First Report on Molecular Characterization of *Taenia multiceps* Isolates From Sheep and Goats in Faisalabad, Pakistan

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Coenurus cerebralis is the larval stage of *Taenia multiceps* commonly found in the brain (cerebral form), intramuscular and subcutaneous tissues (non-cerebral form) of ungulates. Globally, few reports exist on the molecular characterization and genetic diversity of *C. cerebralis* with none available for Pakistan. The current study molecularly characterized 12 *C. cerebralis* isolates surgically recovered from sheep (*n* = 4) and goats (*n* = 8) from a total of 3,040 small ruminants using a portion of the cytochrome *c* oxidase subunit 1 (*cox1*) mitochondrial (*mt*) gene. NCBI BLAST search confirmed the identity of each isolate. A high haplotype and a low nucleotide diversity with three haplotypes from the 12 isolates were observed. The findings suggest the existence of unique haplotypes of *C. cerebralis* in Pakistan. The negative value of Tajima’s *D* and the positive value of Fu’s *Fs* were inconsistent with population expansion, however, the sample size was small. Bayesian phylogeny revealed that all Pakistani isolates alongside the Chinese sequences (obtained from GenBank) constituted a cluster while sequences from other regions constituted another cluster. This is the first molecular study to determine the genetic diversity of *C. cerebralis* in Pakistan and serves as a foundation for prospective studies on the prevalence and population structure of *C. cerebralis* in the country. Furthermore, in this study, we amplified only a partial segment of the *cox1* gene from a limited sample size. This could have implications on the interpretation of the actual population structure in reality. Thus, we recommend future studies to consider a larger sample size in a massive epidemiological survey for further insights.

**Keywords:** *Coenurus cerebralis*, *cox1*, *Taenia multiceps*, genetic diversity, phylogeny, Pakistan
INTRODUCTION

The genus *Taenia* is a diverse group of helminths with more than 40 known species that infect livestock and humans causing cysticercosis and/or taeniasis (1, 2). *Coenurosis cerebralis* refers to the larval stage of *T. multiceps* (an obligate intestinal tapeworm of dogs) infecting sheep and goats population across the world especially in tropical countries in Asia, Africa, and the Middle East (3). Sheep and goats are the common intermediate hosts but infection has also been reported in cattle and humans (4). Intermediate hosts including sheep and goats often become infected following feeding on vegetation and water contaminated by eggs from dogs (5, 6). This is followed by the development of the larval stage in different tissues including the brain, muscles, and skin (7, 8). The parasite completes its life cycle when the definitive hosts consume poorly discarded infected carcasses, (9). Predominantly, *Coenurosis* cysts in the brain (10) present as either acute or chronic form. Acute clinical manifestations include fever, muscle tremors, hemorrhagic retinal lesions, ataxia, blindness, nystagmus, dysmetria, and scoliosis (11, 12). In chronic stages, in addition to acute signs, painful response to pressure over the cystic area, paralysis, blindness, lack of coordination, lethargy, and lack of response to stimuli are evident (13–15). Cyst formation is also frequently seen in subcutaneous and intramuscular tissues (16–21) where they possibly impair normal organ functioning and induce muscular pain (4).

Coenurosis is more prevalent in developing countries (22) with ovine coenurosis varying between 1 and 40% across countries in Africa, Asia, and the Middle East. In Asia and the Middle East, the prevalence in Bangladesh, India, and Jordan has been reported to be 2.5, 2.88, and 3% (13, 23, 24) while in Iran, prevalence in sheep and goat sits between 1.7 and 18.7% (16, 25–28). In countries like Ethiopia, Mozambique, Tanzania, Kenya, and Egypt, the prevalence may range from 1.5 to 42.1% (29–33). In the developed world like the United Kingdom, the infection is uncommon. However, abattoir-based surveys conducted during the 1980s put the prevalence between 0.5 and 5.58% (9). Low prevalence has also been reported in countries like France and Italy (34). Although the infection is cosmopolitan, to the best of our knowledge, only two necropsy-based case reports are available for Pakistan (35, 36).

