Validation of Lingual and Carcass Cyst with Enzyme Linked Immunoelectrotransfer Blot (EITB) for the Diagnosis of Porcine Cysticercosis

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ABSTRACT

Background: Cysticercosis is a major public health problem worldwide. The objectives of this study were to determine prevalence by lingual examination, carcass examination and enzyme-linked immunoelectrotransfer blot (EITB) and to identify predilection sites of T. solium cysticercosis in pigs as well as to validate the lingual examination, carcass examination and EITB for the diagnosis of porcine cysticercosis.

Methods: A study was conducted among 320 pigs from four different slaughter slabs of Chitwan and Kathmandu valley during June to November 2006. Lingual and carcass examination were carried out in all pig samples. Serological EITB diagnosis was carried out in 100 pig serum samples.

Results: The prevalence rate of cysticercosis by lingual examination, carcass examination and EITB was 0.63%, 0.94% and 19% respectively. The incidence of cysticercosis was not significantly different (P>0.05) between the Chitwan and Kathmandu valley and in the three diagnostic test as lingual (P>0.05), carcass examination (P>0.05) and EITB (P>0.05).

Conclusion: The sensitivity, specificity, positive and negative predictive values of Lingual examination and EITB, taking carcass examination as gold standard was 50, 100, 100, 98.99 and 100, 82.65, 10.53 and 100 percent respectively.

Key words: cysticercosis; porcine; carcass; lingual

INTRODUCTION

Neurocysticercosis is a severe disease of human in which cysticerci can survive for many years in the brain. The parasite usually develops in the ventricles of the brain. Sporadically clinical examination such as pigs tongue used as diagnostic method for cysticercosis. Therefore, main goals were to identify recent prevalence of porcine cysticercosis in pigs in Nepal using lingual examination, carcass examination as well as Taenia solium cyst antigen EITB test. The adult T. solium occurs in the small intestine of man. Taenia cysts collected from pigs in Nepal were reported as Asian genotype first time in history of Nepal through DNA multiplex PCR method[1]. Meat inspection has been using as the most common diagnostic method for the detection of cysticerci in pigs. A survey of porcine cysticercosis was conducted where by 200 pigs were subjected to lingual examination, antibody detection by ELISA and Post-mortem carcass examination with 10.5%, 22.5% and 20.5% found positive respectively[2].

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Objectives of the present study are:

I.   To determine prevalence and identify predilection site of *T. solium* cysticercosis *Cysticercus cellulosae* in pigs by ante and post mortem examination.

II. To determine sero-prevalence of cysticercosis using *T. solium* cyst wall glycoprotein antigen EITB.

III. To compare the result of lingual examination, carcass examination and EITB.

METHODS

This was a comparative diagnostic study between lingual, carcass examination and EITB test. In this study stunted pig were used for lingual examination before slaughtering, blood sample collection was done from slaughtered pig after stunting and pig carcass were examined for cyst. Therefore this study does not need any ethical clearance. This study was conducted at Chitwan and Kathmandu valley. The Kathmandu valley consists of three districts as Kathmandu, Lalitpur and Bhaktapur. The sample size determination was done by the standard formula which is mentioned below.

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

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

The estimate frequency of Cysticercus cellulosae in Chitwan valley = 6.66 % estimated[3].

So,   p = 0.0666   q = 1-0.0666 = 0.9334
        At 5 % level of error, d = 0.05

So,

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

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

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. The individual pig was the sampling unit, which was selected randomly at the time of slaughter. Total 320 pigs were examined for cysticercosis with an equal number sampling unit (80) in each slaughter slab.

About 10ml blood sample was collected in a sterile blood-collecting vial without EDTA directly from the heart just before the Slaughtering of pigs.

Lingual Examination

The pig was controlled with the help of a rope and a wooden jack was kept between the jaws. The tongue was exposure and inspected visually and by palpation. After slaughtering the tongue was sliced by a knife and the cut surfaces were examined thoroughly for the detection of any cyst if present.

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.

