Molecular characterization and detection of variants of *Taenia multiceps* in sheep in Turkey

BETUL SONMEZ[1], ERGUN KOROGLU[2] *and* SAMI SIMSEK[2]*

[1] *Department of Animal Sciences, Directorate of Provincial Food Agriculture and Livestock, Tunceli, Turkey*
[2] *Department of Parasitology, Faculty of Veterinary Medicine, University of Firat, 23119, Elazig, Turkey*

(*Received 22 June 2016; revised 15 August 2016; accepted 21 August 2016; first published online 6 October 2016*)

SUMMARY

Taenia multiceps is a cestode (family Taeniidae) that in its adult stage lives in the small intestine of dogs and other canids. The metacestode, known as *Coenurus cerebralis*, is usually found in the central nervous system including brain and spinal card in sheep and other ruminants. The presence of cysts typically leads to neurological symptoms that in the majority of cases result in the death of the animal. Coenurosis could cause high losses in sheep farms because the disease commonly affects young animals. A total of 20 *C. cerebralis* isolates collected from naturally infected sheep in Mardin province of Turkey were characterized through the polymerase chain reaction and sequencing of a fragment of cytochrome c oxidase subunit 1 (CO1) gene. The results showed that the CO1 gene sequences were highly conserved in *C. cerebralis* isolates. Phylogenetic analysis based on partial CO1 gene sequences revealed that *C. cerebralis* isolates were composed of three different variants.

Key words: *Taenia multiceps*, *Coenurus cerebralis*, sheep, variant, CO1, PCR, Turkey.

INTRODUCTION

Taenia multiceps inhabits the small intestine of wild and domestic canids while the larval stage develops in the central nervous system of sheep and other ungulates. Sheep, cattle or sometimes goats, deer, antelope and less commonly, human beings act as intermediate hosts of the parasites following ingestion of the eggs. The larvae hatch in the intestine through the blood stream towards different organs. The larvae, which reach the brain and spinal cord grow to the metacestode stage. *Coenurus cerebralis* will further mature in the brain and spinal cord. The clinical disease occurs in sheep and rarely in cattle (Soulsby, 1982). The presence of cysts typically leads to neurological signs that cause the infected animals. As the location and depth of *T. multiceps* cysts are quite variable, there are no unique clinical signs. Ataxia, incoordination, paresis, head pressing, circling, blindness and coma may be observed in affected sheep. The disease may last several months and is commonly fatal (Abo-Shehada *et al*. 2002).

Turkey has a long tradition of rearing sheep and there is extensive local information on the management of sheep. Sheep are the main livestock of nomadic people in the east and southeast regions of Turkey. Although it is an offence to slaughter animals outside the abattoirs, illegal slaughtering of sheep is a common practice. Sheep are usually kept in large flocks, and mainly under free-range feeding. The prevalence of *C. cerebralis* in Turkey has been recorded in sheep, goat and cattle (Gicik *et al*. 2007). The prevalence in sheep has been reported to be 15·5% in Kars, 1·3% in Istanbul and 16·3% in Konya (Akkaya and Vuruşaner, 1998; Gicik *et al*. 2007; Uslu and Guclu, 2007). The prevalence of *T. multiceps* in dogs has been estimated to range between 0·32 and 12% in Turkey (Umur and Arslan, 1998).

Phenotypic differences in many instances reflect genotypic variability (McManus, 2005). There have been very limited investigations about genetic variability within *T. multiceps* (Varcasia *et al*. 2006; Al-Riyami *et al*. 2016). The presence of some clinical variation in coenurosis can indicate the existence of genetic intraspecific variability within the species (Varcasia *et al*. 2016). Thus, for the aim of a molecular perspective, we described an investigation of genetic variability within Mardin isolates of *T. multiceps* in Turkey. DNA sequence variability was investigated within the cytochrome c oxydase subunit 1 (CO1) mitochondrial gene that it has been commonly used for the distinction of species and strains of other cestodes (McManus, 2005).