Genetic variability among many species found within the family taeniidae has been well-documented especially for the genus *Echinococcus*. Nonetheless, the importance and degree of intraspecific variations within some species of *Taenia* remain to be fully understood. However, a good understanding of the genetic diversity and population structure of cestodes remains an important aspect that is essential for the implementation of control and prevention programs as it provides insights related to host specificity or geographical peculiarities. For *T. multiceps*, the genetic variability is poorly studied and there are arguments whether genetic variation may exist between isolates from different hosts, geographical location, or cerebral or non-cerebral forms. In a recent study, the phylogenetic analysis of *nad1*, *cox1*, and 12S rRNA mtDNA sequences showed that no monophyletic groups were based on geographical origin, organ location in the intermediate host (cerebral or non-cerebral), or species of the intermediate host (37). Meanwhile, from Sardinia sheep in Italy, Varcasia et al. (38) reported three variants of *C. cerebralis* using the *cox1* and *nad1* genes. In Iran, a high scale genetic diversity was reported in *C. cerebralis* isolates obtained from sheep and goats (12, 39) with the *cox1* gene demonstrating 11 segregation sites resulting in seven haplotypes in sheep (39). Five distinct haplotypes of *C. cerebralis* cysts recovered from sheep, goats and cattle based on partial *cox1* gene have also been documented in Greece (40). In China, the *cox1*, *cytb*, *nad1*, and *nad4* genes have been employed in investigating the genetic diversity of *C. cerebralis* in Sichuan, Gansu, and Inner Mongolia (41–44) and the evidence suggests very low sequence variation 0–0.8%. Li et al. (41) further demonstrated that the *cox1* mitochondrial gene of *C. cerebralis* obtained from sheep and goats from Gansu province of China were highly conserved.

On the other hand, small ruminant farming is one of the major determinants of advancement in developing countries. However, infectious diseases including parasitic infections have been known to constitute major obstacles to livestock production. Parasitic diseases cause enormous financial losses to owners of sheep and goat population as a result of poor growth, morbidity, poor carcasses value and mortality (34, 45). Mortality in infected sheep and goats reaches about 5% in Ethiopia (32), 25% in Istanbul, Turkey (46) while in Iran losses amounting to 10.2 million rials (27) due to condemnation of infected carcasses highlights the economic setbacks by tapeworm metacestodes. While Pakistan hosts a very large population of small ruminants, little is known on the prevalence, molecular and phylogenetic characteristics of *C. cerebralis* infecting small ruminants. Therefore, the current study was designed to molecularly identify and determine the genetic variability of *C. cerebralis* isolates surgically removed from sheep and goats using the mt cytochrome oxidase subunit 1 (cox1) gene to provide preliminary data for Pakistan besides contributing to our understanding of the global molecular epidemiology and population structure of *C. cerebralis*.

MATERIALS AND METHODS

Study Area

Faisalabad is one of the three most populous cities in Pakistan. Geographically, the city is located 186 meters above sea level lying between 73° and 74° East (longitude), and 30° and 31.5° North (latitude). An extensive canal system and the presence of Ravi and Chenab rivers in the outskirts make the agricultural land highly fertile, fetching the attention of peasants toward agriculture farming and livestock rearing to earn their livelihoods.

Parasite Material

A total of 3,040 sheep and goats presented to the outdoor patient department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan (irrespective of their disease or purpose of visiting) during May–December, 2019 were palpated thoroughly using digital palpation. Sheep and goats having subcutaneous or intramuscular palpable (cystic) swelling or bulge giving asymmetry to the infested organ were subjected to ultrasound scan procedure for the diagnosis of non-cerebral
coenurosis. Hair from the bulging area was clipped and shaved and then animals (sheep and goats) were anesthetized with a combination of xylazine and diazepam at a dose rate of 1 mg/kg (47) and 0.25 mg/kg (48), respectively. Further, local infiltration of 3 ml of 2% lignocaine was also administered subcutaneously. Sterile Aquasonic® 100 Ultrasound Gel (Parker Laboratories) was applied and scanning was performed using 2–5 MHz convex probe (transducer) that was directly placed in the transverse plane over the cystic area using Convex scanner HS-1500 (HONDA® Electronics) and then was spun/moved in cranial and caudal planes for more enhanced visualization of the coenurus cysts. Transcutaneous ultrasonographic confirmation of cysts using convex array was carried out in <1 min followed by ultrasound-guided aseptic aspiration of the cystic fluid using fine needle aspiration technique (FNA). Each cyst was surgically removed and placed carefully in sterile falcon tubes and transferred to the In-House Preventive Veterinary Medicine Public Health Laboratory of the Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan. Gross morphological identification of the cysts was carried out as described previously by Soulsby (49), Faruk et al. (50), and Oryan et al. (45). The walls of the cysts were translucent and thin filled with a fluid having water like consistency. Protoscolices were firmly adhered to the germinal layer (45).

