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Molecular detection of gene encodes a β-tubulin protein in *Haemonchus contortus* in sheep in Al-Qadisiyah province, Iraq

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Abstract

β-tubulin protein is essential to parasitic resistance against widely used anthelmintic drugs. Many world countries are facing major challenges due to the failure of anthelmintic drugs, due to resistance, to eliminate parasitic infection in different animals, mainly sheep. So, there is a significant need to increase research focusing on parasites' resistance components, especially β-tubulin. Accordingly, the present study was conducted to identify and examine the genetic evolution of the β-tubulin gene responsible for synthesizing the β-tubulin protein in *Haemonchus contortus* in sheep. Here, 250 slaughtered sheep were explored, and 21 nematodes were collected and utilized in microscopic (10 nematodes) by placing each worm on a glass slide and exploring it under a light microscope at 10X magnification. In addition, 11 nematodes were set for molecular (PCR and sequencing) methods. The microscopic detection demonstrated the identification of the worm. The results revealed the amplification of the gene region in 11 worms. The sequencing of the nematodes showed the identification of 10 isolates closely similar to isolates from Sweden, with a similarity rate of up to 98%. The study's data report the major presence of the β-tubulin gene, which might be responsible for the drug due to drug resistance, and reveal important information about the genetic evolution of this gene that presents critical data about drug resistance development in the current study isolates of *Haemonchus contortus*.

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Introduction

Several diseases can lead to the outbreak of anemia. Haemonchosis is one of these diseases that cause anemia caused by the parasitic nematode *H. contortus*. *H. contortus* is a parasitic nematode classified in the Phylum Nematoda, Class Secernentea, Order Strongylida, and Family Trichonstrongylidae. *H. contortus* is a species adapted to thrive in various climatic zones, especially where the weather is warm and humid, especially in areas with high levels of rainfall (1-5). Historically, haemonchosis was acknowledged to occur in established fit and reference zones. Still, climate change (i.e., the warming of areas that were previously not considered to be high-risk) has seemingly allowed *H. contortus* to survive and develop in zones that were supposed to be low-risk. Therefore, it is advisable for veterinarians and producers not only to consider haemonchosis when confronted with a case of anemia or mortality of sheep in so-called high-risk areas but also in low-risk or anywhere in between zones (6-10). If *H. contortus* remains in moist, warm conditions (above 12ºC) for 7-10 days, it completes its embryonic development. If conditions remain satisfactory for longer, the free-living larvae will progress through increased developmental stages, up to the fourth. Haemonchosis can occur throughout the year if the needed conditions are met. An example of the impact of climate on the potential for disease caused by *H. contortus* is that the maximal prevalence of this disease was recorded in the tropical zones located at 23.5º latitudes of North and South (11-13). The tropics are regions between 23.5º N and 23.5º S latitudes. These parts of the earth are considered tropical because they remain hot and humid all

year round. In these tropical regions, climatic conditions favor the survival of *H. contortus*. They are associated with areas with the highest disease prevalence worldwide, such as Southeast Asia, India, and Africa. Haemonchosis is uncommon in drier parts of the world, i.e., regions where sufficient moisture is not present to support the independent larval phases of *H. contortus*, but this can change with increasing precipitation or irrigation, promoting the survival of *H. contortus* in hotter, drier regions (14-17). *H. contortus* eggs hatch best between 22 and 26ºC when humidity is close to 100% in the microclimate of the vegetation. Larvae can survive in dried feces in the harsh climate of deserts and emerge following the rain. As a consequence, a higher infection rate emerges suddenly after rainfall. In optimal environmental conditions, the development of *H. contortus* from egg to a third-stage larva (L3) can be completed within four days (18-20).

β-tubulin protein is essential to parasitic resistance against widely used anthelmint drugs, such as Benzimidazole derivatives, such as albendazole and mebendazole. The present study was carried out to identify and examine the genetic evolution of the β-tubulin gene, responsible for the synthesis of β-tubulin protein in *H. contortus* in sheep.

Materials and methods

Ethical approve

All the authors of the present work ensure that all procedures of our experiment were performed under the Ethical Norms approved by the scientific board of the College of Veterinary Medicine, University of Al-Qadisiyah (committee approval number 2111 on 16/10/2023).

