

A New Tapeworm *Taenia solium* Asian Genotype Recorded First Time in Nepal Through DNA Multiplex PCR Method

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Abstract

Introduction The disease in human and pigs in an ancient parasitic disease rooted in developing countries and emerging as a major health problems of global dimensions. The infection is common in low socio-economic and poor sanitary community. Cysticercosis cellulosa usually name as cysticercosis is produced by the attack human tissues or organs by the larval form of *Taenia solium*.

Objectives The study was conducted to collect *Taenia* cyst from pig meat and to carryout DNA multiplex PCR diagnosis on *Taenia* cyst fluid for testing genotype.

Methods Total 200 pigs were examined one by one for lingual examination and post mortem examination for cysts. Then cysts were preserved in 70 percent ethanol and kept at 5°C in refrigerator before testing for DNA complex PCR analysis. The test was carried out using multiplex PCR protocol provided by the Department of Parasitology, Asahikawa Medical College, Asahikawa, Japan.

Results Each pig carcass was examined thoroughly to look for taenid cysticerci. Out of 200 carcasses, only 41 (20.5%) had cysts. Some of them had several thousand cysts and only few had few cysts. Taeniid cysticerci colleted from pigs in Nepal diagnosed first time by DNA multiplex PCR method were all found to be of *Asian genotype*. This is the new *T. solium* Asian genotype diagnosed and recorded first time in history.

Conclusion Taeniid cysticerci (*Taenia* cyst) were collected from pigs of Kathmandu Valley which were imported from mainly Sunsari district and periphery of Kathmandu Valley. So far *Taenia solium* American genotype, African genotype and Asian genotype have been found in different countries of the world. But in Nepal this is the first time *Taenia solium* Asian genotype has been recorded.

Key words Porcine, Lingual, Cysticerci

Introduction

Cysticercosis cellulosa usually name as cysticercosis is produced by the attack human tissues or organs by the larval form of *Taenia solium*. It can locate not only in skin muscles, brain and eye orbits but also in other important organs in man. Human can be infected Cysticercus cellulosa by eating eggs or the gravid segments of adult tapeworms of *T. solium*, and also become infected metacestode of *T. solium* by consuming raw or insufficiently cooked measly pork, which contain C. cellulosa. So, man is the intermediate host and the definitive host of *T. solium*¹ (Chen et al., 2005).

The disease in human and pigs in an ancient parasitic disease rooted in developing countries and emerging as a major health problems of global dimensions. The infection is common in low socio-economic and poor sanitary area of central and southern Mexico and central and southern America. The infection is also present in India, Pakistan, North China, Thailand and Nepal.

Epidemiological Cycle of Cysticercosis

The disease in humans and pigs is an ancient parasitic disease rooted in developing countries and emerging

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as a major health problem of global dimensions² (Sciutto *et al.*, 2000). The infection is also present in India, Pakistan, North China, Thailand and Nepal³ (Scantz *et al.*, 1992). *Taenia* cysts were first time observed in pig meat slaughtered in Kankeshwori, Kathmandu^{4,5} (Joshi, 1973 and Joshi, 1991).

Taeniasis refers to a human infection with the adult tapeworm of *Taenia solium* and *Taenia saginata*. The infective stage of *T. solium* called *Cysticercus cellulosae* develops in the pig muscles while that of *T. saginata* called *Cysticercus bovis* develops in muscles of cattle and buffaloes. The adult stages of *T. solium* and *T. saginata* are obligatory intestinal parasites for man. The infection with the larval stage of *T. solium* is called cysticercosis.

Humans become infected by ingesting uncooked or poorly cooked water buffalo or pork infected with cysticerci, and the mature tapeworms develop in the intestines. Eggs and gravid proglottids release from adult worms and excrete to the environment. The eggs are ingested, and the larval stages, *Cysticercus bovis* of *T. saginata* is found in the muscles of water buffaloes and *Cysticercus cellulosae* of *T. solium* in those of pigs.

Human infection by *Taenia solium* metacestodes is recognized, when it is a systemic infection, as cysticercosis, and when focused in the brain, as neurocysticercosis (NCC). The infection frequently occurs in populations living in poor sanitary conditions. Persons infected with *T. solium* will initiate the spread of proglottids into an endemic environment. Cysticercosis is, therefore, a communicable infectious disease among humans residing in poor and unhygienic communities. The most important human cestodes in Nepal are *T. solium*, *T. saginata*, and *T. asiatica*, known to be found in several districts of the country.

