Molecular differentiation of *Thysaniezia* (*Helicometra*) *giardi* and *Moniezia* species based on 18s rRNA gene in small ruminants

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**Abstract**

This study was conducted to investigate Anoplocephalidia Cestoda in sheep and goat and evaluate the 18s rRNA to genetically differentiate the genera of this family. Sixty sample tapeworms were collected from small intestines of 30 sheep and 30 goats from different slaughterhouses in Al-Najaf and Al-Qadisiyah provinces, during September, 2016 to February, 2017. Based on polymerase chain reaction (PCR) and 18s rRNA gene partial sequencing (18sGPS) methods used, tapeworm infection of sheep and goat’s intestines was 32.9% and 31.4%, respectively. The partial gene sequencing of the 18s rRNA gene showed two closely related isolates of *M. benedeni* which are aligned distinctly to an NCBI isolate of the same species from China. For *T. giardia*, the outcomes of the phylogenetic analysis unveiled three distinct local isolates which were similar to an NCBI database isolate from China. The current data ensure the importance of the molecular techniques in differentiating between *Thysaniezia* (*Helicometra*) *giardi* and *Moniezia* species that were identified for their presence in the small intestines of sheep and goats.

**Introduction**

Small ruminant livestock has social wealth and economic values of rural households in Iraq. *Anoplocephalidae* are a group of the most common parasites that infect sheep and goat, which remain the leading infectious agents that affect the productivity of the small ruminant livestock sector (1). *Thysaniezia* are globally high-prevalent cestodes currently assigned within the family *Anoplocephalidae*. These tapeworms, especially the adult cestodes, are greatly recognized to infect the ruminant small intestines of sheep and goats. Even though these parasites are well-known for their world incidence, their occurrence can hugely be correlated with some factors such as climate geographical and climate criteria, husbandry management services, and the density of livestock (2).

*Thysaniezia ovilla* (*T. giardia*), *Moniezia expansa*, and *Avitellina centripunctata* have been shown to have incidence rates at 3.59% to 89.92% of the entire intestinal parasite infections in sheep (3,4). Small ruminants have a prominent role for being socially, economically, and politically significant at national and international levels for disease control and prevention. *Thysaniezia* genus infection of this family occurs in small intestine of ruminants (5).

Although some literatures reported small intestine infections by some species of parasites, studies are needed to complete the identification pictures of the relevant parasites by applying techniques to differentiate between those organisms for better diagnosis and treatment. Therefore, the herein study was conducted to investigate *Anoplocephalidida cestoda* in sheep and goat and evaluate the 18s rRNA to genetically differentiate the genera of this family.

**Materials and methods**

**Tapeworm sampling**

A total of 60 samples of tapeworms were collected randomly from small intestines of sheep and goats in the main slaughterhouses of Al-Najaf and Al-Qadisiyah...
provinces, Iraq, from September, 2016 to February, 2017. The sampled worms were transmitted to the parasitology laboratory, College of Veterinary Medicine, University of Al-Qadisiyah. The adult stage of the tapeworms was gently washed with PBS twice and placed separately in 70% ethanol-preloaded containers, then stored at -20 °C until further work, started with DNA extraction, was conducted (6).

DNA extraction
Small pieces of the worms were utilized for genomic DNA extraction after they were rinsed thoroughly with distilled water to remove any remnant of the ethanol. KAPA Express Extract Kit (R&D, Cape Town, South Africa) was employed for the DNA extraction process that depended on the instructions of the manufacturer. Quantity and quality of DNA were measured with a NanoDrop.