MATERIAL AND METHODS

Sample collection

The *C. cerebralis* samples, were collected by a registered veterinary surgeon from sheep ($n = 20$) in Mardin province, which is situated in the southeast part of Turkey between the northern latitudes of 37° to 45° and eastern longitudes of 40° to 22°. Mardin has a hot-summer mediterranean climate

* Corresponding author. Department of Parasitology, Faculty of Veterinary Medicine, University of Firat, 23119, Elazig, Turkey. E-mail: ssimsek@firat.edu.tr

 © Cambridge University Press 2016
doi:10.1017/S0031182016001669

https://doi.org/10.1017/S0031182016001669 Published online by Cambridge University Press

with hot, dry summers and cold, wet and occasionally snowy winters. Temperatures in summer usually increase to 40 °C (104°F). Snowfall is quite common between the months of December and March, snowing for a week or two. Mardin has over 3000 h of sun per year. The highest recorded temperature is 42·5 °C (108·5°F). Average rainfall is about 641·4 mm (25 inches) per year (Turkish State of Meteorological Service). Cysts were obtained from the brain of individual sheep following either post mortem examination of clinically affected animals (by euthanizing of individual sheep) or following the routine slaughtering (by investigating the brain of all slaughtered sheep) in the abattoir during 2014. All cysts had protoscoleces according to morphological and microscopical examinations. Protoscoleces and/or membrane from individual cysts were fixed in 70% ethanol and then stored at −20 °C till used.

Genomic DNA isolation and polymerase chain reaction (PCR) amplification

Total genomic DNA was isolated from the protoscoleces and germinal membrane of the individual cysts with the use of a commercially available DNA extraction kit (MBI Fermentas, Vilnius, Lithuania) according to the manufacturer's instructions. A fragment (446 bp) of the CO1 gene was amplified with the use of the JB3 (5′-TTTTTTGGGCA TCCTGAGGTTTAT-3′), and JB4.5 (5′-TAAAG AAAGAACATAATGAAAATG-3′) primer set (Bowles *et al*. 1992) under the following conditions: Pre-denaturating step at 95 °C for 5 min and 35 cycles (50 s 94 °C, 50 s 45 °C, 50 s 72 °C) followed by a final extension step (10 min 72 °C). Amplicons were electrophoretically separated on an ethidium bromide stained 1·4% agarose gel and visualized under UV. The CO1 bands were excised from the gel and purified with the use of QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). The sequences were automatically obtained by ABI PRISM Sequence Detection System, and their identity was determined by an NCBI BLAST search. Chromatograms' quality was evaluated, the edges were trimmed and the ambiguities were corrected in FinchTV 1.4.0 (Geospiza Inc., Seattle Washington, USA) (http://www.geospiza.com).

Phylogenetic analyses

Multiple sequence alignment and phylogenetic tree construction of the obtained sequence of the mt-CO1 gene were performed with the use of CLC Main Workbench software (Knudsen *et al*. 2007). Unidirectional DNA sequence analysis of the mt-CO1 gene of all samples was performed. Unreliable ends of the raw sequences were trimmed and then the phylogenetic tree was built using the neighbour-joining method (Saitou and Nei, 1987). Based on pairwise comparisons, sequence differences were calculated using the CLC Main Workbench software. The reliability of the inferred tree was evaluated by bootstrap analysis on 1000 replicates. Reference sequences of *T. multiceps* (JQ710587; FJ886783) were also included for phylogenetic analysis. *Taenia saginata* (JQ756979) and *Echinococcus granulosus sensu stricto* (EU006777) were used as an out group.

