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Comparative molecular characterization and phylogenetic analysis of cerebral and non-cerebral coenurosis in Indian goats

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ABSTRACT

Coenurus cerebralis is the larval stage of *Taenia multiceps*, which infects the muscles and brain of goats and, to a lesser extent, sheep. The resulting cerebral and non-cerebral infections caused by the larval form (metacestode) of this cestode are commonly known as coenurosis. A weak emaciated carcass of five months old female goat, on necropsy, revealed numerous parasitic cysts ($n = 56$, grossly visible) in the visceral cavity including heart, diaphragm, thoracic cavity, abdominal cavity and pelvic inlet. A large number of variable sized parasitic cysts were also observed embedded in the pericardium and myocardium causing functional damage to the heart. The parasite caused extensive tissue damage at gross and microscopic levels in the heart including traumatic destruction of the myocardium with degenerative and necrotic changes and infiltration of mononuclear cells. On parasitological examination, the cysts were identified as *Coenurus cerebralis*, as the scolices had characteristic four suckers and a rostellum with a double crown of hooks. Further confirmation was done using polymerase chain reaction targeting specific ND1 and CO1 genes. Phylogenetic analysis of CO1 and ND1 genes showed a major branch comprising two clades of *T. multiceps* grouped as separate entities with the first clade showing *T. multiceps/Coenurus cerebralis* native CIRG strain (cerebral) being placed in proximity to *T. multiceps/Coenurus cerebralis* CIRG strain (non-cerebral/visceral) compared to the Chinese strains of *T. multiceps*. The phylogenetic analysis of ND1 and CO1 genes of *C. cerebralis* of cerebral and non-cerebral isolates revealed close proximity but expressed in two different disease forms (i.e., visceral coenurosis and neural coenurosis) which indicated that they were very close divergent from a common ancestor. On the basis of the observations it was concluded that goat died due to cardiac dysfunction resulting from severe systemic infection of *metacestode* of *T. multiceps* was closely related to isolate that caused neural coenurosis in another goat. Based on the sequencing analysis and phylogenetic information, the possible differences in the clinical manifestation (neural or visceral) could be attributed to the pathogenesis.

1. Introduction

Coenurosis or Gid is a parasitic disease caused by the larval/metacestode stage of tapeworm *Taenia multiceps* (Cestoda, Taeniidae) commonly called *Coenurus cerebralis* (Rostami et al., 2013). The metacestode affects sheep and goats throughout the world (Sadarnashipur and Lalgola, 1991; Oryan et al., 2010) and causes severe enormous economic losses (Oryan et al., 1994, 2014; Deressa et al., 2012). The adult parasite inhabits the small intestine of domestic and wild canids (Hall, 1919; Varcasia et al., 2015), which excrete the eggs in pasture through their faeces. These eggs upon ingestion by the intermediate host (herbivores including sheep, goat, horse, camel, deer, pigs etc.) release the oncospheres in the intestine which then enters into blood stream and reaches the central nervous system and other organs (Oryan et al., 2010; Paltrinieri et al., 2010; Avcioglu et al., 2012). Coenurosis, caused by *C. cerebralis* which is a bladder metacestode stage of *Taenia multiceps,*

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predominantly develops in the brain and spinal cord of many mammal species, including human (Christodoulopoulos, 2007; Ing et al., 1998; Sharma and Chauhan, 2006; Varcasia et al., 2013). However, there have also been rare reports of Coenurus cyst occurrence in the intramuscular or subcutaneous tissues and in the abdominal cavity in sheep and goats earlier referred to as *Coenurus gaigeri* (Patro et al., 1997; Christodoulopoulos, 2007; Oryan et al., 2010). Coenuri are frequently found in the nervous system including brain and spinal cord of many herbivores including goats resulting in neurological signs such as gid, ataxia, head deviation and blindness. Such neurological signs, in the majority of cases, result in the death of the affected animals (Avcioglu et al., 2011). Acute coenurosis occurs in young goat kids (3–6 months) due to migration of the oncospheres and is characterized by pyrexia, listlessness, head aversion to convulsions and death within 4–5 days (Evangelisti et al., 2018). In chronic cases, the disease progress towards quiescent phase, with parasite growing into cystic lesions, usually encountered in adult/older animals leading to the 'Gid', which is the consequence of cyst development. The symptoms depend on the size and location of the cyst, with infected animals exhibiting circling, head tilt, ataxia, blindness and death due to weakness and starvation (Paltrinieri et al., 2010; Varcasia et al., 2016).