DNA Extraction, Amplification, and Sequencing
Genomic DNA from each cyst was extracted using Qiagen Blood and Tissue Kit (Qiagen, Hilden, Germany). A reaction mixture of 25 μl consisting of Ex Taq™ (12.5 μl) from Takara Bio, Kusatsu, Japan, forward (10 pmol) and reverse primers 1(10 pmol), extracted genomic DNA (0.5 μl), and RNase free water up to the final volume of 25 μl was used to carry amplification of the portion of the cox1 gene (1,073 bp) using the forward (5′-CTTTGACTGGCTGTGTGTTGT-3′) and reverse (5′-AGAACCTACAGGTGACACAAAT-3′) primers. In place of DNA, RNase free water was added as a negative control. The PCR program was as follows: an initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation (95°C for 30 sec), annealing (55°C for 40 sec), and extension (72°C for 1 min) and a final extension step at 72°C for 10 min. The PCR products were observed under UV light transilluminator using an agarose gel (1.5% w/v) stained with GelRed™. The sizes of the amplicons were estimated by running a 2,000-bp ladder in each gel. The PCR products were sent to Tsingke Biotechnology Company, Beijing for sequencing.

Molecular Analysis
DNA sequences were corrected manually for any misread nucleotides using Unipro UGENE v1.32.0 software. Using the same software, multiple sequence alignment was carried out. The identities of isolates were confirmed by NCBI BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Population Genetics and Phylogenetic Analyses
DnaSP 4.5 software was used to calculate nucleotide diversity (π), number of haplotypes (h), and haplotype diversity (Hd). Fu’s Fs (51) and Tajima’s D (52) were estimated by Arlequin software (53). A median-joining network (54) was constructed based on the partial mt cox1 sequences to examine intraspecific variation between haplotypes.

A dataset consisting of cox1 gene (639 bp) sequences of the representative haplotypes and other Taenia spp. (T. asiatica, T. crassiceps, T. hydatigena, T. ovis, T. pisiformis, T. saginata, T. solium, and T. taeniaeformis) retrieved from GenBank were used to construct a phylogenetic tree using the Bayesian method in MrBayes v.3.1.1 software. Echinococcus granulosus whose larvae can infect sheep and goats was used as an out-group.

RESULTS
Ultrasonographic Findings
During the study period (May–December, 2019), 4 sheep and 8 goats were found infected with T. multiceps. The cysts were observed in the face, neck, trunk, and groin regions (Figure 1A). All cysts showed characteristics morphology containing clear fluid of varying volume with many protoscolices. Many scolices were attached to the internal wall of the cyst (Figure 1B). Ultrasound examination showed a hypoechoic cyst wall (Figure 1C, blue arrow) with anechogenic cystic fluid (Figure 1C, white arrow) and protoscolices (Figure 1C, red arrow). The cysts appeared as hypoechoic structures with acoustic enhancement at the distal edge.

Nucleotide Polymorphism and Population Indices
A PCR product of ~1,073 bp was obtained. After multiple sequence alignment, polymorphism analysis resulted in three parsimony informative sites from six mutation sites producing three haplotypes. According to the median-joining network of the partial cox1 gene sequences, PAK-Hap3 haplotype comprised only goat isolates, PAK-Hap2 of sheep origin, while PAK-Hap1 of both hosts (Figure 2A). The examined nucleotide polymorphism resulted in amino acid changes (PAK-Hap2: 103L-103S; PAK-Hap1: 145N-145D). A nucleotide diversity of 0.00297 and haplotype diversity of 0.718 were observed for the overall T. multiceps population. The overall values of Tajima’s D (−0.06198) and Fu’s Fs (2.248) were not significant (p > 0.05). A similar observation was also found for T. multiceps from the separate populations (sheep and goats) (Table 1).