RESULTS

During this study 20 coenurus cysts of various sizes and locations from sheep were collected from slaughterhouses in Mardin province of Turkey. Among these, the most remarkable cerebellum cyst of sheep was showing the clinical signs. When skull was opened a massive nut size *C. cerebralis* cyst embedded into the cerebellum tissue was detected (Fig. 1). Distribution of cysts according to the age, sex and cyst localization has been shown in Table 1. We collected 19 cysts from brain however only one was obtained from cerebellum. Brain localized fluid-filled *C. cerebralis* cysts are shown in Fig. 2. All of those examined with the PCR method was also observed in the 446 bp band. Ten of them was shown in Fig. 3. Nucleotide sequences were deposited in GenBank under the accession nos: KT217577-596. According to the alignment results the most radical change was seen in two sites of the sequences. A/G and T/C nucleotide changing were observed in between BC10, BC11, BC22, *T. multiceps* reference sequences and the other samples in 150th and 252nd nucleotides, respectively (Fig. 4). Genetic tree view of alignment samples was done using neighbour-joining method of measurement with Kimura 80 (Fig. 5).

According to the statistical evaluation of sequence data C + G rate was 121 and A + T rate was 249 in BC3 sample while these rates were 119 and 261 in BC10 and BC1 samples, respectively. First was 122 and the second was 258 in the other samples. Both the excess change rate of nucleotides and alignment results showed that BC3, BC10 and BC11 could be different variants.

DISCUSSION

Although coenurosis is common in almost every part of the world, it is more widespread in underdeveloped countries in sheep and goats (Scala *et al*. 2007). This study provides the first approach into the genetic characterization of *T. multiceps* from sheep in Turkey. A molecular-phylogenetic analysis of the coenurus cyst samples using mt-CO1 sequences was evaluated for the first time in Turkey. Mitochondrial genes are the most popular identification markers in population genetics and

https://doi.org/10.1017/S0031182016001669 Published online by Cambridge University Press

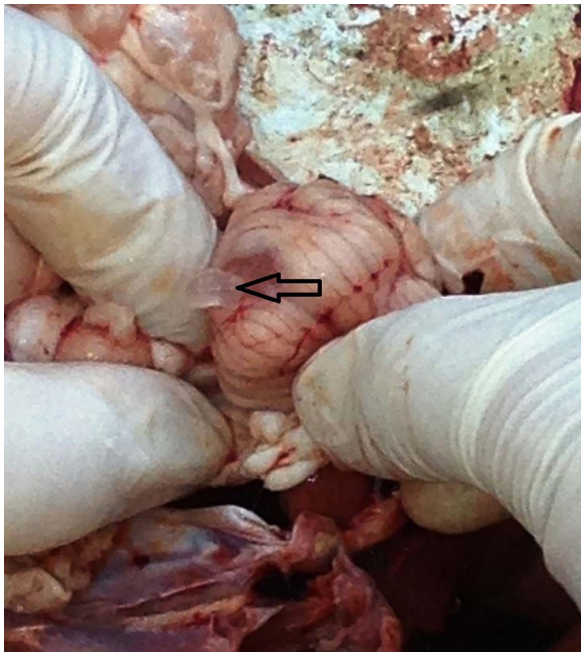

Fig. 1. Coenurus cerebralis cyst in cerebellum.

Table 1. Distribution of cysts according to the age, sex and cyst localization

Sample no (accession no)	Age	Sex	Cyst localization
BC1 (KT217577)	7 months	Male	Brain
BC2 (KT217578)	1 year	Female	Brain
BC3 (KT217579)	5 months	Female	Brain
BC5 (KT217580)	1 year	Male	Brain
BC6 (KT217581)	8 months	Female	Brain
BC7 (KT217582)	2 years	Female	Right and left lobes of brain
BC8 (KT217583)	1 year	Male	Cerebellum
BC9 (KT217584)	1 year	Female	Brain
BC10 (KT217585)	8 months	Female	Brain
BC11 (KT217586)	10 months	Female	Brain
BC12 (KT217587)	7 months	Female	Brain
BC13 (KT217588)	9 months	Female	Brain
BC15 (KT217589)	1 year	Female	Brain
BC17 (KT217590)	9 months	Male	Brain
BC18 (KT217591)	7 months	Female	Brain
BC19 (KT217592)	9 months	Female	Brain
BC20 (KT217593)	1 year	Female	Brain
BC22 (KT217594)	8 months	Female	Brain
BC23 (KT217595)	1·5 year	Female	Brain
BC24 (KT217596)	9 months	Female	Brain