Joshi^{6,7} *et al.*, in 2001 and 2004, have studied and found the prevalence of porcine cysticercosis to be 32.5 percent (419 pigs' lingual inspection); in male pigs, it was 31.5 percent (92) in individuals below six months of age and 22.5 percent (120) in those above six months of age; in female pigs, it was 27.3 percent (99) in individuals below six months of age and 51.0 percent (108) in those above six months of age; prevalence of porcine cysticercosis was 23.5 percent (204 pigs examined by antibody ELISA). Prevalence of cysticercosis in Tindobate, Tulsibhanjyang, Jagatradevi VDCs and Waling municipality were 37.5, 43.7, 17.1 and 29.1 percentages respectively⁸ (Maharjan, 2002).

Objectives

- 1 To collect *Taenia* cyst from pig meat
- 2 To conduct DNA multiplex PCR diagnosis on *Taenia* cyst fluid for testing genotype.

Methodology

The study was conducted in outer Kathmandu and Lalitpur cities areas in a pig rearing community in Kathmandu Valley where these pigs had been imported from Sunsari, Morang, Jhapa, Dhankuta and Makwanpur by trucks.

This part of the study has been focused on two places. The first one was "Dharane Kalo Bangurko Meat Centre" or NIPPON in Talchhikhel, Lalitpur and the second one was Safety and Healthy Meat Shop, Talchhikhel, Lalitpur. Usually crossbred 'Pakhrabas black' pigs are slaughtered and sold in these two pork shops. But some Hampshire crossbreds and Hurrath crossbreds are also found to be sold from these centres.

Sample size

200 pigs between age group 3 months to one year were proposed in the proposal but 200 pigs were examined one by one for lingual examination and test serum collection and post mortem examination for cysts followed this.

Taenia cyst collection

After lingual inspection and blood collection, all pigs one by one transported to the two slaughterhouses, namely National Industry of Pork Processing Nepal (NIPPON), also called Kalo Dharane Bangur Meat Shop, and "Safety and Healthy Meat Shop", Talchhikhel, Lalitpur. Thorough post-mortem examination of all relevant organs including the tongue, neck, diaphragm, psoas muscles was carried out for *T. solium* cysts and the number of cysts in the organs were counted by individuals by visual inspection. Then cysts were preserved in 70 percent ethanol and kept at 5°C in refrigerator before testing to Japan for DNA/PCR analysis. The author himself went to Asahikawa Medical College, Asahikawa, Japan for laboratory diagnostic test.

Results

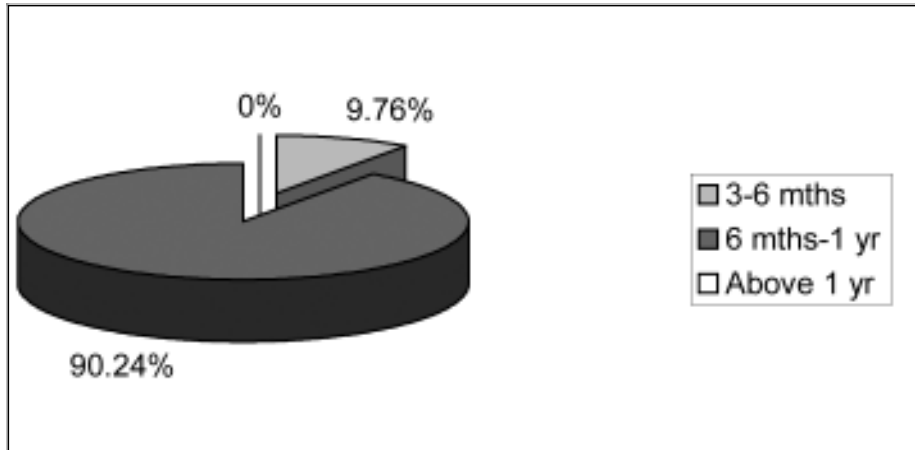
Each pig carcass was examined thoroughly to look for taenid cysticerci. Out of 200 carcasses, only 41 (20.5%) had cysts. Some of them had several thousand cysts and only few had few cysts. In some cases, that had several hundred cysts in the carcass,

cysts were also seen even in tongue. Age wise pig carcass examination results for Taenia cysts is presented in table no. 1 and graph no. 1.

Table 1: Age wise Pig Carcass Examination Results for Taenia cysts.