In case of supposedly aberrant migration of the oncospheres, the cyst develops in different visceral organs (Godara et al., 2011) and the symptoms depends on the type of organ affected, position and size of the cyst. There had been many conflicting reports about the taxonomy of *T. multiceps* (Cestoda, Taeniidae). Previously, *C. cerebralis* in brain was referred to be the larval stage of *T. multiceps*, while *C. gaigeri* in other organs was referred to be the larval stage of *T. gaigeri*. But, lately the molecular data and phylogenetic analysis suggested that these two are not separate but a single species. However, the reason for aberrant migration of oncospheres in later case still remains unexplained. Information regarding visceral coenuruses infection is very scanty (Sharma et al., 1995, 1998; Gharagozlou et al., 2003) in goats. Visceral form of *C. cerebralis* (earlier *C. gaigeri*), was phylogenetically same as cerebral form of *C. cerebralis*, commonly localizes in subcutaneous regions or skeletal muscles such as thigh, biceps femoris, forelimb muscles, e.g., triceps, and muscles of the head, neck, thorax and abdomen of goats (Ghosh et al., 2005). Necropsy finding of cyst, Clinical signs, CT, ultrasound and X-ray are the diagnostic method of coenurosis (Roy et al., 2007). Mitochondrial DNA is widely used for molecular characterization of many parasites because of its high evolutionary rate more than the nuclear DNA (Gasser et al., 1999). Molecular characterization of *T. multiceps* was firstly studied in sheep using cytochrome *c* subunit 1 (CO1) and NADH dehydrogenase 1 (ND1) mitochondrial genes by Varcasia et al. (2006), in Italy. Genetic variation was reportedly possible for *T. multiceps* based on these mitochondrial genes with recorded genetic variability ranging from 0.22 to 0.67% into three different variants viz., Tm1, Tm2 and Tm3 (Varcasia et al., 2006). As far as we know, no sequence analysisis available from the Indian isolates of *T.multiceps* from goats with clearly defined genetic variations like that of Italian (Varcasia et al., 2006, 2016) or Iranian isolates (Rostami et al., 2013).

A long time ago, Hall directly demonstrated *Taenia gaigeri* as a valid taxon, based on the morphological observation of the larval form observed in the muscular tissues of goats in India, but this revision was not accepted widely (Hall, 1916). The taxonomy of tapeworms belonging to the family Taeniidae has been controversial because of the paucity of adult phenotypic characters and the great plasticity of larvae in intermediate hosts (Nakao et al., 2010). The taxonomic status of *T. gaigeri* still remained controversial, because morphologically similar taxa were inadequately proposed based mainly on the host specificity (Rostami et al., 2013). Varcasia et al. (2016) studied the genetic variability of various *Taenia multiceps* coenuri isolated from cattle and small ruminants and found 10 haplotypes based on the *cox1* partial gene belonging to the Tm1 variant. Therefore, this article describes the pathology and comparative molecular characterization of metacestodes of *T. multiceps* isolated from two different forms of coenurosis (Visceral and Neural) in goats in India using mitochondrial genes viz., CO1 and ND1.

2. Materials and methods

A carcass of five months old female goat was subjected to detailed post mortem examination. The affected organs were thoroughly examined and gross pathologic lesions were recorded. The parasitic cysts were removed carefully along with their membranes and the parasitological examination was carried out to identify the parasites and their molecular characterization. Representative tissue specimens were collected in 10% buffered neutral formalin solution for histopathological studies.

Formalin-fixed tissues were routinely processed and paraffin embedded tissue sections were cut at 5–6 µm thickness with the help of semi-automatic rotary microtome (Leica RM 2145, Germany). The sections were stained with conventional Haematoxylin and Eosin (H&E) stain following procedure (Luna, 1972) and examined under light microscope for histopathology.

2.1. Molecular study

The *C. cerebralis* cysts collected from the visceral form were compared with cysts obtained from the cerebral form from necropsy of goats at CIRG. DNA samples were isolated from scolices excised from the inner wall of the coenurus cysts from both the forms of coenurosis. Molecular characterization was done to compare the genetic details with respect to the mitochondrial gene sequences of these two distinct manifestations in goats.

DNA isolation was done from separated scolices from the coenurus cysts using QIAamp® DNA mini kit (Qiagen, USA, Cat# 51304) following protocol provided by the manufacturer. The parasite was further confirmed by PCR amplification of two species specific genes NADH Dehydrogenase 1(ND1) and Cytochrome-C Oxidase subunit 1 (CO1). In brief, 1 µl of the template DNA was used in a 25 µl reaction containing Emerald GT amp master mix (DSS TaKaRa, Japan), 10 picomole of sense and antisense primers, JB11 (5′-AGATTCGTAAGGGGCCTA ATA-3′) and JB12 (5′-ACCACTAACTAATTCACTTTC-3′), respectively for ND1 gene (Varcasia et al., 2012), and the same concentration of each sense and antisense primers viz., JB3 (5′-TTTTTTGGGCATCCTG AGGTTTAT-3′) and JB4.5 (5′-AAAGAAAGAACATAATGAAAATG-3′) for the CO1 gene (Varcasia et al., 2006). Further, thermocycling was carried out with the initial denaturation of 98 °C for 1 min followed by 30 cycles of denaturation with 98 °C for 15 s, annealing at 55 °C for 20 s and extension at 72 °C for 30 s, and the final extension at 72 °C for 5 min.

The PCR products were purified and sequenced using Sanger's dideoxy method on both the DNA strands using Bigdye® terminator v1.1 cycle sequencing kit (Applied biosystems). The raw sequences were aligned and subjected to phylogenetic analyses using Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). For CO1 gene, the raw sequences were aligned and subjected to phylogenetic analyses using Maximum Likelihood method based on the Tamura-Nei model. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood (−961.4151) was computed. The percentage of trees in which the associated taxa clustered together was shown next to the branches. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining methods to a matrix of pairwise distances were estimated using the Maximum Composite Likelihood (MCL) approach. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 15 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 390 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013). In the same manner, the dataset for ND1 was computed

using Maximum Likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood (−1469.4801) was constructed during the current analysis. The percentage of trees in which the associated taxa clustered together was shown next to branches. Initial tree (s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pair wise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 16 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 338 positions in the final dataset. A sequence identity plot was constructed to compare nucleotide variations in the various *Taenia* spp. with respect to ND1 and CO1 genes.