To evaluate the geographical relatedness of the 12 Pakistani isolates from this study with those from different parts of the globe, a dataset of 25 cox1 sequences (339 bp) was used to draw a median-joining network (Figure 2B). The analysis showed 13 haplotypes, with a high haplotype diversity (0.883), and nucleotide diversity (0.23691). Non-significant Tajima’s D = 2.24407 and Fu’s Fs = 14.002 were obtained.
Haplotype Data Availability
Sequences of the representative haplotypes from this study have been deposited in the GenBank database under the accession numbers MT863708–MT863710.

Phylogenetic Analysis
The phylogenetic tree was inferred based on the mt cox1 gene sequences of the representative haplotypes along with those retrieved from GenBank. The Pakistani and Chinese isolates formed a cluster and were found at a reasonable distance to another cluster comprising isolates from other countries (Figure 3). In the phylogenetic tree, other Taenia species (T. asiatica, T. crassiceps, T. hydatigena, T. ovis, T. pisiformis, T. saginata, T. solium, and T. taeniaeformis) were represented while Echinococcus granulosus sensu stricto (genotype 1,3) were used as outgroups.

DISCUSSION
Progress in the caprine and ovine rearing sector is thought to be an important indicator of protracted advancement of developing countries. Parasitic diseases pose a serious threat to livestock production all over the world leading to hampered economic progress (55, 56). Coenurosis is one of the production limiting diseases of ungulates and the exact prevalence is almost impossible to evaluate since the animals are sent for slaughter without prior knowledge of infection by the farmers. Meanwhile, molecular analyses have demonstrated huge importance in the identification and characterization of taeniids cysts (57), as well as the epidemiology, population genetics and phylogeny (58, 59).

Cerebral and non-cerebral coenurosis are terms used to describe infection caused by T. multiceps based on the location of the coenuri. T. multiceps is regarded as the causative agent for cerebral coenurosis while the larval stages of Multiceps gaigeri and M. skrjabini were previously believed to be responsible for non-cerebral coenurosis in goats and sheep until recently.
when modern studies have adopted *T. multiceps* to refer to causative agents for both cerebral and non-cerebral coenurosis (37). Information related to non-cerebral coenurosis is scanty and mainly available in countries like Bangladesh (60), India (4, 61, 62), and Iran (63). It has also been reported in Sudan, Oman and Namibia (17).

Here, we detected non-cerebral coenurosis in 12 small ruminants (eight goats and four sheep) out of 3,040 sheep and goats examined from May–December, 2019 which demonstrate a prevalence of 0.39%. Similar low prevalence has been observed in Iran where the prevalence recorded were 0.48 and 1.79% in sheep and goats, respectively (45). A study conducted in Greece to determine the prevalence of non-cerebral coenurosis in sheep also demonstrated a low prevalence of 0.008% (7/90,415) (18).

In this study, a molecular description of *C. cerebralis* isolates from sheep and goats was reported for the first time in Pakistan based on *mt* cox1 gene as *mt* DNA remains an important marker in exploring intraspecific variation due to maternal inheritance, conserved structure, higher evolution rate, high genetic divergence, and absence of recombination (64–68). The result demonstrates a high haplotype and low nucleotide diversity comparable to the population indices reported in Italy (Hd = 0.664, *π* = 0.004) (69) as well as in Egypt, where a considerable number of SNP in the *cox1* gene, mostly non-parsimony informative with low genetic diversity and fewer haplotypes were reported (70). Although this observation is mostly characteristic of an expanding population, our study revealed inconsistency with population expansion as demonstrated by the population indices: an insignificant negative Tajima’s *D* and a positive Fu’s *Fs*, unlike the Italian study where negative but insignificant values were observed for both Tajima’s *D* and a positive Fu’s *Fs* (69).

