Iraqi Journal of Veterinary Sciences

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

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Article information

Article history:
Received December 15, 2019
Accepted January 20, 2020
Available online November 1, 2020

Keywords:
Cestodes
Sheep
Goats
Iraq
PCR

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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* (*Helictometra*) *giardi* and *Moniezia* species that were identified for their presence in the small intestines of sheep and goats.

DOI: 10.33899/ijvs.2020.126407.1313,

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 *Anoplocephalidia 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 ℃ 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-GTTTACAAACTACCACCACGGATCG-3 and R: 5-CTGATTACGTCCCTGCCCTTTG-3, were designed and synthetized 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).

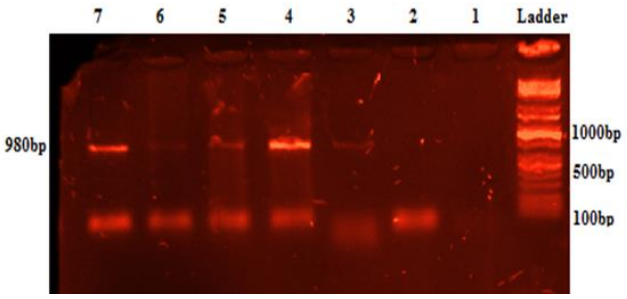

Figure 1: Reveals the 18s rRNA gene PCR products on the agarose gel (1%) image. Lanes, 3 to 7, represent PCR positive product is at 980bp. Lanes, 1 and 2, are negative samples.

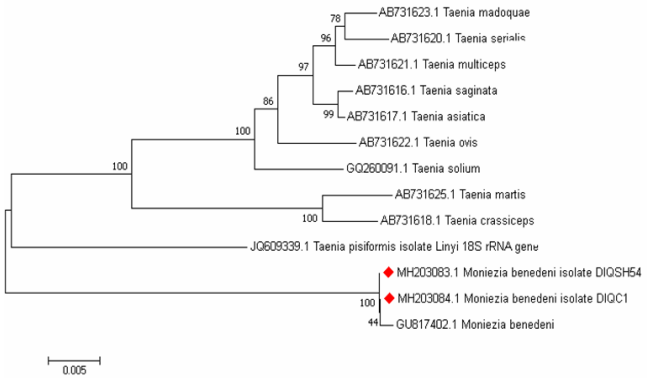

Figure 2: Displays the phylogenetic tree according to the 18s rRNA partial gene sequencing from the M. benedeni worm samples. The Neighbor-Joining and the Maximum Composite Likelihood methods were utilized for this purpose devoted in NCBI databases and MEGA v7 software. The both isolates (red dots) are aligned closely to the NCBI Chinese isolates; GU817402.1.

Discussion

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 *-σκοπία* = 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.

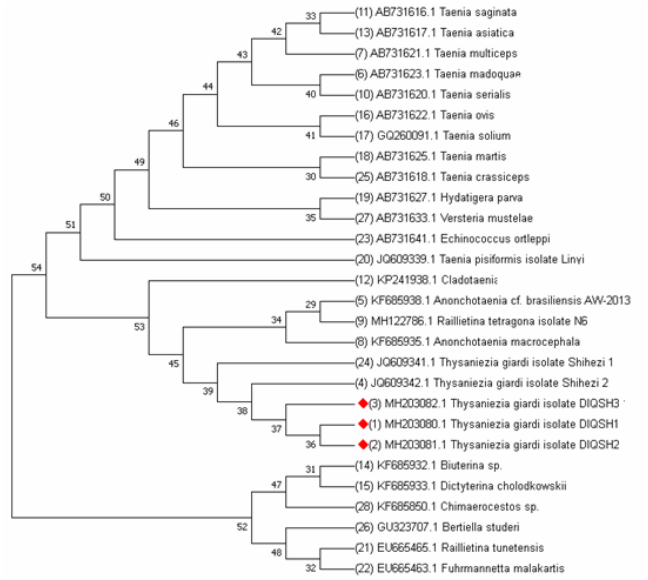

Figure 3: Displays the phylogenetic tree according to the 18s rRNA partial gene sequencing from the M. benedeni worm samples. The Neighbor-Joining and the Maximum Composite Likelihood methods were utilized for this purpose devoted in NCBI databases and MEGA v7 software. The both isolates (red dots) are aligned closely to the NCBI Chinese isolates; GU817402.1. Moniezia benedeni MH203083.1,

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 1 (*cox1*) 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. giardia*, Ndom *et al.* (14) have morphologically and genetically (*cox1* 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 molecular techniques revealed two different clades of *T. ovilla* 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 current data ensure the importance of the molecular techniques in differentiating between *Thysaniezia* (*Helictometra*) *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* (*Helictometra*) *giardi* and *Moniezia* species that were identified for their presence in the small intestines of sheep and goats.

Acknowledgments

The authors thank Professor Jabbar Ahmed Alssady Dean of College of Veterinary Medicine, University of Al-Qadisiyah, Iraq, for technical assistance.

Conflict of interests

The authors have not received any funding or benefits from industry, agency of financing, or elsewhere to conduct this study.

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التمايز الجزيئي لأنواع ثيسانيزيا (هليكتوميترا) الجياردي ونوع مونيزيا على أساس جينات rRNA s18 في المجترات الصغيرة

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الخلاصة

أجريت الدراسة لاستقصاء شريطية الأنوبلسفيديا في الأغنام والماعز وتقييم 18s rRNA للتمييز الوراثي بين أجناس هذه الأسرة. تم جمع ستين عينة من الديدان الشريطية من الأمعاء الدقيقة المكونة من ٣٠ خروفًا و ٣٠ من الماعز من مختلف المسالخ في محافظتي النجف والقادسية، خلال الفترة من سبتمبر ٢٠١٦ إلى فبراير ٢٠١٧. بناءً على تفاعل سلسلة البلمرة المتسلسل (PCR) وطرق التسلسل الجزئي لجينات rRNA (18sGPS) المستخدمة، كانت إصابة الدودة الشريطية بأمعاء الأغنام والماعز ٣٢,٩٪ و ٣١,٤٪ على التوالي. أظهر التسلسل الجيني الجزئي لجين 18S rRNA عزلتين وثيقتي الصلة من نوع *M. benedeni* والتي تتماشى بشكل واضح مع عزل الـ NCBI من نفس النوع من الصين. بالنسبة إلى *T. giardia*، كشفت نتائج التحليل النشوئي عن ثلاث عزلات محلية متميزة كانت مشابهة لقاعدة بيانات الـ NCBI المعزولة من الصين. تضمن البيانات الحالية أهمية التقنيات الجزيئية في التمييز بين أنواع ثيسانيزيا (هليكتروميترا) الجياردية وأنواع المونيزيا التي تم تحديدها لوجودها في الأمعاء الدقيقة للأغنام والماعز.