3. Results

The goats were reared under organized semi-intensive farming system in standard hygienic and managemental condition at ICAR-Central Institute for Research on Goats, Makhdoom, India. A carcass of five months old Barbari female goat was presented to Post-mortem House in the Division of Animal Health, ICAR-Central Institute for Research on Goats, Makhdoom, India for necropsy. The external appearance of the carcass was weak and emaciated. On opening the carcass, numerous parasitic cysts (n = 56, grossly visible) were present in the visceral cavity including heart, diaphragm, thoracic cavity, abdominal cavity and pelvic inlet (Fig. 1). Interestingly, large number of variable sized cysts were observed, attached to the pericardium and embedded in the heart musculature (Fig. 1A). Transverse section of the heart revealed numerous parasitic cysts embedded in the myocardium signifying obvious destruction of the organ to the extent of rendering it non-functional (Fig. 1B).

Microscopically, the cysts caused severe myocardial destruction by compressing the surrounding musculature causing pressure atrophy, necrosis and inflammatory reaction. Cardiac muscles depicted severe necrosis and infiltration of mononuclear inflammatory cells (Fig. 1D), comprising predominantly of macrophages, lymphocytes and plasma cells admixed with variable degree of fibroblasts. Dead necrosed parasitic cysts with calcification eliciting inflammatory reaction around it were also observed in the vicinity of atrophied myocardium. Liver showed areas of centrilobular necrosis of hepatocytes, whereas kidneys exhibited severe necrosis of tubular epithelium, especially in cortical region. Other organs did not show any significant changes.

The cysts were removed along with their membranes which were filled with transudate of host tissue fluid and numerous scolices were attached to the inner surface. The sizes of the cysts were comparable to that of a pea to a size of a lemon. Detailed parasitological examination resulted in identification of cysts as being of *Coenurus cerebralis,* as scolices had four suckers and a rostellum with a double crown of hooks (Fig. 1C). It was the larval stage of *Taenia multiceps*, which infests the muscles of goats. Molecular confirmation of parasite was done by PCR amplification of the two genes ND1 and CO1 on specific feature yielded specific product size of 471 bp and 396 bp respectively (Fig. 2).

The tree information for CO1 gene was compared with various *Taenia* species affecting the domestic animals including *Taenia multiceps*, *T. saginata*, *T. solium* and *T. ovis* (Fig. 3). There were two major branches, with the top branch comprising of *T. multiceps* in one sub-branch and *T. saginata* in another sub-branch; whereas the second major branch consisted of two clades in which *T. solium* and *T. ovis* are

