Identification of non-cerebral cyst: Zoonotic *Taenia multiceps* in domestic goat in Bangladesh

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**Abstract**

**Aim:** This study was performed to identify the non-cerebral *Taenia multiceps* cyst through molecular phylogeny of the 12S rRNA gene.

**Materials and Methods:** Eight cyst samples were collected from 385 examined slaughtered goats during October 2015-September 2016 from three slaughterhouses in Chittagong City Corporation. Cysts were removed from the thigh muscle, and scoleces were collected for light microscopic examination and molecular identification. The DNA was extracted and analyzed by polymerase chain reaction using 12S rRNA gene primers. Cyst samples were also preserved in 10% buffered formalin for histopathological study.

**Results:** *T. multiceps* non-cerebral cyst is 2.1% prevalent in goat in this area. Under light microscopic examination, scolex was found with four suckers and a rostellum with the double crown of 32 hooks and hooklets. Molecularly, all the samples were amplified with 12S rRNA gene fragments yielded 270 base pair amplicon. Zenker’s necrosis with focal to diffuse infiltration of lymphocytes and eosinophil was also found around the cyst wall in histopathological examination.

**Conclusion:** Although the non-cerebral form of the cysts produced by *T. multiceps* is genetically identical with the cerebral cyst, previously published data indicated that cerebral *T. multiceps* cyst is predominant in other parts of the world as well as in Bangladesh. This study showed that non-cerebral cyst is also prevalent in this country which is very important for public health concern. This study depicts an idea of non-cerebral form of zoonotic *T. multiceps* cyst which will be helpful in taenia cyst control and prevention.

**Keywords:** 12SrRNA, Bangladesh, goat, non-cerebral cyst, phylogeny, *Taenia multiceps*.

**Introduction**

The cestode *Taenia multiceps* is a causative agent for coenurosis in sheep and goat affect the central nervous system (CNS) particularly the brain of sheep and goats but rarely in the spinal cord also called Coenurus cerebralis [1,2]. Coenurosis is widely recognized as zoonotic parasite [3] and is presents as an endemic condition in small ruminant in Bangladesh [4]. The disease is recorded as 2.4-5.6% in goat in Bangladesh [5,6]. Environmental factors such as rainfall, relative humidity, and air temperature are considered to be the risk factors for coenurosis in this country [7].

Besides this, a lack or limited use of anticestodal drug, uncontrolled backyard slaughtering, and improper offal disposal are also responsible for transmission of this cestode [8]. The cerebral form of the cysts produced by *T. multiceps* in sheep is genetically identical with the non-cerebral cysts of *T. multiceps gaigeri* [9,10] in intramuscular and/or subcutaneous tissues in goats [11,12]. Recent molecular phylogenetic analysis of nad1, cox1, and 12S rRNA showed that *T. multiceps* can cause cerebral coenurosis in sheep and non-cerebral forms in goats but rarely in sheep [13]. Information on intramuscular and subcutaneous (other organs) coenurosis in goats is limited and incomplete. Molecular study of *T. multiceps* has not been done yet, but *Echinococcus granulosus* has been identified molecularly in cattle in Bangladesh [14].

The aim of the present study was to determine the prevalence, morphological features of parasite scolex, and molecular detection of *T. multiceps* besides phylogenetic analysis of the goat isolates sequence with NCBI reference sequences for characterization of cyst in this area.

**Materials and Methods**

**Ethical approval**

All samples were collected and complies with relevant legislation. It follows the international
guiding principles for biomedical research involving animals.

Collection of T. multiceps cysts

Eight cyst samples were collected from 385 examined slaughtered goats in three slaughterhouses located in Chittagong City Corporation during October 2015-September 2016. After gross examination cysts were removed from the parasitized organs and preserved in 10% buffered formalin for histopathological study and the scoleces or larval tissue material from cysts was preserved in 90% ethanol for molecular study. Scoleces were collected through scraping from the sides of germinal layer of the cyst also stained with eosin stain for light microscopic examination.

