pigs, 25% were examined in the Chitwan valley and 75% in Kathmandu valley. According to area of examination 1.25% pigs were positive in the Chitwan valley

where as 0.42% were positive on Kathmandu valley on lingual examination. An equal number of pigs were examined in each slaughter slab. According to slaughter slabs wise distribution slaughter slab 1 and 2 shows the equal distribution of cysticercosis positive on lingual examination. No cyst was found in the slaughter slab 3 and 4 during the study period.

Carcass Examination

Out of 320 pigs examined only 3 pigs were found positive for *Cysticercus cellulosae* during meat inspection. So the prevalence of *Cysticercosis* on meat inspection is as below

$$P_1 = \frac{\text{Total number of cysticercosis observed on meat inspection}}{\text{Total number of pigs examined by meat inspection}} \times 100$$

$$= \frac{3}{320} \times 100 = 0.94\%$$

Table 2: The distribution of *Cysticercosis* positive carcasses during carcass examination by area of origin and slaughter slabs in Chitwan and Kathmandu valley

S.N.	Particulars	Total carcass	Percentage +ve	+ve	-ve	χ ² (P)
1	Area of examination					
	Chitwan	80	1.25	1	79	0.112
2	Kathmandu	240	0.83	2	238	(P0.738)
	1	80	1.25	1	79	
	2	80	2.5	2	78	3.701
	3	80	0	0	80	(P0.296)
	4	80	0	0	80	

The distribution of *Cysticercosis* carcass positive by area of examination and slaughter slabs is shown in the table 4. Out of total 320 pigs, 25% were examined in the Chitwan valley and 75% in Kathmandu valley. According to area of examination 1.25% pigs were positive in the Chitwan valley where as 0.83% were positive on Kathmandu valley. An equal number of pigs were examined in each slaughter slab. According to slaughter slabs wise distribution the highest percentage (1.25%) of *cysticercosis* positive carcass was seen in slaughter slab 2 which was followed by slaughter slab 1 (1.25%). No cyst was found in the slaughter slab 3 and 4 during the study period.

Animal Factors

Age and Sex wise distribution of Slaughtered pigs

Out of 320 slaughtered pigs 71% (226) were male and 29% (94) were female. The pigs between the age of 7-10 months were mostly (62.5%) slaughtered, followed by 11-14 months

(19.06%), 3-6 months (12.5%) and >14 months (5.94%).

Table 3: Age and Sex wise distribution of Slaughtered pigs

S.N.	Age (month)				Sex	
	3-6	7-10	11-14	>14	Male	Female
1	40	200	61	19	226	94
2	12.50%	62.50%	19.06%	5.94%	71%	29%

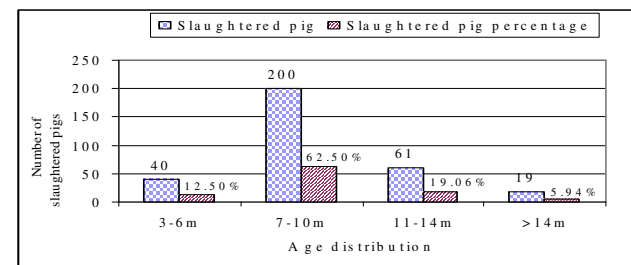


Fig. 1: Age wise distribution of slaughtered pigs.

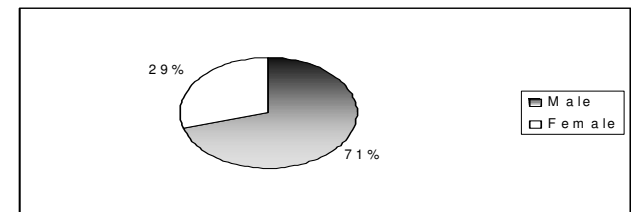


Fig. 2: Sex wise distribution of slaughtered pigs

Discussion

The study showed that most of the farmers are illiterate (56%) so they don't have adequate knowledge about the sanitation and hygiene. Low level of sanitation and hygiene are considered risk factors for the transmission of zoonotic diseases (Ratala, 2005). Still now 32% of the farmers don't have the toilet and are using the open ground for the defecation which is the risk factors for the spread of disease as Ngowi *et al.* (2004) in Tanzania and Diaz *et al.* (1992) in Peru reported a statistically significant association between latrines and incidence of porcine cysticercosis. The housing and feeding pattern shows that 6% of the pigs are rearing in the outdoor housing with scavenging feeding system and 11.5% in the mixed housing of indoor and outdoor with mixed type of feeding habit both scavenging and stall feeding. In free range husbandry system, Pigs have a much higher access to human faeces in communities with few or no latrines was the main risk factors associated with the porcine cysticercosis (Sikasunge *et al.*, 2007, Sarti *et al.*, 1997 and Pouedet *et al.*, 2002). Most of the pigs (51%) are rearing without deworming. These entire factors are responsible for the incidence of cysticercosis.

This study was conducted at four slaughter slabs of Chitwan

Comparison of carcass and lingual examination

and Kathmandu Valley. The result shows that the incidence rate of cysticercosis was not significantly different ($P>0.05$) between the area of examination in the entire diagnostic test as lingual examination ($P=0.413$), carcass examination ($P=0.738$). Similarly the incidence of cysticercosis was not significantly ($P>0.05$) different among the slaughter slabs by lingual examination ($P=0.570$), carcass examination ($P=0.296$). Cysts were mostly found in the muscle and diaphragm followed by heart, oesophagus and tongue. The hind limb muscle, forelimb muscle and the masseter muscles were heavily infected with the *Cysticercus cellulosae* followed by abdominal, cervical and intercostals muscles in all positive carcasses (Pandey, 2007).