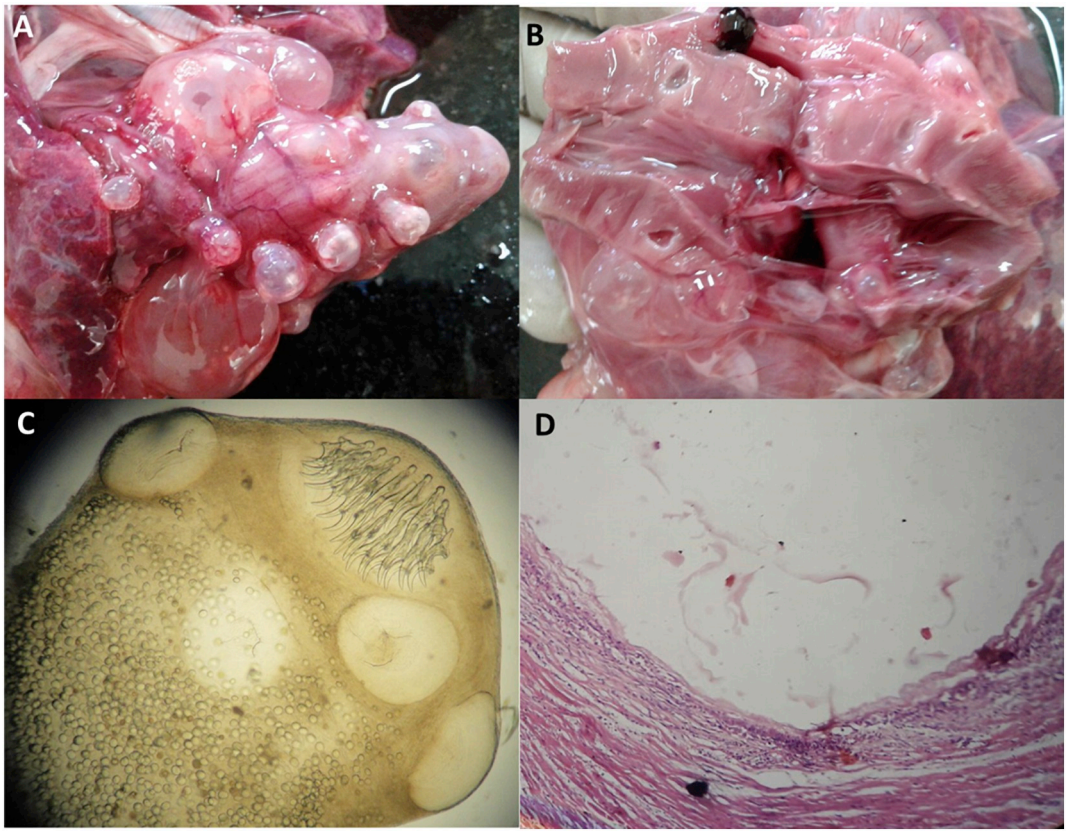

Fig. 1. Visceral coenurosis in goats caused by *Coenurus cerebralis* in goats. A-Heart showing numerous attached and embedded parasitic cysts with visible whitish scolices inside them; B-Myocardium of heart showing several parasitic cysts embedded in cut surfaces causing cystic spaces, necrosis and considerable muscular destruction; C-A Single scolice of *C. cerebralis* showing four suckers and a rostellum with a double crown of hooks; D- Cyst in the cardiac muscles showing cellular infiltration.

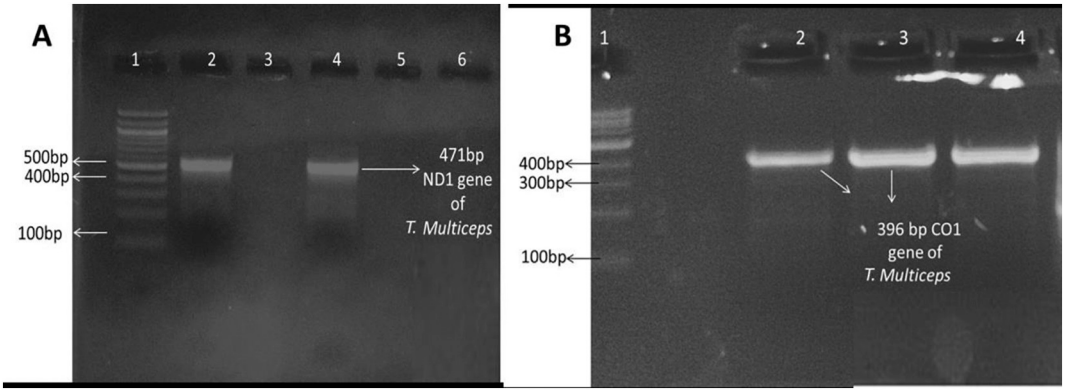

Fig. 2. A- PCR confirmation of *C. cerebralis* by ND1 gene. Lane 1: 100 bp DNA ladder, Lane 2: Positive control, Lane 3&4: test samples, Lane 5: negative control, Lane 6: no template control. B-PCR confirmation of *C. cerebralis* by *CO1* gene. Lane 1: 100 bp DNA ladder, Lane 2: Positive control, Lane 3&4: test samples.

grouped separately. The first major branch of the CO1 phylogeny has two clades of *T. multiceps* grouped as separate entities. The most striking feature of the first clade of the top sub-branch is that it has *T. multiceps* native CIRG taxa from cerebral and non-cerebral forms were placed in proximity. In contrast, the Chinese strains of *T. multiceps*' CO1 sequences are phylogenetically distant compared to the CIRG strains. The sequence identity plot showed a picture with few point mutations at the CO1 gene coding sequence. The native CIRG strains of *T. multiceps* compared with other major *Taenia* spp. showed mutations at nucleotide 973rd position from T → C for the two CIRG strains isolated from neural coenurosis and visceral coenurosis respectively (Fig. 4).

The tree information for ND1 gene was compared with various *Taenia* species affecting the domestic animals including *Taenia multiceps*, *T. saginata*, *T. solium*, *T. ovis*, *T. asiatica* etc. There were two branches, with the top branch comprising of *T. multiceps* and other *Taenia* spp. except *T. hydatigena*, which was separately placed in another branch. The major branch consisted of two sub-branches with the first on comprising of various clades and subclades of *T. solium*, *T. ovis*, *T. saginata*, *T. asiatica*, *T.serialis* and *T. krabbei*, whereas the second sub-branch comprised of *T. multiceps*. There were two clades wherein the first clade consisted of exclusively chinese strains of *T. multiceps*, and the second clade having the CIRG strains of *T. multiceps* of visceral and cerebral origin arranged together in the same subclade (Fig. 5). The nucleotide picture in the ND1 gene of CIRG strains of *Taenia multiceps* (visceral + neural strains), when compared with other *Taenia* species showed mutations G → T, A → T, GT → AG, G → A at nucleotide positions 165th, 169th, 172-173rd and 181st positions, respectively (Fig. 6).

4. Discussion

In a majority of coenuri affected goats, the cysts anchor, develop, mature and cause asymptomatic focal lesions in extra cranial aberrant sites. The lesions often persist throughout the life span of the host (Sharma and Chauhan, 2006). It is governed by multiple factors, including the quantum and periodicity of infection intake, subsequent in-situ ongoing events of the host-parasite interaction, age and acquired immune status of the goat, etc. Chronic infections are more prevalent in the goats, aged between one to two years (Palmer, 1976; Sharma et al., 1998). Poor awareness about the use of infected/incinerators for disposal of contaminated carcass left over contaminated forage or grazing pastures further aggravates the situation. Goats, being intermediate host usually get the infection from the dog's excreta; therefore, the treatment of dogs in and around the farms for tapeworm should be done to control the disease.