PCR and partial gene sequencing
The primers of the current work regarding the 18S rRNA gene, F: 5-GTTTACAAACTACCAACCGGATCG-3 and R: 5-CTGATTACGTCCCCGCTTTTG-3, were designed and synthesized with Primer Quest Tool (Integrated DNA Technologies, Inc., Belgium) for detecting Thysaniezia and Moniezia species. For the PCR thermocycler conditions, the initial denaturation was 94°C for 5mins followed by 35 cycles of (a denaturation step 94°C for 1min, an annealing step 57°C for 1min, and an extension step 72°C for 2mins) that were finished with a final extension step for 7mins. A one-percentage agarose-gel electrophoresis was followed to examine the PCR products which were later UV-imager-visualized (6).

The partial gene sequencing was intended to sequence five positive PCR products. Phylogenetic analyses were performed using NCBI-related data bases, and the phylogenetic trees were drawn using MEGA v7 software depending on the Neighbor-Joining and the Maximum Composite Likelihood methods (6,7).

Results
The PCR and 18sGPS findings demonstrated that tapeworm infection incidence rates of small ruminant, sheep and goats, intestines were 32.9% and 31.4%, respectively. Figure (1) reveals the 18s rRNA gene PCR products on the agarose gel. The PCR positive product is at 980bp.

The partial gene sequencing of the 18s rRNA gene showed two closely related isolates of M. benedeni (MH203083.1 and MH203084.1) which are aligned distinctly to an NCBI isolate of the same species from China, GU817402.1, figure 2.

For T. giardia, the outcomes of the phylogenetic analysis unveiled three distinct local isolates, MH203082.1, MH203080.1, and MH203081.1, which were similar to an NCBI database isolate, JQ609342.1, from China (Figures 3).
The current data ensure the importance of the molecular techniques in differentiating between *Thysaniezia (Helicometra) giardi* and *Moniezia* species that were identified for their presence in the small intestines of sheep and goats.

### Conclusion

To investigate the importance of the molecular techniques in differentiating between *Thysaniezia (Helicometra) giardi* and *Moniezia* species that were identified for their presence in the small intestines of sheep and goats.

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### Conflict of interests

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### References

1. Ndom M, Diop G, Quilichini Y, Yanagida T, Ba CT, Marchand B. Prevalence and scanning electron microscopic identification of anoplocephalid cestodes among small ruminants in Senegal. J Parasitol Res. 2016;4(1):1-9. Doi: 10.1155/2016/5937292

2. Squire SA, Robertson ID, Yang R, Ayi I, Ryan U. Prevalence and risk factors associated with gastrointestinal parasites in ruminant livestock in the Coastal Savannah zone of Ghana. Acta Trop. 2019;199:105-126. Doi: 10.1016/j.actatropica.2019.105126

3. Southworth J, Harvey C, Larson S. Use of praziquantel for the control of Moniezia expansa in lambs. New Zealand Vet J. 1996;44(3):112-115. Doi: 10.1080/00480169.1996.35947

4. Meradi S, Cabaret J, Bentousni B. Sheep enteric cestodes and their influence on clinical indicators used in targeted selective treatments against gastrointestinal nematodes. Onderstepoort J of Vet Res. 2019;86(1):1-3. Doi: 10.1080/00207020.2019.1657286

5. Squire SA, Yang R, Robertson I, Ayi I, Squire DS, Ryan U. Use of praziquantel and ivermectin for the control of Moniezia expansa in lambs. New Zealand Vet J. 1996;44(3):112-115. Doi: 10.1080/00480169.1996.35947

6. Saitou N, Nei M. The neighbor joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4(4):406-425. Doi: 10.1093/molbev/sle016

7. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 2013;30(12):2725-2729. Doi: 10.1093/molbev/ms376

8. Roeker J, Jex AR, Gasser RB. Impact of gastrointestinal parasitic nematodes of sheep, and the role of advanced molecular tools for molecular techniques revealed two different clades of *T. ovilia* from sheep and *T. connochaeti* from cattle. This suggests that using morphological methods is not useful for all cases in which certain molecular approaches should be followed for complete and accurate diagnosis (15-17).