To date, the importance of the nucleotide substitutions and corresponding amino acid changes within *C. cerebralis* is unclear (44) though genetic variability leading to phenotypic differences in many other cestodes has been documented (57).

The phylogenetic analysis of the *cox1* sequences from these isolates revealed a separate cluster suggesting the existence of unique *C. cerebralis* haplotypes in Pakistan. This pattern was also observed in the population network and it is also similar to the cluster pattern in a study conducted in Greece (40). All the sequences obtained from GenBank were found to be in one cluster irrespective of the country of origin, predilection site and host while Pakistani isolates occupied a second cluster with the Chinese sequences. This network pattern was also observed by Rostami et al. (22, 39) and Al-Riyami et al. (40) unlike Varcasia et al. (69). Nonetheless,
this clustering pattern may indicate a genetic distinction between cerebral and non-cerebral coenurosis agents or intermediate host-specific specificity. Howbeit, further investigation is still warranted. The phylogenetic tree also confirmed *T. asiatica* and *T. saginata* as the closest to *T. multiceps* as reported previously (41).

**CONCLUSION**

In this study, *C. cerebralis* surgically isolated from sheep and goats in Faisalabad, Pakistan were characterized in what we believe is the first attempt to molecularly identify *T. multiceps* in Pakistan. Despite the sample size limitation, this study
Abo-Shehada MN, Jebreen E, Arab B, Mukbel R, Torgerson PR. Prevalence of Taenia multiceps in sheep in northern Jordan. Prev Vet Med. (2002) 55:201–7. doi: 10.1016/S0167-5877(02)00056-9

2. Hoger EP. Phylogeny of Taenia: species definitions and origins of human parasites. Parasitol Int. (2006) 55:23–30. doi: 10.1516/j.parint.2005.11.049

3. Amrabadi O, Oryan A, Moazeni M, Shari-Fiyazdi H, Akbari M. Morphological and molecular evaluation of the experimentally infected goats by the larval forms of Taenia multiceps. Iran J Parasitol. (2019) 14:95. doi: 10.18502/ijp.v14i1.722

4. Sharma DK, Chauhan PPS. Coenurosis status in Afro-Asian region: a review. Small Ruminant Res. (2006) 64:197–202. doi: 10.1016/j.smallrumres.2005.05.021

5. Sami M, Mirzaei M, Kheirandish R. Intramuscular and subcutaneous coenurosis in goats and sheep in south-east Iran. Comp Clin Path. (2012) 23:141–4. doi: 10.1007/s00580-012-1585-4

6. Tanowitz HB, Weiss LM, Wittmer M. Tapeworms. Curr Infect Dis Rep. (2001) 3:77–84. doi: 10.1007/s11908-001-0062-z

7. Avcioglu H, Yildirim A, Duzlu O, Inci A, Terim KA, Balkaya I. Prevalence and molecular characterization of bovine coenurosis from Eastern Anatolian region of Turkey. Vet Parasitol. (2011) 176:59–64. doi: 10.1016/j.vetpar.2010.10.033

8. El-Neweshy MS, Khalafalla RE, Ahmed MMS, Al Mawly JH, El-Manakhly EM. First report of an outbreak of cerebral coenurosis in Dhofari goats in Oman. Rev Bras Parasitol Vet. (2019) 28:479–88. doi: 10.1590/1984-29612019054

9. Scott PR. Diagnosis and treatment of coenurosis in sheep. Vet Parasitol. (2012) 189:75–8. doi: 10.1016/j.vetpar.2012.03.034

10. Christodouloupolous G, Theodoropoulos G, Petarakos G. Epidemiological survey of cestode-larva disease in Greek sheep flocks. Vet Parasitol. (2008) 153:368–73. doi: 10.1016/j.vetpar.2008.02.002

11. Giadinis N, Brellou G, Pouriotsis K, Papazahariadou M, Sofianidis G, Poutahidis T, et al. Coenurosis in a beef cattle herd in Greece. Vet Rec. (2007) 161:697. doi: 10.1136/vr.161.20.697