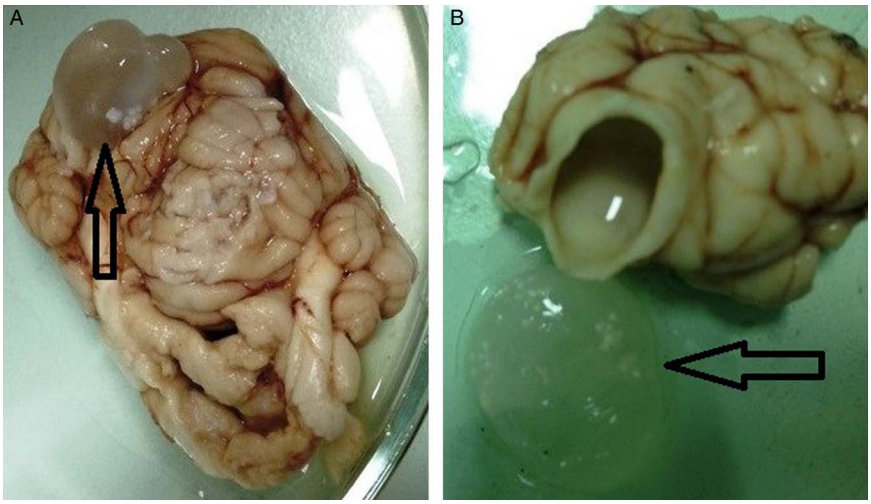

Fig. 2. (A) Coenurus cerebralis cyst localized in the brain tissue; (B) The cyst cavity formed in the brain tissue and the appearance of the protoscoleces in the cyst fluid.

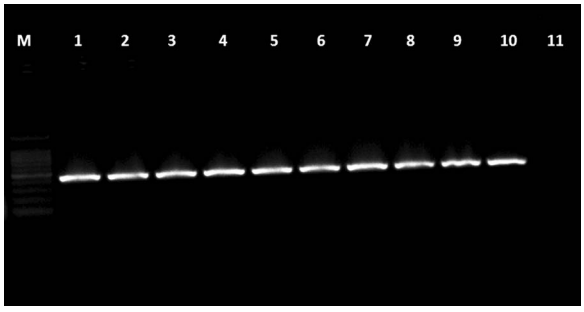

Fig. 3. mt-CO1 PCR results of Coenurus cerebralis cysts. M: Marker (100 bp), 1–9: randomly selected samples, 10: positive control, 11: negative control.

ecological studies of helminth parasites (Hebert and Gregory, 2005). Some genetic markers like 28S rRNA, CO1, nad1, nad4, ITS rDNA and other nuclear protein-coding genes as *rpbz*, *pepck* and *pold* are used for the molecular characterization and phylogenetic studies in Taeniid tapeworms. However, multiplex-PCR and PCR-restriction fragment length polymorphism techniques were used for the identification of Taenia species and often CO1 and cytb genes that are conserved gene regions were preferred (Zhang *et al*. 2007). But, we used mt-CO1 sequence for genotyping of sheep coenurus isolates in the current study.

https://doi.org/10.1017/S0031182016001669 Published online by Cambridge University Press