Joshi *et al.* (2006) reported 10.5% and 20.5% of porcine cysticercosis on lingual and carcass examination of 200 pigs in Kathmandu valley in 2005 respectively. Sapkota, (2005) reported 0.99% of porcine cysticercosis on carcass examination in Kathmandu valley. The prevalence rate of porcine cysticercosis on carcass examination in Chitwan valley has been reported as 6.66% (Rana and Dhakal, 2006).

In this study, lingual examination showed the sensitivity (50%) and specificity (100%) similarly the EITB showed the sensitivity (100%) and specificity (82.65%). This finding is nearly similar to the finding of the Gonzalez *et al.* (1990) where he reported lingual examination with a sensitivity of 70% and specificity of 100%, the EITB sensitivity and specificity of 100%. Dorny *et al.* (2004) reported the performance of lingual examination as sensitivity (21%) and specificity of 100%, Meat inspection sensitivity of 22.1% and specificity of 100%. Lingual examination has 50% sensitivity.

The ages of the slaughter pigs ranged from 3 to 60 months. The pigs between the age of 7-10 months were mostly (62.5%) slaughtered, followed by 11-14 months (19.06%), 3-6 months (12.5%) and >14 months (5.94%). Out of total 320 slaughtered pigs 71% (226) were male and 29% (94) were female. The incidence rate of cysticercosis wasn't significantly ($P>0.05$) associated with the age ($P=0.176$) and sex ($P=0.880$) however significantly associated ($P<0.05$) with the breed ($P=0.048$).

Taenia cysts collected from pigs in Nepal were reported as Asian genotype first time in history of Nepal through DNA multiplex PCR method (Joshi, 2007).

Cysts are essentially found in the following organs and muscles: heart, tongue, masseters and diaphragm muscles, shoulder and intercostals muscles and esophagus.

Porcine Cysticercosis in Asian countries varies from 0.02 to 32.5%. In China, the infection in pigs is highly variable

ranging from 0.84 to 15% and in some areas as high as 40% (Rajshekhhar *et al.*, 2003). Cysticercosis in a pig farming community in India was found to be 26% and the human taeniosis 38% (Prasad *et al.*, 2002). In Nepal, few reports are available about porcine cysticercosis, human taeniosis and cysticercosis. Joshi *et al.* (2004a) reported 14.28% of porcine cysticercosis in meat inspection in Kathmandu in 1997 and by tongue palpation 32% pigs were positive for cysticercosis at the Magar ethnic community of the Syangja district in 2000. The slaughter prevalence of porcine cysticercosis in Kathmandu valley was found as 0.99% (Sapkota, 2005). The prevalence rate of porcine cysticercosis in Chitwan valley has been reported as 6.66% (Rana and Dhakal, 2006).

Prevalence of Taeniosis/cysticercosis in certain ethnic groups of Nepal is very high due to close relationship between pig and man and frequent consumption of measly pork (Ratala, 2006). The prevalence of human taeniosis in the Sarki and Magar communities of the Syangja district was found as 47.7% (Gaire, 2000). In a study of 152 stool samples from Bote and Darai communities of Tanahun district, taeniosis was found as 18.42% (Joshi & Thapa, 2006). Dorny *et al.* (2004) in a study of 868 slaughtered pigs at Lusaka (Zambia) found the sensitivity and specificity of lingual examination, meat inspection, Ab-ELISA and Ag-ELISA as 0.210, 0.221, 0.358, 0.867 and 1.000, 1.000, sp 0.917 and 0.947 respectively. Souza & Hafez, (1999) reported serodiagnosis as the major reliable technique than meat inspection. Joshi *et al.*, (2006) conducted a survey of porcine cysticercosis where by 200 pigs were subjected to lingual examination, Post-mortem carcass examination with 10.5, 22.5 and 20.5% found positive, suggesting lingual examination method for detecting porcine cysticercosis is easy, inexpensive and could be utilized as a surveillance tool in developing countries like Nepal where technical resources and technological capacity are very limited.

Conclusion

This study shows that a zoonotic disease like porcine cysticercosis is prevalence in pig and human population of some districts of Nepal. There are very few programs for its prevention, control and eradication. The current study findings not merely reflect the situation in the study area but it may be representative situation of all the intensive farming areas. Lingual examination could predict the disease before slaughtering. The meat inspection act and regulation should be followed strictly to avoid the suffering from such a zoonotic disease. Parasitic diseases control, disease surveillance, awareness and treatment or deworming human tapeworm carrier, toilet facilities and pig husbandry

practices should be improved. Access of pigs to human faeces should be controlled. Recent survey also showed that human population is also suffering from Neurocysticercosis thus long term control strategy involve health education, modernization of pig farming, rigorous inspection of pork in slaughter slabs, sanitary and hygienic condition in the community, proper disposal of human faeces as well as sewage management system to break the cyclozoonosis of the parasite, active epidemiological surveillance system to identify tapeworm carrier and to activate potential eradication.

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Comparison of carcass and lingual examination

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