The metacestodes of goat such as *Coenurus cerebralis*, *Cysticercus tenuicollis*, *Cysticercus ovis* and hydatid cysts have been extensively

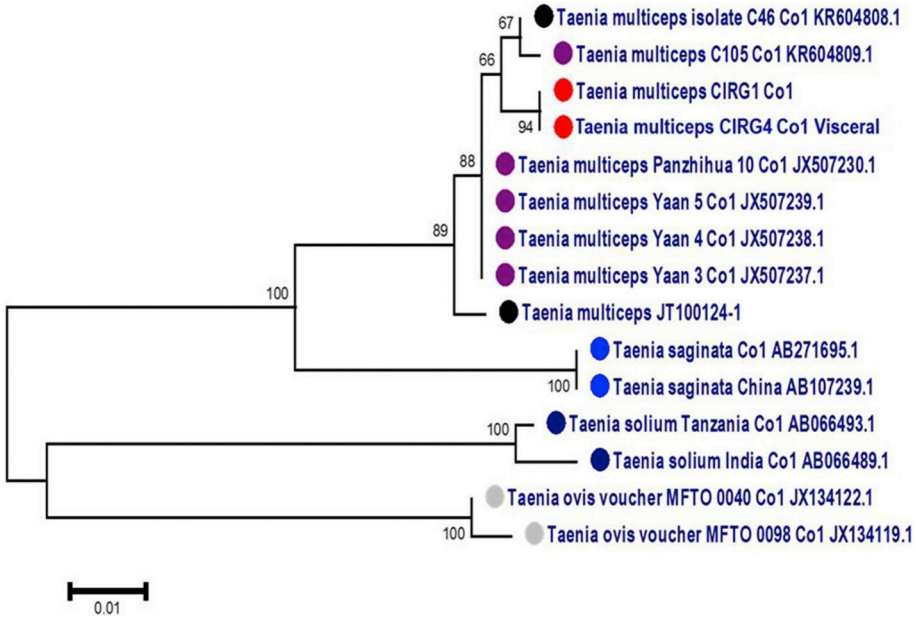

Fig. 3. Molecular Phylogenetic analysis of CO1 gene of various *Taenia* spp. by Maximum Likelihood method.

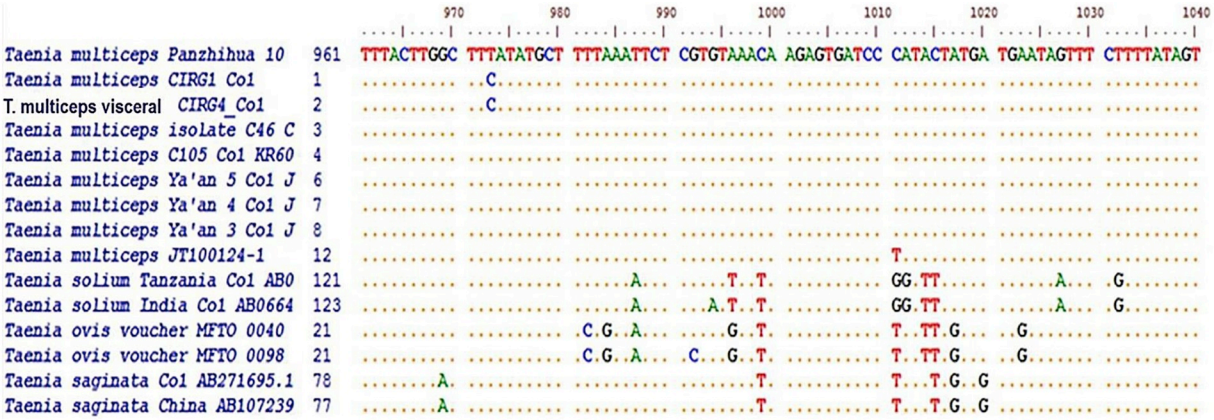

Fig. 4. Sequence identity plot of the 3′ end of the CO1 gene. The native CIRG strains of *T. multiceps* compared with other major *Taenia* spp. showing mutations T → C at nucleotide positions 973rd position respectively for the two CIRG strains isolated from cerebral and visceral coenurosis respectively.

studied but *C. cerebralis of* visceral origin have very few records due to its uncommon and sporadic occurrence. Coenuri are frequently found in the nervous system including brain and spinal cord. They are commonly localized in subcutaneous regions or skeletal muscles such as thigh, biceps, forelimb muscles and muscles of the head, neck, thorax and abdomen of goats (Patro et al., 1997; Oryan et al., 2010; Varcasia et al., 2012), but extensive presence of coenuri in the heart in large numbers has not been reported so far. Location of the cysts in cardiac muscles may cause pain, muscular degeneration, necrosis and pressure atrophy which may result in impaired cardiac function in severe infection. No such case has been recorded on severe cardiac damage caused by huge number of cysts of *C. cerebralis*. Therefore, the present study was important to evaluate the pathological profiles of *C. cerebralis* in the heart of five months old goat.