Age Group	Total pigs examined	Positive	Positive (%)
3-6 mths	16	4	9.76
6 mths-1 yr	181	37	90.24
Above 1 yr	3	-	-
Total	200	41	20.5

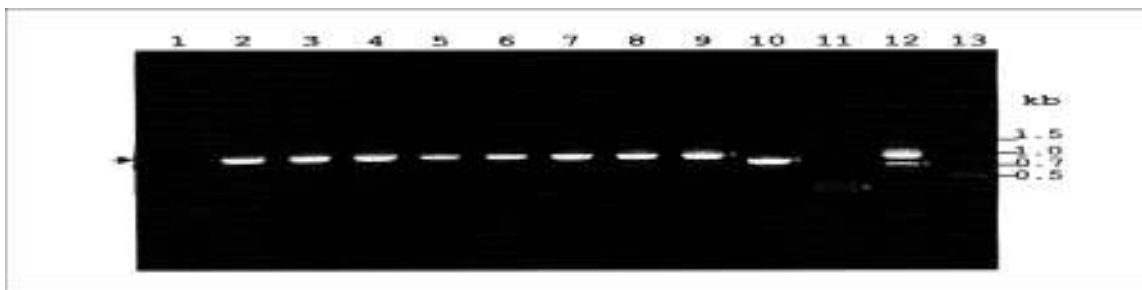
Graph 1: Age wise pig carcass examination results for Taenia cysts.



Thus it's clear from the above table that only 20 of the total 200 pigs examined were positive for all the three diagnostic tests. Out of the 21 pigs positive on lingual examination for porcine cysticercosis, only one did not show positive for cyst in the carcass in spite of being ELISA positive. On the other hand, out of the 45

serum samples positive for porcine cysticercosis as exhibited by ELISA, four were negative for the pig carcass examination for Taenia cysts. DNA multiplex PCR diagnosis result of porcine Taeniid cysticerci is presented in graph no. 2.

Graph 2: DNA multiplex PCR diagnosis result of porcine Taeniid cysticerci.



Multiplex PCR diagnosis of taeniid cyaticerci collected from pigs in Nepal.

- Lane 1. blank without template DNA
- Lane 2-8. cysticerci from Nepal 1
- Lane 9-12. *T. solium* (Asian genotype), *T. saginata*, *T. asiatica*, and *T. solium* (American/African genotype) as controls
- Lane 13. 100 bp ladder DNA size markers
- *Diagnostic band

Taeniid cysticerci collected from pigs in Nepal diagnosed first time by DNA multiplex PCR method were all found to be of *Asian genotype*. This is the new *T.solium* Asian genotype diagnosed and recorded first time in history.

Discussion and Conclusion

Taeniid cysticerci (*Taenia* cyst) were collected from pigs of Kathmandu Valley which were imported from mainly Sunsari district and periphery of Kathmandu Valley. So far *Taenia solium* American genotype, African genotype and Asian genotype have been found in different countries of the world. But in Nepal this is the first time *Taenia solium* Asian genotype has been recorded.

Consumption of mealy pork from infected pigs with cysts may cause taeniasis and trichinellosis. Cysticercosis may result from autoinfection or accidental ingestion of eggs of tapeworm. Human cysticercosis is an emerging public health problem in Nepal. Development of larva of *Taenia solium* in the brain is called cerebral cysticercosis or Neurocysticercosis, which is responsible for fatal outcome. The larva may also develop in the anterior chamber of the eye, which is known as ocular cysticercosis, and this may even lead to blindness. In severe conditions, three phases of trichinellosis (intestinal, muscle invasion and convalescent) are reported which occasionally leads to death. Pork from most of the shops has been found to be contaminated. Shrestha⁹ *et al.* (2003) has reported that pork sold from various shops in Kathmandu was contaminated with nine different types of bacteria of which *E. coli*, *Salmonella typhi* and *Staphylococcus aureus* are of public health importance. The other challenging aspect that has always been in shade is the effect of drug residues in pork in the health of the consumers as meat withdrawal period of various drugs has been ignored and no organization prevails to monitor it.

Out of 21 lingual positive, only one pig sample did not show positive for cyst in the carcass whereas out of 41 positive for serology ELISA test, only four pigs were negative for cyst in the carcass. This has been validated that lingual, ELISA and cyst examination result showed that almost all lingual positive were also positive for serology and cysts in muscle.

Taenia solium is a predominant food-borne parasitic zoonosis (FBPZ) in South East Asia Region, India and Nepal. Based on behavioral and environmental assessments (Steps 1 and 2 of PRECEDE-PROCEED), following strategies should be adopted to prevent and control taeniasis¹⁰ (Joshi *et al.*, 2001).