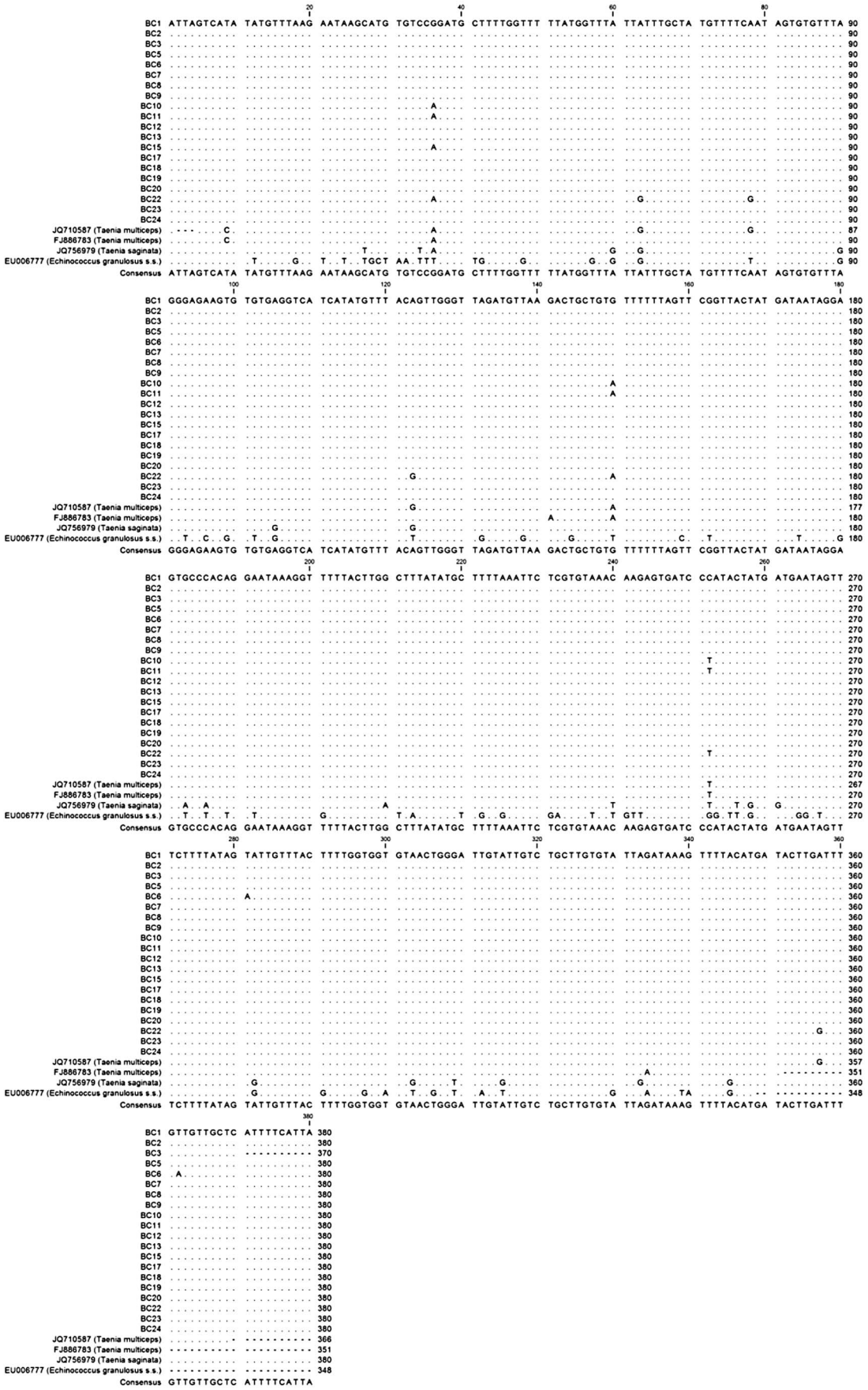

Fig. 4. Sequence alignment of samples analysed in this study using the reference sequences (JQ710587 and FJ886783). *Taenia saginata* (JQ756979) and *E. granulosus sensu stricto* (EU006777) were used as an out group.

https://doi.org/10.1017/S0031182016001669 Published online by Cambridge University Press

Fig. 5. Genetic tree view of the aligned sequences.