In present study, molecular diagnosis was done for confirmation of *T. multiceps* by employing previously published primers (Varcasia et al., 2006, 2012), which yielded gene products of 471 bp and 396 bp sizes for ND1 and CO1 genes, respectively that are specific to the parasite. Varcasia et al. (2006, 2012) carried out complete molecular characterization of ND1 and CO1 genes of *Coenurus cerebralis* infestation in subcutaneous and muscular regions of 300 goats and constructed a phylogenetic tree by maximum composite likelihood method. The results showed that the pair-wise distances (between various *T. multiceps* strains) for ND1 and CO1 genes were 2.4–4.1% and 1.0–1.3%, respectively. Nevertheless, based on gross appearance, microscopic findings, parasitological studies and molecular diagnosis, the present case was confirmed as systemic *C. cerebralis* infection.

The sequencing studies were done to compare the various *Taenia* species by phylogenetic analysis. The neural cyst from CIRG (earlier published data, Shivasharanappa et al., 2017) obtained during necropsy were compared with the current case of visceral coenurosis. The idea of this analysis is to compare the closely related parasite from the same geographical area involved the vicious disease cycle of visceral coenurosis and neural coenurosis in goats.

As per the phylogenetic tree, the *T. Multiceps* native CIRG strain isolated from cerebral form of coenurosis was placed in close proximity to *T. multiceps* CIRG strain of visceral origin, compared to the Chinese strains of *T. multiceps*' COI sequences which are being phylogenetically distant compared to the CIRG strains. From, this it can be inferred that both the *C. cerebralis* isolated from visceral and neural forms could have diverged from a common ancestor. In another perspective, it can also be construed that the same strain might behave differently due to some unknown reasons causing two different forms of coenurosis viz. cerebral or visceral coenurosis in goats. In the current study, the animal succumbed to visceral coenurosis is 5 months old, while in earlier cases of cerebral coenurosis, mostly adult animals were affected

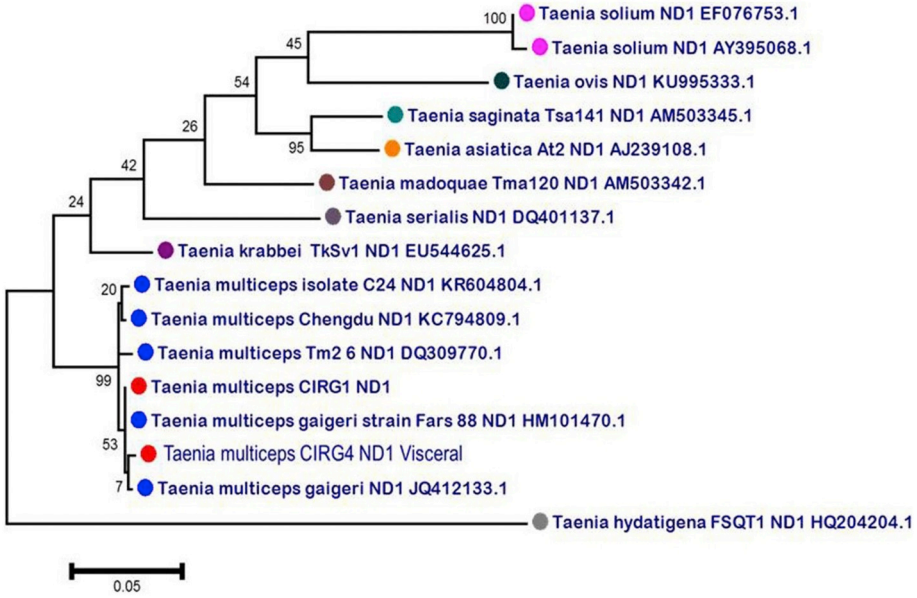

Fig. 5. Molecular Phylogenetic analysis of ND1 gene of various *Taenia* spp. by Maximum Likelihood method.

Fig. 6. Sequence identity plot of the 5′ end of the ND1 gene.The native CIRG strains of *T. multiceps* compared with other major *Taenia* species showing mutations G → T, A → T, GT → AG, G → A at nucleotide positions 165th, 169th, 172-173rd and 181st positions respectively.

(Shivasharanappa et al., 2017). On the contrary, even pre-weaned lambs less than 30 days of age were diagnosed with cerebral coenurosis was reported (Pintus et al., 2018)

In the same manner, the nucleotide sequences of ND1 gene were phylogenetically analyzed, which presented two clades in the tree with the first clade comprising of chinese strains of *T. multiceps*, and the second clade having the CIRG strains of *T. multiceps* of visceral and neural origin placed together in the same subclade. This phylogeny of the CIRG native strains being placed in close proximity but expressing as two different disease forms (viz. cerebral and visceral coenurosis) shows that they are very close divergent from a common ancestor.

Varcasia et al. (2012) studied the sequencing analysis and molecular characterization of non-cerebral coenurosis cases from abattoirs of Emirates, and they reported the divergence of mitochondrial genes ND1 and CO1 between the isolates from cerebral coenurosis. Based on the findings including morphological and molecular characterization of CO1 gene, they concluded that the non-cerebral coenuri from abattoir of Emirates could belong to a different genotype due to the fact that they are unique compared to other genotypes of *T. multiceps.*

Interspecies variation plays a pivotal role in epidemiology and biology of members of Taeniidae family. Formulation and implementation of appropriate species specific control programmes also depend on evaluating the genetic variation among the species. In most of the olecular phylogenetic studies the conserved mitochondrial DNA are used owing to their high evolutionary rate. Previous phylogenetic studies regarding genetic characterization of T. multiceps using CO1 and ND1 genes (Oryan et al., 2010; Varcasia et al., 2016; Varcasia et al., 2012; Akbari et al., 2015) corroborates well with the present study.