Serological Study by EITB

EITB was conducted with the help of National Zoonoses and Food Hygiene Research Centre, Kathmandu and Neurology Laboratory of Christian Medical College, Vellore, India. This assay used purified glycoprotein antigens in an EITB format to detect infection-specific antibodies in serum.

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

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

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

The distribution of cysticercosis lingual positive by area of examination and slaughter slabs is shown in the table 1. 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

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

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

According to area of examination 1.25% pigs were positive in the Chitwan valley where as 0.83% were positive on Kathmandu valley (See table 2).

Serum Examination (EITB)

Out of 320 pigs examined 100 serum samples (25 from each slaughter slab) were examined by EITB method. Out of 100 serum sample 19 were found positive for Cysticercus cellulosae during EITB. So the prevalence of Cysticercosis on EITB is as below

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

$$= \frac{19}{100} \times 100 = 19\%$$

Out of total 100 pigs' sera examined. 25% were collected from Chitwan valley and 75% from Kathmandu valley. According to area of examination 20% pigs were positive in the Chitwan valley where as 18.67% were positive on Kathmandu valley

Sensitivity, Specificity and Predictive Values of Lingual Examination and EITB

The sensitivity, specificity and predictive values for lingual examination and EITB were calculated taking carcass examination as the gold standard. Each test was considered true positive when the particular test and carcass examination were positive.

The result showed highest specificity and positive predictive value both 100%, high negative predictive values (98.99%) and medium sensitivity (50%) for lingual examination where as highest sensitivity and Negative predictive values both 100%, high specificity (82.65%) but

Table 1. The distribution of cysticercosis positive pig during lingual examination by area of origin in Chitwan and Kathmandu Valley.

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

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	x^2 (P)
1	Area of examination	Chitwan	80	1.25	1	79	0.112
		Kathmandu	240	0.83	2	238	(P0.738)
2	Slaughter Slabs	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	

lowest positive predictive value (10.52%) was recorded for EITB.

Table 3. Breakdown of test results with respect to carcass examination as gold standard (n = 100)

Test Results			Gold Standard Carcass Examination	
			+ve	-ve
	Lingual examination	+ve	1	0
		-ve	1	98
	EITB	+ve	2	17
		-ve	0	81

Table 4. Sensitivity, Specificity and Predictive values of Lingual examination and EITB taking Carcass examination as gold standard.

Parameters	Tests	
	Lingual examination	EITB
Sensitivity	50%	100%
Specificity	100%	82.65%
Positive Predictive Values	100%	10.53%
Negative Predictive Values	98.99%	100%

DISCUSSION

In Nepal, there is no standardized method which could permit reliable, unbiased epidemiological data. The pigs are the main hosts of the metacestode. Eggs can pass through sewage treatment plants and be released into rivers with the effluent, contaminate pasture treated with sludge or be disseminated by birds feeding on raw sewage.[4] It has been 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.[5] The cysticerci are palpable on lingual examination at an anti-mortem examination. Cysticercosis endemic areas have been using lingual examination to reject the pigs that are recognized having *T. solium* larvae under the tongue for fear of losing money due to carcass condemnation in meat inspection.[6]

Serological tests for animals have not reached the stage where commercialization for individual diagnosis is possible. It has been reported 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.[7] It has suggested that 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.[5, 8-10]

The prevalence rate of porcine cysticercosis was found as 0.63, 0.94 and 19% based on lingual and carcass examination of 320 pigs and serum examination (EITB) of 100 pigs respectively. It has been reported that 10.5% and 20.5% of porcine cysticercosis on lingual and carcass examination of 200 pigs in Kathmandu valley in 2005 respectively.[6] It has been reported 0.99% of porcine cysticercosis on carcass examination in Kathmandu valley.[11] The prevalence rate of porcine cysticercosis on carcass examination in Chitwan valley has been reported as 6.66%.[12] 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) and serum examination i.e. EITB (P=0.883). 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) and serum examination i.e. EITB (P=0.056).

CONCLUSIONS

In conclusion the sensitivity, specificity, positive and negative predictive values of lingual examination and EITB, taking carcass examination as gold standard, was 50, 100, 100, 98.99 and 100, 82.65, 10.53 and 100 percent respectively.

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