- a. Train meat producers and sellers to detect contaminated pork and avoid selling it.
- b. Improve pig husbandry to limit the animals' access to human faeces.
- c. Construct hygienic model slaughterhouses.
- d. Enforcement of Animal Slaughtering and Meat Inspection Act.
- e. To carry out more validation comparative diagnostic tests of porcine cysticercosis in the country.

The present available data on both taeniasis, human and porcine cysticercosis indicated that the disease is very severe and developing as a serious public health concern.

It has been found that the comparative diagnostic results of porcine cysticercosis between ELISA, lingual examination and pig meat with taeniasis results of this study has provided the basis of lingual examination validated with serology and taenia cysts found in muscle of same group of pig population. This has suggested carrying out further national surveys on porcine cysticercosis in bigger scale¹¹ (Joshi *et al.*, 2006).

The review of the Taeniasis and cysticercosis research in Nepal showed that, parasitic infection has existed since past many years. But recording on the basis of research is still in the beginning stage. The available data on both taeniasis in humans and cysticercosis in humans and porcine indicated that the disease is very severe and developing as a serious public health importance⁷ (Joshi *et al.*, 2005).

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References

1. Chen Y, Xu L. and Zhou X. Cysticercosis cellulosae in China. In: Akira, HW and Hiroshi, Y (Eds.). Taeniasis, Cysticercosis and Echinococcosis in Asia. The Federation of Asian Parasitologists. 2005; Vol. 2. pp. 37-83.
2. Sciuto E, Fragoso G, Fleury A, Lacleite JP, Sorelo J, Aluja A, Vargas L and Larralde C.. *Taenia solium* disease in humans and pigs: an ancient parasitosis disease rooted in developing countries and emerging as a major health problem of global dimensions. *Microbes and Infection* 2000; 2(15): 1875-90.
3. Schantz PM, Annc C, Moore JL, Munoz BJ, Hartman JA, Schaefer AM, Aron DP, Sarti E, Wilson M and Flisser A. Neurocysticercosis in an orthodox Jewish community in New York City, *N. Engl. J. Med.* 1992; 327(10): 629-95.
4. Joshi D.D. Veterinary Public Health Hazards in Nepal. Published by K.D. Joshi, Tahachal, Kathmandu, Nepal. 1973
5. Joshi DD. Current Practices of Livestock Slaughtering and Meat Marketing in Kathmandu, Lalitpur and Bhaktapur. *Published by NZFHRC, Tahachal, Kathmandu, Nepal.* 1991
6. Joshi DD, Poudyal PM, Jimba M, Mishra PN, Neave LA and Maharjan M.. Epidemiological status of *Taenia/cysticercosis* in pigs and humans in Nepal. *Journal of Institute of Medicine* 2001; 23: 1-12.
7. Joshi DD and Maharjan M.. Taeniasis and Cysticercosis Situation in Nepal. In: Akira, HW and Hiroshi, Y (Eds.). Taeniasis, Cysticercosis and Echinococcosis in Asia. The Federation of Asian Parasitologists. 2005; Vol. 2. pp. 141-53.
8. Maharjan M.. An epidemiological study of Porcine Cysticercosis among Magar Communities of Syangja District, Nepal. Final Report. National Zoonoses and Food Hygiene Research Centre Kathmandu, Nepal. 2002.
9. Shrestha RD, Adhikari DR and Manandhar P.. Study of Bacterial Contamination in Raw Meat of Different Species of Animals (Buffalo, Goat, Pig, Chicken) in Kathmandu Valley. Proceedings of the 7th National Conference of Nepal Veterinary Association (VETCON '03), 5-7 November 2003, Kathmandu, Nepal. Nepal Veterinary Association. 2003; p. 121
10. Joshi DD, Poudyal PM, Jimba M, Mishra PN, Neave LA and Maharjan M.. Controlling *Taenia solium* in Nepal using the PRECEDE-PROCEED model. *Southeast Asian J Trop Med Public Health* 2001; 32 Suppl 2: 94-7.
11. Joshi D. D., Ito A., Yamasaki H., Willingham A.L.. Epidemiological Diagnostic Status of Porcine Cysticercosis in Nepal. Present Situation Challenges in Treatment and Elimination of Taeniasis/Cysticercosis in Nepal. Edited by Dr. Durga Datt Joshi, Ms. Minu Sharma and Dr. Sujan Rana, *NZFHRC, Kathmandu, Nepal.* 2005; PP 3-11.