However, there is still no explanation to the fact that what triggers the migration of oncospheres to CNS and to aberrant sites like other visceral organs. It may be speculated that there are two types of oncospheres with different spatial distribution of surface antigens. Also there may be some different receptor interaction at cellular level thus channeling one type towards CNS and the other type towards the viscera. Even several host factors like age, immune status, previous exposure, infectivity of the inoculum might be involved in cerebral and visceral coenurosis. Even difference in the species of the intermediate host may be defining factor in the two different predilection sites of the cyst.

To support this fact, Oryan et al. (2015), which studied the sequences of ND1 and CO1 genes of mitochondrial DNA and found that, they were more similar to each other from cerebral and non-cerebral affections. It is also emphasized based on the findings that *T. gaigeri* could not be considered as a distinct species from *T. multiceps*, but a single species belong to the same origin.

Similarly, Abbas and Elbeskawy (2016) reported the phylogenetic analysis of NDI and CO1 gene from cases of *Coenurus cerebralis* from sheep. They found that both CO1 and ND1 phenograms evidenced the existence of genetic variants within *T. multiceps* from variable hosts within different geographical regions. Further they found more specificity between the isolates from sheep and goat compared to cattle isolates.

On the basis of gross pathology (widespread cyst in myocardium and thorax) and histopathological findings, it may be concluded that the goat died due to cardiac dysfunction resulted from severe *C. cerebralis* infection of the myocardium, confirmed by molecular study.

Based on the thorough scrutiny of the available literature, it appeared to be the first case of severe *C. cerebralis* infection in the cardiac muscle leading to death in a five month old Barbari goat. Further, the reason behind intensive predilection of *C. cerebralis* cysts for myocardium remains unknown and may require additional studies on the life cycle of *Taenia multiceps* in the goats reared under semi-intensive conditions.