The life-cycle of the *T. multiceps* is very large extent rural, in which the dog-sheep cycle is the most important transmission dynamic. However, the farmer could often facilitate the contamination of the environment by opening the skull of infected sheep for interest or to confirm his own 'diagnosis', leaving the *Coenurus* cyst free to be eaten by dogs or, feeding them directly with it (Scala and Varcasia, 2006). Similarly, every year hundreds of thousands sheep are slaughtered in festival of sacrifices without veterinary inspections and the infected brains may be given to stray dogs in Turkey. Therefore, the prevalance of parasite is higher in the country.

The differences in the clinical and pathology appearances in coenurosis suggest the presence of genetic intraspecific variability within the species, such as in other members of the genus *Taenia* and *Echinococcus*. For these reasons DNA sequence variability was investigated within the mt-CO1 gene in this study. Twenty samples were obtained from Mardin province of Turkey and examined through the sequencing of the mt-CO1 gene. A/G and T/C nucleotide changing were observed in three samples. Considering the analysed gene, it is possible to define three specific genetic variants in the current study.

Coenurosis affects sheep during their first year of age, mainly when small lambs of 3–4 months are left in the grass at the beginning of the spring season, when their immune system and rumen activity are not yet well developed (Hebert and Edwards, 1984). Although 1 or 2-years-old sheep (15 and 21·7%, respectively) were more susceptible to the parasite (Gicik *et al*. 2007) we detected the infection rate as 60% in under 1 year old lamb. This may be explained by the inadequate of an acquired immunity. It was reported that low amount of taenid eggs are capable to develop into mature metacestodes. Although 93% of *Taenia hydatigena* eggs cannot develop into a cyst, it can stimulate the immunity for the next exposure (Gemmell *et al*. 1987). It can also be possible for the *T. multiceps*. Relatively low prevalence in older sheep may be due to the maternal immunity.

The coenurosis cyst can localize in any part of the brain, mostly in the cerebral hemispheres (Parihar, 1988). The great majority of the cysts (96·7%) was located in the cerebral hemispheres in another study (Gicik *et al*. 2007). Scala *et al*. (2007) reported that the brain cortex localization rate was 80·7 while 7·3% was for cerebellum. In the present study we detected only one cyst in the cebellum (5%). However we could not find any correlation between cyst localization and genotypic differences.

Concluding remarks

This is the first approach into the genotyping of *C. cerebralis* from sheep in Turkey. A molecular-phylogenetic analysis of coenurus cyst samples using mt-CO1 sequences was conducted and we detected three haplotypes for the first time. It is evident that intraspecific variations within *T. multiceps* may have biological or epidemiologic significance warrants for further studies. Thus the current study provides an insight for future studies of the genotyping of *T. multiceps* in possible hosts in Turkey.

ACKNOWLEDGEMENTS

The authors would like to thank all veterinarians and technicians for their kind help during sample collection.

FINANCIAL SUPPORT

This study has been supported by a grant from Firat University Scientific Research Projects Unit (Project No: VF.14.05, to S. S.).

REFERENCES

Abo-Shehada, M. N., Jebreen, E., Arab, B., Mukbel, R. and Torgerson, P. (2002). Prevalence of *Taenia multiceps* in sheep in northern Jordan. *Preventive Veterinary Medicine* **55**, 201–202.

https://doi.org/10.1017/S0031182016001669 Published online by Cambridge University Press

Akkaya, H. and Vuruşaner, C. (1998). Coenurosis in slaughtered sheep and calves in Istanbul. *Acta Parasitologica Turcica* **22**, 320–324 (in Turkish).

Al-Riyami, S., Ioannidou, E., Koehler, A. V., Hussain, M. H., Al-Rawahi, A. H., Giadinis, N. D., Lafi, S. Q., Papadopoulos, E. and Jabbar, A. (2016). Genetic characterisation of *Taenia multiceps* cysts from ruminants in Greece. *Infection Genetics and Evolution* **38**, 110–116.