12. Kommenou A, Dessiris A, Giadinis N. Surgical treatment of coenurosis (gid) in sheep. Vet Rec. (2000) 147:242–4. doi: 10.1136/vr.147.9.242

13. Oryan A, Nazifi S, Shari-fiyazdi H, Ahmadnia S. Pathological, molecular, and biochemical characterization of Coenurus gaigeri in Iranian native goats. J Parasitol. (2010) 96:961–7. doi: 10.1645/GE-2399.1

14. Schuster RK, Sivakumar S, Wieckowsky T. Non-cerebral coenurosis in goats. Parasitol Res. (2010) 107:721–6. doi: 10.1007/s00430-010-1919-6

15. Christodouloupolous G, Kassab A, Theodoropoulos G. Occurrence of non-cerebral coenurosis in sheep. J Helminthol. (2013) 87:125–7. doi: 10.1017/S0022149X110085X

16. Giadinis N, Brellou G, Pouriotsis K, Papazahariadou M, Sofianidis G, Poutahidis T, et al. Morphometric analysis of larval rostellar hooks in Coenurus serialis 21. Gaiger SH. Coenurus serialis found in two goats in India. J Trop Vet Sci. (1907) 2:316–21.

17. Rostami S, Beech RN, Salavati R, Baneshi MR, Kamyabi H, Harandi MF. Morphometric analysis of larval rostellar hooks in Taenia multiceps in sheep and Iran and its association with mitochondrial gene variability. Iranian J Parasitol. (2013) 8:579. Available online at: https://ijpa.tums.ac.ir/index.php/ijpa/article/view/452

18. Islam AW, Rahman MS. A report on incidence of gid of calves of Bangladesh. Indian J Anim Health. (1997) 36:187–8.

19. Varma TK, Malviya HC. Prevalence of coenuriosis in sheep, goat and pigs in Bareilly, Uttar Pradesh. J Vet Parasitol. (1989) 3:69–71.

20. Kheirandish R, Sami M, Azizi S, Mirzaei M. Prevalence, predilection sites and pathological findings of Taenia multiceps coenuri in slaughtered goats from south-east Iran. Onderstepoort J Vet Res. (2012) 79:436. doi: 10.4102/ojvr.v79i1.436

21. Taavassoli M, Malekifard F, Soleimanzadeh A, Tajik H. Prevalence of Coenurus cerebralis in sheep in Northwest of Iran. Vet Res Forum. (2011) 2:274–6. Available online at: https://scholar.google.com.pk/scholar?q=Prevalence+of+Coenurus+cerebralis+in+sheep+in+Northwest+of+Iran.&hl=en&as_sdt=0&as_vis=1&oi=scholar