The findings obtained in the present investigation included the use of PCR and sequencing methods which showed the presence of *T. giardi* and *M. benedeni* in the intestinal content samples of sheep and goats. Diop et al. (12) have employed PCR and DNA sequencing methods that targeted cytochrome c oxidase subunit I (coxI) gene from the mitochondria and small subunit ribosomal RNA (SSU rDNA) gene from the nuclei of their 64 adult intestinal cestodes sampled from cattle, sheep, and goats and morphologically found that the worms of cattle belonged to *M. benedeni*. In contrast, the samples of the cestodes gathered from sheep and goats were recognized as *M. expansa*; however, lacking morphological determinants such as interproglottidal glands left a group of unassigned worms without identification until it was completely resolved using the sequencing which classified those cestodes as *M. expansa* (12). This confirms the reliability of the morphological methods but with certain limits that can be broken using advanced techniques involving the use of the genetic materials (13).

For the *T. giardi*, Ndom et al. (14) have morphologically and genetically (coxI and SSU rDNA genes) examined 52 adult *Thysaniezia* cestodes collected from sheep and cattle from Senegal and detected using the morphological feature identification that tapeworms from both animal categories were similar. Nevertheless, the
9. Demeler J, Gill JH, Von SG, Sangster NC. The in vitro assay profile of macrocyclic lactone resistance in three species of sheep trichostrongyloids. Int J Parasitol Drugs Resist. 2013;3:109-118. Doi: 10.1016/j.ipdr.2013.04.002

10. Cringoli G, Rinaldi L, Maurelli MP, Utzinger J. Flo tac: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nat Protoc. 2010;5(3):503-515. Doi: 10.1038/nprot.2009.235

11. Cringoli G, Rinaldi L, Veneziano V, Capelli G, Scala A. The influence of flotation solution, sample dilution and the choice of McMaster slide area (volume) on the reliability of the McMaster technique in estimating the faecal egg counts of gastrointestinal strongyles and Dicrocoelium dendriticum in sheep. Vet Parasitol. 2004;123(1-2):121-131. Doi: 10.1016/j.vetpar.2004.05.021

12. Diop G, Yanagida T, Hailemariam Z, Menkis R, Nakao M, Sako Y, Ba TC, Ito A. Genetic characterization of Moniezia species in Senegal and Ethiopia. Parasitol Int. 2015;64(5):256-260. Doi: 10.1016/j.parint.2015.02.008

13. District MS, Ambedkar B. Morphological and molecular studies of Moniezia Sp. (Cestoda Anaplocephalidea) a parasite of the domestic goat Capra hircus (L.) in Aurangabad district. Zool. 2015;5(8):10-14. Doi: 10.36106/zjari

14. Ndom M, Yanagida T, Diop G, Quilichini Y, Ba A, Sako Y, Nakao M, Marchand B, Dieye A, Ba TC, Ito A. Genetic and morphological characterization of Thysaniezia tapeworms from cattle and sheep in Senegal. Vet Parasitol Reg Stud Reports. 2018;11:27-31. Doi: 10.1016/j.vprsr.2017.11.008

15. Budischak SA, Hoberg EP, Abrams A, Jolles AE, Ezenwa VO. A combined parasitological molecular approach for noninvasive characterization of parasitic nematode communities in wild hosts. Mol Ecol Resour. 2015;15(5):1112-1119. Doi: 10.1111/1755-0998.12382

16. Abdulla RG, Mageed SN, Obed CE, Jumaa JA. Molecular characterization of fertile hydatid cysts from the liver of the sheep and cows and associated environmental influence factors. Iraqi J of Vet Sci. 2020; 34(2):321-327. Doi: 10.33899/ijvs.2019.126036.1213

17. Jarad NI. Molecular detection of Cryptosporidium parvum in chicken in Al-Diwaniya province. Iraqi J of Vet Sci. 2020;34(2):441-445. Doi: 10.33899/ijvs.2019.126159.1249