Bowles, J., Blair, D. and McManus, D. P. (1992). Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. *Molecular and Biochemical Parasitology* **54**, 165–174.

Gemmell, M. A., Lawson, J. R. and Roberts, M. G. (1987). Population dynamics in echinococcosis and cysticercosis-evaluation of the biological parameters of *Taenia hydatigena* and *Taenia ovis* and comparison with those of *Echinococcus granulosus*. *Parasitology* **94**, 161–180.

Gicik, Y., Kara, M. and Arslan, M. O. (2007). Prevalance of *Coenurus cerebralis* in sheep in Kars province. Turkey. *Bulletin of the Veterinary Institute in Pulawy* **51**, 379–382.

Hebert, L. V. and Edwards, G. T. (1984). Some host factors which influence the epidemiology of *Taenia multiceps* in sheep. *Annals of Tropical Medicine and Parasitology* **78**, 243–248.

Hebert, P. D. N. and Gregory, T. R. (2005). The promise of DNA barcoding for taxonomy. *Systematic Biology* **54**, 852–859.

Knudsen, B., Knudsen, T., Flensborg, M., Sandmann, H., Heltzen, M., Andersen, A., Dickenson, M., Bardram, J., Steffensen, P. J., Mansted, S., Lauritzen, T, Forsberg, R., Thanbichler, A., Bendtsen, J. D., Gorlitz, L., Rasmussen, J., Tordrup, D., Vaerum, M., Ravn, M. N., Hachenberg, C., Fisker, E., Dekker, P., Schultz, J., Hein, A. M. K., Sinding, J. B. (2007). *CLC Main Workbench*. Version 5.5. CLC bio, Aarhus, Denmark.

McManus, D. P. (2005). Molecular discrimination of taeniid cestodes. *Parasitology International* **55**, 31–37.

Parihar, N. S. (1988). Pathology of *Coenurus cerebralis* in ovine subclinical infections. *Indian Journal of Animal Science* **58**, 539–543.

Saitou, N. and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**, 406–425.

Scala, A. and Varcasia, A. (2006). Updates on morphobiology, epidemiology and molecular characterization of coenurosis in sheep. *Parassitologia* **48**, 61–63.

Scala, A., Cancedda, G. M., Varcasia, A., Ligios, C., Garippa, G. and Genchi, C. (2007). A survey of *Taenia multiceps* coenurosis in Sardinian sheep. *Veterinary Parasitology* **143**, 294–298.

Soulsby, E. J. L. (1982). *Helminths, Arthropods and Protozoa of Domesticated Animals*, 7th Edn. Bailliere and Tindall, London.

Umur, S. and Arslan, M. O. (1998). The prevalence of helminth species in stray dogs in Kars province. *Acta Parasitologica Turcica* **22**, 188–193.

Uslu, U. and Guclu, F. (2007). Prevalence of *Coenurus cerebralis* in sheep Turkey. *Medicina Weterinaria* **63**, 678–680.

Varcasia, A., Lightowlers, M. W., Cattoli, G., Cansedda, G. M., Canu, S., Garippa, G., Scala, A. (2006). Genetic variation within *Taenia multiceps* in Sardinia. Western Mediterranean (Italy). *Parasitology Research* **99**, 622–626.

Varcasia, A., Pipia, A. P., Dessi, G., Zidda, A., Tamponi, C., Pau, M., Scala, A. and Boufana, B. (2016). Morphology and genetic variability within *Taenia multiceps* in ruminants from Italy. *Veterinary Parasitology* **223**, 181–185.

Zhang, L., Hu, M., Jones, A., Allsopp, B. A., Beveridge, I., Schindler, A. R. and Gasser, R. B. (2007). Characterization of *Taenia madoquae* and *Taenia regis* from carnivores in Kenya using genetic markers in nuclear and mitochondrial DNA, and their relationships with other selected taeniids. *Molecular and Cellular Probes* **21**, 379–385.

https://doi.org/10.1017/S0031182016001669 Published online by Cambridge University Press