Genomic DNA extraction and amplification by polymerase chain reaction (PCR)

Genomic DNA was extracted from protoscolices using iNtRoN Patho gene Spin DNA/RNA Extraction Kit according to the manufacturer’s recommendations. Genomic DNA concentrations were determined spectrophotometrically by Qubit 2.0 (Invitrogen, Korea), and the DNA samples were stored at −20°C until used. 12S rRNA gene primers were designed according to the mitochondrial genome sequence of T. multiceps as follows: Tae-F (5’-GATTCTTTTTAGGGGAAG G-3’) and Tae-R (5’-GCGGTGTGTACMTGAGCTAAAC-3’). 50 µl of PCR reaction was performed using 10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM of each deoxynucleotide triphosphate, 20 pmol of each primer, 100 ng template gDNA, and 2 U Taq DNA polymerase (Tokyo, Japan) in each reaction under the following cycling conditions: After an initial denaturation at 94°C for 5 min, then 94°C for 30 s (denaturation), 55°C for 1 min (annealing), and 72°C for 30 s (extension) for 35 cycles, followed by a final extension at 72°C for 10 min. Samples without genomic DNA were included in each amplification run as negative controls. PCR products were separated on a 1% agarose gel and detected by ethidium bromide staining.

Sequencing and phylogenetic analysis

Four PCR products were purified by PCR purification kit (FavorPrep™ PCR Clean-Up Mini kit, Favorgen Biotech Corporation, www.favorgen.com) according to the manufacturer’s instructions, and then, these purified products send to Bioneer, Korea, for sequencing. Sequence chromatograms were analyzed using the Chromas Pro 2 (South Brisbane and Mega 6.0 computer software programs [15]. Available reference sequence of T. multiceps was retrieved from NCBI GenBank, and E. granulosus (as outgroup) also inferred from other publications [16]. Sequences were compared with each other using Chromas and BLASTn program. After multiple alignments by ClustalW, phylogenetic analyses of the sequences data were performed using 12S rRNA gene sequences, and phylogeny tree was drawn using sequences obtained in this study as well as reference sequences of all described T. multiceps by MEGA6 software.

Histopathology

Tissue specimens collected from the affected muscular area were fixed in 10% neutral buffered formalin. The paraffin blocks were prepared, and sections of 4-5 µm thickness were obtained on glass slides with a rotatory microtome. These paraffin sections were stained with hematoxylin and eosin stain for routine histopathology [17].

Results

In this study, a total of 385 domestic goats were examined during postmortem. Eight animals (2.1%) were found infected with taeniid cysts with an average diameter of 2.7 cm (Table-1). The entire cysts were observed in the thigh muscle, inguinal region, and in the prescapular region (Figure-1).

The characteristics morphology of T. multiceps larvae was seen in all of the cysts containing clear fluid with numerous fertile protoscolices. A large number of scoleces as white clusters were attached to the internal cyst wall (Figure-2). Under the microscope, larvae or scolex was examined at 10× magnification (Figure-3). Most of the scoleces had four suckers and a rostellum with a double crown of 32 hooks with hooklets (Figure-4).

Histopathological examination of the cyst

The thin fibrous cyst wall causes mechanical destruction in muscle layer associated with degenerative and necrotic changes including hyaline degeneration and Zenker’s necrosis (Figure-5). Diffuse infiltration of lymphocytes and eosinophil was also found around the cyst wall and between muscle fibers (Figure-6).

Molecular study and phylogenetic analysis

Eight samples were amplified with 12S rRNA gene fragments yielded 270 base pair amplicon (Figure-7). These samples were identified as T. multiceps. Four sequences were submitted to the GenBank under following accession numbers - KX977119, KX977120, KX957746, and KX984366. Phylogenetic tree constructed through using the sequence data indicates that all the isolates from the present study were grouped into a distinct cluster of sheep and goat originated T. multiceps sequences (Figure-8).

Table-1: Occurrence of Taenia spp. cyst.

| Species | Number of examined | Number of infected animal | Prevalence % | Total number of cyst | Average diameter of the cyst (cm) | Mean or average number of cyst/animal |
|---------|--------------------|----------------------------|--------------|---------------------|----------------------------------|-------------------------------------|
| Goat    | 385                | 8                          | 2.1          | 42                  | 2.7                              | 5.25                                |