22. Oryan A, Moghaddar N, Gaur SNS. Metacestodes of sheep with special reference to their epidemiological status, pathogenesis and...
economic implications in Fars Province Iran. Vet Parasitol. (1994) 51:231–40. doi: 10.1016/0304-4017(94)90160-0
28. Javadi A, Hashemzadeh H, Shahbazi P. Study of Cerebral coenurosis rate in slaughtered sheep and goat brains in slaughterhouse in Tabriz. Iran Vet J. (2010) 6:59–63. Available online at: https://www.sid.ir/en/journal/ViewPaper. aspx?id=176325
29. Asmare K, Sibhat B, Abera M, Haile A, Degefu H, Fentie ND, et al. Abattoir prevalence, organ distribution, public health and economic importance of major metacestodes in sheep, goats and cattle in Fars, southern Iran. Trop Biom. (2012) 29:349–59. Available online at: https://pubmed.ncbi.nlm.nih.gov/23018497/
30. Afonso SMS, Mukaratirwa S, Hajovska K, Capece BPS, Cristofol C, Arboix M, et al. Genetic variation within Taenia multiceps from Abattoir-Slaughtered and experimentally infected goats. J Neuroparasitol. (2011) 2:1–5. doi: 10.4303/jnp/225352
31. Miran MB, Nizalwaje J, Kassouk AA, Swai ES. Prevalence of coenurosis in sheep and goats at three slaughter slabs in Ngorongoro District, Tanzania. Trop Anim Health Prod. (2015) 47:1591–7. doi: 10.1007/s11250-015-0903-7
32. Achenef M, Markos T, Feescha G, Hibret A, Tembley S. Coenurus cerebralis infection in Ethiopian highland sheep: incidence and observations on pathogenesis and clinical signs. Trop Anim Health Prod. (1999) 31:15–24. doi: 10.1023/A:1005253162775
33. Abedl-Maogood SZ. Studies on metacestode of sheep with reference to serodiagnosis of Coenurus multiceps Cysts (Coenurus cerebralis) from Abattoir-Slaughtered and experimentally infected goats. J Parasit Dis. (2011) 2:28–30. Available online at: https://www.indianjournals.com/jpd2011/paper/201109.pdf
34. Boyko Y, Polischuk N, Ragimov K, Strunin P, et al. Characterization of Taenia multiceps from Abattoir-Slaughtered and experimentally infected goats. J Vet Parasitol. (2010) 24:429–41. doi: 10.1016/j.cvfa.2008.07.001
35. Oryan A, Goorgipour S, Moazeni M, Shirian S. Abattoir prevalence, organ distribution, public health and economic importance of major metacestodes in sheep, goats and cattle in Fars, southern Iran. Trop Biom. (2012) 29:349–59. Available online at: https://pubmed.ncbi.nlm.nih.gov/23018497/
36. Aleixo R, Nogueira RM, Jorge P, Almeida P, Nogueira F, et al. Genetic characterization of Taenia multiceps from Abattoir-Slaughtered and experimentally infected goats. J Vet Parasitol. (2011) 24:429–41. doi: 10.1016/j.cvfa.2008.07.001
37. Garcia-Villar R, Toutain PL, Alvinerie M, Ruckebusch Y. The pharmacokinetics of xylazine hydrochloride: an interspecific study. J Vet Pharmacol Therap. (1981) 4:478–92. doi: 10.1111/j.1748-070X.1981.tb00715.x
38. Soulsby EJL. Ruminant field anaesthesia. Vet Clin Food Anim. (2008) 4:249–41. doi: 10.1016/j.cvfa.2008.07.001
39. Alvi et al. Genetic Diversity of Taenia multiceps in Sardinian sheep. (2015) 47:1591–7. doi: 10.1007/s11250-015-0903-7
40. Biyikoglu G, Bagci O, Oncel T. A coenurosis outbreak of sheep in Istanbul, Turkey. Pendik Veteriner-Mikrobiyoloji-Dergisi. (2005) 32:7:30–3. Available online at: https://www.researchgate.net/publication/284676366_A_coenurosis_outbreak_of_sheep_in_Istanbul_Turkey
41. Abdel-Maogood SZ. A study on Coenurus cerebralis with reference to serodiagnosis of Coenurus multiceps Cysts (Coenurus cerebralis) from Abattoir-Slaughtered and experimentally infected goats. J Parasit Dis. (2011) 2:28–30. Available online at: https://www.indianjournals.com/jpd2011/paper/201109.pdf
42. Christodoulopoulos G, Dinkel A, Romig T, Ebi D, Mackenstedt U, Loos-Frank B. Cerebral and non-cerebral coenurosis: on the genotypic and pathogenesis and clinical signs. Vet Parasitol. (2011) 17:1591–7. doi: 10.1007/s11250-015-0903-7
43. Almean M, Al-Saoud A, Al-Shaya A, Al-Saloom R, et al. Genetic diversity of Taenia multiceps isolated from sheep in Iran. Iran Vet J. (2010) 55:31–7. doi: 10.1016/j.parint.2005.11.004
44. Guo A, Li L, Jia Y, Guo W. Genetic characterization of Taenia multiceps from Abattoir-Slaughtered and experimentally infected goats. J Vet Parasitol. (2010) 24:429–41. doi: 10.1016/j.cvfa.2008.07.001
45. Garcia-Villar R, Toutain PL, Alvinerie M, Ruckebusch Y. The pharmacokinetics of xylazine hydrochloride: an interspecific study. J Vet Pharmacol Therap. (1981) 4:478–92. doi: 10.1111/j.1748-070X.1981.tb00715.x
46. Afonso SMS, Mukaratirwa S, Hajovska K, Capece BPS, Cristofol C, Arboix M, et al. Genetic variation within Taenia multiceps from Abattoir-Slaughtered and experimentally infected goats. J Parasit Dis. (2011) 2:1–5. doi: 10.4303/jnp/225352
47. Garcia-Villar R, Toutain PL, Alvinerie M, Ruckebusch Y. The pharmacokinetics of xylazine hydrochloride: an interspecific study. J Vet Pharmacol Therap. (1981) 4:478–92. doi: 10.1111/j.1748-070X.1981.tb00715.x
48. Garcia-Villar R, Toutain PL, Alvinerie M, Ruckebusch Y. The pharmacokinetics of xylazine hydrochloride: an interspecific study. J Vet Pharmacol Therap. (1981) 4:478–92. doi: 10.1111/j.1748-070X.1981.tb00715.x
49. Almean M, Al-Saoud A, Al-Shaya A, Al-Saloom R, et al. Genetic diversity of Taenia multiceps isolated from sheep in Iran. Iran Vet J. (2010) 55:31–7. doi: 10.1016/j.parint.2005.11.004
50. Afonso SMS, Mukaratirwa S, Hajovska K, Capece BPS, Cristofol C, Arboix M, et al. Genetic variation within Taenia multiceps from Abattoir-Slaughtered and experimentally infected goats. J Parasit Dis. (2011) 2:1–5. doi: 10.4303/jnp/225352
51. Miraftab A, Kheirabadi RR, Alizadeh H, Almasi R, et al. A study on Coenurus cerebralis with reference to serodiagnosis of Coenurus multiceps Cysts (Coenurus cerebralis) from Abattoir-Slaughtered and experimentally infected goats. J Parasit Dis. (2011) 2:28–30. Available online at: https://www.indianjournals.com/jpd2011/paper/201109.pdf
64. Brown WM, George M, Wilson AC. Rapid evolution of animal mitochondrial DNA. *Proc Natl Acad Sci USA*. (1979) 76:1967–71. doi: 10.1073/pnas.76.4.1967

