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 *Anoplocephalidida 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.
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-GTTTACAAACTACCACCCGGATCG-3 and R: 5-CTGATTACGTTCCCTGCCCCCTTG-3, were designed and synthetized with Primer Quest Tool (Integrated DNA Technologies, Inc., Belgium) for detecting Thysaniezia and Montezia 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).

Even though the presence of highly advanced biological techniques such as genomics based methods including metagenomics and proteomics that are supported by bioinformatics (8), the diagnosis of gastrointestinal parasitic organisms in ruminants is still highly depending on coprological examination (9,10). Indeed, coproscopy (from the Greek words κόπρος = feces and σκοπέω = examine) means exploring fecal samples for the occurrence of any parasitic components (e.g. eggs). The method is the most widely used diagnostic procedure in veterinary parasitology (11). However, exploring fecal samples for such presence of intestinal parasites requires additional methodology for drawing complete differentiation picture between cestodes for enhancing diagnosis and managing infections caused by those organisms.

Figure 1: Reveals the 18s rRNA gene PCR products on the agarose gel (1%) image. Lanes, 3 to 7, represent PCR positive product is at 980bp. Lanes, 1 and 2, are negative samples.

Figure 2: Displays the phylogenetic tree according to the 18s rRNA partial gene sequencing from the M. benedeni worm samples. The Neighbor-Joining and the Maximum Composite Likelihood methods were utilized for this purpose devoted in NCBI databases and MEGA v7 software. The both isolates (red dots) are aligned closely to the NCBI Chinese isolates; GU817402.1.
molecular techniques revealed two different clades of *T. ovila* from sheep and *T. connochaetii* 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 current data ensure the importance of the molecular techniques in differentiating between *Thysanobia* (*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 *Thysanobia* (*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**

8. Rober F, Jex AR, Gasser RB. Impact of gastrointestinal parasitic nematodes of sheep, and the role of advanced molecular tools for...