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Coenurosis is an endemic disease of Bangladesh although very few studies have been conducted in this country and all of them are only epidemiological or case study [4]. The present study was carried to determine the prevalence of *T. multiceps* in goat with their molecular detection and characterization. A total of 385 goats were examined postmortem, and 2.1% prevalence was recorded. All of the examined cysts were found in muscle and subcutaneous tissue of slaughtered goat. This is the first molecular study of the non-cerebral form of *T. multiceps* in goats in the country. Although the cerebral form of
T. multiceps (C. cerebralis) in goats was reported in some area, 2.5% at Patuakhali district [18] and 35 cases found in Mymensingh district during the period 2010-2011 [19]. Coenurosis in calves is also reported as 2.47% in Bangladesh [20]. In comparison with this study, C. cerebralis cyst is found in the thigh muscle [21] and subcutaneous tissue [22] of goat in Uttar Pradesh, India. In another study in Southern Iran, it is found that same parasite produced both cerebral and non-cerebral forms in sheep and goats [23]. In sheep, the common predilection sites of these parasites are the CNS. The coenurus cyst that occurred outside the CNS in goats was recorded as T. multiceps [2,24] or T. gaigeri [25]. Genetically, the cerebral cysts in sheep were identical with the non-cerebral cysts in goats [10]. Histopathological findings in the skeletal muscle infected with coenurus included degenerative and necrotic changes, hyalinization, and myositis which are in agreement with other research [11,26].

Although coenurosis particularly in cerebral form in goat was reported in different area of Bangladesh, the molecular identification of T. multiceps was not done yet. Previously reported studies in other countries were based on amplification of several genes such as 12S rRNA gene, cox1 gene, and nad1 gene. We used only 12S rRNA gene marker. The present molecular genetic approach was employed to characterize the DNA sequence of the non-cerebral metacestodal stage of T. multiceps and compared it with other available data from other countries. Goat non-cerebral thigh muscle cyst isolated sequences of this study were aligned with Greece sheep and goat, Iranian sheep, and complete 12S rRNA gene of China.

Conclusions

This study showed that non-cerebral cyst is prevalent in this country which is very important for public health concern. This study depicts an idea of the non-cerebral form of zoonotic T. multiceps cyst which will be helpful in taenia cyst control and prevention.

Authors’ Contributions

Research work was done by MOF and partly MSI. Research design was carried out by AMAMZS, MAH, and SC. All the authors participated in data analysis, while MM, AR, and MSI revised the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