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References

Abbas, I., Elbeskawy, M., 2016. Molecular and phylogenetic status of Coenurus cerebralis infecting sheep from Dakahlia province. Egypt. J. Adv. Parasitol. 3, 117–124.
Akbari, M., Moazeni, M., Oryan, A., Sharifiyazdi, H., 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.
Avcioglu, H., Yildirim, A., Duzlu, O., Inci, A., Terim, K.A., Balkaya, I., 2011. Prevalence and molecular characterization of bovine coenurosis from Eastern Anatolian region of Turkey. Vet. Parasitol. 176, 59–64.
Avcioglu, H., Terim, K.A., Yildirim, A., 2012. Clinical, morphological and histopathological features of bovine coenurosis: case reports. Rev. Med. Vet. 163, 295–298.
Christodoulopoulos, G., 2007. Two rare clinical manifestations of coenurosis in sheep. Vet. Parasitol. 143, 368–370.
Deressa, A., Tilahun, T., Tadesse, A., Beyene, M., Gebrewold, G., Pal, M., 2012. Assessment of coenurus cerebralis and its economic impact in sheep brain harvested at Ethiopian Health and Nutrition Research Institue. Ethiopian Int. J. Livestock Res. 2, 217–226.
Evangelisti, M.A., Deiana, R., Melosu, V., Burrai, G.P., Ballocco, I., Varcasia, A., Scala, A., Manunta, M.L., 2018. Relationships among neuroscore, magnetic resonance imaging features, and intracranial pressure in sheep affected by slow-growing brain lesions. Vet. Radiol. Ultrasound. 59, 305–311.
Gasser, R.B., Zhu, X., McManus, D.P., 1999. NADH dehydrogenase subunit 1 and cytochrome c oxidase subunit I sequences compared for members of the genus Taenia (Cestoda). Int. J. Parasitol. 29, 1965–1970.
Gharagozlou, M.J., Mobedi, I., Akhavan, P., Helan, J.A., 2003. A pathological and parasitological study of *Coenurus gaigeri* infection of goats from Iran. Indian J. Vet. Pathol. 27, 95–97.
Ghosh, R.C., Dubhey, S., Mandal, S.C., Sharma, N., 2005. Occurrence of *Coenurus gaigeri* cyst in a goat. Indian Vet. J. 82, 90–91.
Godara, R., Borah, M.K., Sharma, R.L., Jangir, B.L., 2011. Caprine coenurosis with special reference to hepatic coenurosis. Comp. Clin. Pathol. 20, 277–280.
Hall, M.C., 1916. A new and economically important tapeworm, *Multiceps gaigeri* from the dog. J. Am. Vet. Med. Assoc. 50, 214–223.
Hall, M.C., 1919. The adult taenioid cestodes of dogs and cats and related carnivores in North America. In: Proceedings of the United States National Museum. vol. 55. pp. 1–94.
Ing, M.B., Schantz, P.M., Turmer, J.A., 1998. Human coenurosis in North America:case reports and review. Clin. Infect. Dis. 27, 519–523.
Luna, L.G., 1972. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd ed. McGraw-Hill Book Company, New York (38–39pp).
Nakao, M., Yanagida, T., Okamoto, M., Knapp, J., Nkouawa, A., Sako, Y., Ito, A., 2010. State-of-the-art Echinococcus and Taenia: phylogenetic taxonomy of human-pathogenic tapeworms and its application to molecular diagnosis. Infect. Genet. Evol. 10, 444–452.
Oryan, A., Moghaddar, N., Gaur, S.N.S., 1994. Metacestodes of sheep with special reference to their epidemiological status, pathogenesis and economic implication sin Fars Province. Iran. Vet. Parasitol. 51, 231–240.
Oryan, A., Nazifi, S., Sharifiyazdi, H., Ahmadnia, S., 2010. Pathological, molecular and biochemical characterization of *Coenurus gaigeri* in Iranian native goats. J. Parasitol. 96, 961–967.
Oryan, A., Akbari, M., Moazeni, M., Amrabadi, O.R., 2014. Cerebral and non-cerebral coenurosis in small ruminants. Trop. Biomed. 31, 1–16.
Oryan, A., Moazeni, M., Amrabadi, O., Akbari, M., Sharifiyazdi, H., 2015. Comparison of distribution pattern, pathogenesis and molecular characteristics of larval stages of Taeniamulticeps in sheep and goats. Small Rumin. Res. 132, 44–49.
Palmer, A.C., 1976. Introduction to Animal Neurology. Blackwell Scientific, London, pp. 74.
Paltrinieri, S., Varcasia, A., Cazzaniga, S., Giordano, A., Pipia, A.P., Marrosu, R., Scala, A., 2010. Brain creatine kinase isoenzyme (CK-BB) as a possible biomarker for the diagnosis in vivo of ovine coenurosis in a naturally infected flock. Small Rumin. Res. 94, 180–184.
Patro, D.N., Suhani, B., Sahoo, D.K., Nanda, S.K., Pradhan, P.K., Nayak, B.C., 1997. Incidence of generalized *Coenurus gaigeri* infection in a goat farm. Indian Vet. J. 74, 68–69.
Pintus, D., Varcasia, A., Dessì, G., Tamponi, C., Manunta, M.L., Carboni, G.A., Cancedda, M.G., Ligios, C., Scala, A., 2018. Atypical outbreak of acute coenurosis by *Taenia multiceps* in a sheep flock. Parasitol. Res. 117, 985–1988.
Rostami, S., Salavati, R., Beech, R.N., Sharbatkhori, M., Babaei, Z., Saedi, S., Harandi, M.F., 2013. Cytochrome c oxidase subunit 1 and 12S ribosomal RNA characterization of *Coenurus cerebralis* from sheep in Iran. Vet. Parasitol. 197, 141–151.
Roy, M.E., Wrigley, R.H., Kraft, S.L., Steyn, P., Zekas, L.J., Park, R.D., Withrow, S.J., 2007. Of the international veterinary radiology association and the American college of veterinary radiology. Vet. Radiol. Ultrasound. 48, 163–188.
Sadarnashipur, P.O., Lalgola, P.S., 1991. Prevalence of coenurus of *Multiceps gaigeri* in goat and its predilection site distribution. Indian J. Comp. Microbiol. Immunol. Infect. Dis. 12, 71–72.
Sharma, D.K., Chauhan, P.P.S., 2006. Coenurosis status in Afro-Asian region: a review. Small Rumin. Res. 64, 197–202.
Sharma, D.K., Sanil, N.K., Agnihorti, M.K., Singh, N., 1995. Subcutaneous coenurosis in Barbari goat. Indian Vet. J. 72, 1203–1205.
Sharma, D.K., Singh, N., Tiwari, H.A., 1998. Prevalence and pathology of coenurosis in organized goat farms. J. Vet. Parasitol. 12, 30–32.
Shivasharanappa, N., Sharma, N., Sharma, D.K., Pawaiya, R.V.S., Vamadevan, B., Mishra, A.K., Paul, S., 2017. Neuropathological lesions of clinical and sub clinical Coenurosis (*Coenurus cerebralis*) in organized goat farms in India. Acta Parasitol. 62, 482–487.
Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10, 512–526.
Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.
Varcasia, A., Lightowlers, M.W., Cattoli, G., Cancedda, G.M., Canu, S., Garippa, G., Scala, A., 2006. Genetic variation within *Taenia multiceps* in Sardinia, Western Mediterranean (Italy). Parasitol. Res. 99, 622–626.
Varcasia, A., Jia, W.Z., Yan, H.B., Manunta, M.L., Pipia, A.P., Garippa, G., Scala, A., Schuster, R.K., 2012. Molecular characterization of subcutaneous and muscular coenurosis of goats in United Arab Emirates. Vet. Parasitol. 190, 604–607.
Varcasia, A., Pipia, A.P., Arru, D., Pes, A.M., Tamponi, C., Dore, F., Garippa, G., Scala, A., 2013. Morphological and molecular characterization of bovine coenurosis in Sardinia, Italy. Parasitol. Res. 112, 2079–2082.
Varcasia, A., Tamponi, C., Tosciri, G., Pipia, A.P., Dore, F., Schuster, R.K., Kandil, O.M., Manunta, M.L., Scala, A., 2015. Is the red fox (*Vulpes vulpes*) a competent definitive host for *Taenia multiceps*? Parasit. Vectors 8, 491.
Varcasia, A., Pipia, A.P., Dessì, G., Zidda, A., Tamponi, C., Pau, M., Scala, A., Boufana, B., 2016. Morphology and genetic variability within Taenia multiceps in ruminants from Italy. Vet. Parasitol. 223, 181–185.