65. Mueller RL, Macey JR, Jaekel M, Wake DB, Boore JL. Morphological homoplasy, life history evolution, and historical biogeography of plethodontid salamanders inferred from complete mitochondrial genomes. *Proc Natl Acad Sci USA*. (2004) 101:13820–5. doi: 10.1073/pnas.0405785101

66. Shen X, Wang H, Ren J, Tian M, Wang M. The mitochondrial genome of *Euphausia superba* (Prydz Bay) (Crustacea: Malacostraca: Euphausiacea) reveals a novel gene arrangement and potential molecular markers. *Mol Biol Rep*. (2010) 37:771–84. doi: 10.1007/s11033-009-9602-7

67. Wei SJ, Tang P, Zheng LH, Shi M, Chen XX. The complete mitochondrial genome of *Evania appendigaster* (Hymenoptera: Evaniidae) has low A+T content and a long intergenic spacer between atp8 and atp6. *Mol Biol Rep*. (2010) 37:1931–42. doi: 10.1007/s11033-009-9640-1

68. Singh BB, Sharma R, Gill JP, Sharma JK. Prevalence and morphological characterisation of *Cysticercus tenuicollis* (*Taenia hydatigena* cysts) in sheep and goat from north India. *J Parasit Dis*. (2015) 39:80–4. doi: 10.1007/s12639-013-0284-7

69. Varcasia A, Pipia AP, Dessi G, Zidda A, Tamponi C, Pau M, et al. Morphology and genetic variability within *Taenia multiceps* in ruminants from Italy. *Vet Parasitol*. (2016) 223:181–5. doi: 10.1016/j.vetpar.2016.04.039

70. Amer S, Elkhatam A, Fukuda Y, Bakr LI, Zidan S, Elsify A, et al. Prevalence and Identity of *Taenia multiceps* cysts *"Coenurus cerebralis"* in Sheep in Egypt. *Acta Trop.*. (2017) 176:270–6. doi: 10.1016/j.actatropica.2017.08.012

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