References

1. Christodouloupolous, G., Theodoropoulos, G. and Petarakos, G. (2008) Epidemiological survey of cestode-larva disease in Greek sheep flocks. *Veter. Parasitol.*, 153(3): 368-373.
2. Oge, H., Oge, S., Gonenc, B., Ozbakis, G. and Asti, C. (2012) Coenurosis in the lumbar region of a goat: A case report. *Vet. Med. Czech.*, 57(6): 308-313.
3. Scalia, A. and Varcasia, A. (2006) Updates on morphology, epidemiology and molecular characterization of coenurosis in sheep. *Parasitologia*, 48: 61-63.
4. Hassouni, S.S., Amin, M.R. and Islam, A.B.M. (1999) Goat production and its breeding strategy in Bangladesh. In: Proceedings of the First National Workshop on Animal Breeding, BAU, Mymensingh. p17-36.
5. Nooruddin, M., Rashid, M.H. and Hashim, M.A. (2000) Extraneuralcoenurosis in Bengal goats: Epidemiology. *Prog. Agric.*, 11(1-2): 141-145.
6. Karim, M.R., Parvin, M.S., Hossain, M.Z., Islam, M.T. and Hussan, M.T. (2014) A report on clinical prevalence of diseases and disorders in cattle and goats at the Upazilla Veterinary Hospital, Mohammadpur, Magura. *Bangladesh J. Vet. Med.*, 12(1): 47-53.
7. Rashid, M.H., Nooruddin, M. and Hashim, M.A. (2000) Extra neural coenurosis in Bengal goats: 2. Clinical pro-paediatrics progress. *Agricultural*, 11(1-2): 147-152.
8. Varcasia, A., Tanda, B., Giobbe, M., Solinas, C., Pipia, A.P., Malgor, R., Carmona, C., Garippa, G. and Scala, A. (2011) Cystic echinococcosis in Sardinia: Farmer’s knowledge and dog infection in sheep farms. *Vet. Parasitol.*, 181(2-4): 335-340.
9. Varcasia, A., Jia, W.Z., Yanb, H.B., Manunta, M.L., Pipia, A.P., Garippa, G., Scala, A. and Schuster, R.K. (2012) Molecular characterization of subcutaneous and muscular coenurosis of goats in United Arab Emirates. *Vet. Parasitol.*, 190: 604-607.
10. Amrabadi, O., Oryan, A., Moazeni, M., Sharifiyazdi, H. and Akbari, M. (2015) Comparison of cerebral and non-cerebral coenurosis by genetic markers of glycolytic enzyme (enolase) and mitochondrial sequences in sheep and goats. *Vet. Parasitol.*, 214: 333-336.
11. Oryan, A., Nazifi, S., Sharifiyazdi, H. and Ahmadnia, S. (2010) Pathological, molecular, and biochemical characterization of *Coenurus gaiigeri* in Iranian native goats. *J. Parasitol.*, 96: 961-967.
12. Oryan, A., Akbari, M., Moazeni, M. and Amrabadi, O.R. (2014) Cerebral and non-cerebral coenurosis in small ruminants. *Trop. Biomed.*, 31(1): 1-16.
13. Christodouloupolous, G., Dinkel, A., Romig, T., Ebi, D., Mackenstedt, U. and Loos-Frank, B. (2016) Cerebral and non-cerebral coenurosis: On the genotypic and phenotypic diversity of *Taenia multiceps*. *Parasitol. Res.*, 115: 4543-4558.
14. Faruk, M.O., Siddiki, A.M.A., Karim, M.F., Masuduzzaman, M., Chowdhury, S., Islam, M.S. and Hossain, M. (2017) Molecular characterization of *Echinococcus granulosus* through amplification of 12S rRNA gene and cox1 gene fragments from cattle in Chittagong, Bangladesh. *World Acad. Sci. Eng. Technol. Int. Sci. Int. J. Biol. Biomol. Agric. Food Biotechnol. Eng.*, 11(4): 301-305.
15. Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.*, 30: 2725-2729.
16. Faruk, M.O., Siddiki, A.M.A., Masuduzzaman, M., Chowdhury, S. and Hossain, M.A. (2017) Identification and molecular characterization of *Echinococcus granulosus* from domestic goat in Chittagong, Bangladesh. *Trop. Biomed.*, 34(4). (In Press).
17. Luna, L.G. (1968) Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. McGraw-Hill Book Company, Blakiston Division, New York. p94-95.
18. Rahman, M.A., Islam, M.A., Rahman, M.A., Talukder, A.K., Parvin, M.S. and Islam, M.T. (2012) Clinical diseases of ruminants recorded at the Patuakhali science and technology university veterinary clinic. *Bangladesh J. Vet. Med.*, 10(1-2): 63-73.
19. Amin, M.N., Hashim, M.A., Hossain, M.A. and Al-Sultan, I.I. (2013) *Coenurus cerebralis* infection (Gid disease) in Black Bengal goats; effects on certain blood values after surgical treatment. *J. Adv. Biomed. Pathobiol. Res.*, 3(1): 1-7.
20. Islam, A.W.M. and Rahman, M.S. (1997) A report on incidence of gid of calves of Bangladesh. *Indian J. Anim. Health.*, 36: 187-188.
21. Madhu, D.N., Mahan, T., Sudhakar, N.R., Maurya, P.S., Banerjee, P.S., Sahu, S. and Pawde, A.M. (2014) *Coenurus gaiigeri* cyst in the thigh of a goat and its successful management. *J. Parasit. Dis.*, 38(3): 286-288.
22. Sharma, D. and Chauhan, P. (2006) Coenurosis status in Afro-Asian region: A review. *Small Rumin. Res.*, 64: 197-202.
23. Akbari, M., Moazeni, M., Oryan, A., Sharifiyazdi, H. and Amrabadi, O. (2015) Experimental cerebral and non-cerebral coenurosis in goats: A comparative study on the morphological and molecular characteristics of the parasite. *Vet. Parasitol.*, 211: 201-207.
24. Sharma, D.K., Sanil, N.K., Agnihotri, M.K. and Singh, N. (1995) Subcutaneous coenurosis in Barabari goat. *Indian Vet. J.*, 72: 1203-1205.
25. Moghaddar, N. (2007) Occurrence of *Coenurus gaiigeri* infection in goats in Shiraz, Iran. *J. Appl. Anim. Res.*, 31(1): 83-84.
26. Kheirandish, R., Sami, M., Azizi, S. and Mirzaei, M. (2012) Prevalence, predilection sites and pathological findings of *Taenia multiceps* coenuri in slaughtered goats from southeast Iran. *Onderstepoort J. Vet. Res.*, 79(1): 436-441.

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