Samples and microscopic examination

The present study was carried out to identify and examine the genetic evolution of the β-tubulin gene, responsible for the synthesis of β-tubulin protein in *H. contortus* in sheep. Here, 250 slaughtered sheep were explored, and 21 nematodes were collected and utilized in microscopic (10 nematodes) and 11 nematodes for molecular (PCR and sequencing) methods. The study collection of samples was done from October 2023 to February 2024. The samples were placed in lactophenol-included containers for microscopic examination. Other parts of the nematodes were placed in 70% ethanol for molecular tests. Each worm was placed on a glass slide and explored under a light microscope with magnification power at 10X.

DNA extraction and PCR

The DNA from the worms was extracted using the protocol and the kit AddBio (Korea). The PCR primers were collected from Zongze *et al.* (21). These are F: GGAACAATGGACTCTGTTCG and R: GAATCGAAGGCAGGTCGT. The 20 µl reaction volume contained the master mix at 10 µl, each direction of the primers at (0.5 pmol/20 µl) at 2 µl, PCR water at 4 µl, and DNA at 2 µl. The conditions for the PCR thermal cycler were one starting denaturing step, 40 steps (principal denaturing step, annealing step, and principal extension step), and one ending extension step at 95ºC, (95ºC, 55ºC, and 72ºC), and 72ºC for 5 mins, (35 s, 30 s, and 30 s), and 5 mins. The electrophoresis was run through a 1.5 agarose gel at 100 volts and 80 AM for 60 s. A gel documentation system was employed to visualize the PCR products.

β-tubulin gene partial sequencing

The PCR-positive products were sequenced at Macrogen Company in Korea. The NCBI websites and MEGA X software were utilized to analyze the sequencing data and produce the phylogenetic tree.

Results

The microscopic detection demonstrated the worm's positive identification. The results revealed the amplification of the gene region in 11 worms. These samples were amplified at 785 bp of length. Figure 1 shows these positive PCR products and their positive amplification bands.

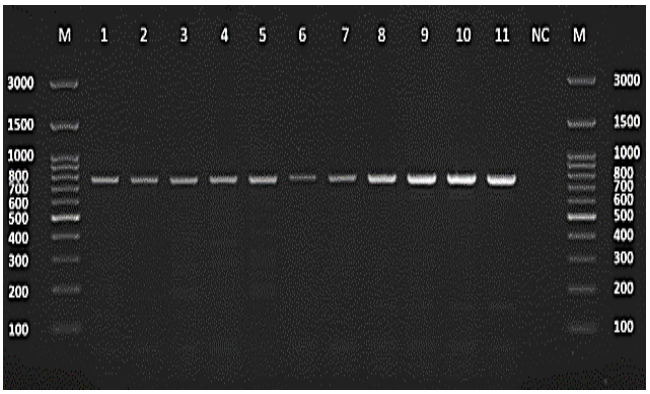

Figure 1: Image of 1.5% Agarose gel electrophoresis of PCR targeting the β-tubulin gene of *Haemonchus contortus* in sheep. M: Ladder, 1-11: Positive bands at 785 bp, and NC: Negative control.

The sequencing of the nematodes identified ten isolates that are closely similar to isolates from Sweden, with a similarity rate of up to 98%. These sequences were PP393524, PP393525, PP393526, PP393527, PP393528, PP393529, PP393530, PP393531, PP393532, and PP393533 as they appeared in the GeneBank depository system (Table 1 and Figure 2).

Table 1: Comparison between the current study isolates and world isolates of *Haemonchus contortus* in sheep

Accession number	GenBank Accession number	Country	Identity (%)
PP393524	MK382798	Sweden	98.85
PP393525	MK382800	Sweden	96.08
PP393526	MK382797	Sweden	95.11
PP393527	MK382761	Sweden	95.92
PP393528	KF483614	Canada	95.92
PP393529	DQ469245	Switzerland	94.61
PP393530	MK382772	Sweden	95.42
PP393531	MK382788	Sweden	95.62
PP393532	KX246652	USA	94.60
PP393533	X80046	Netherlands	94.60

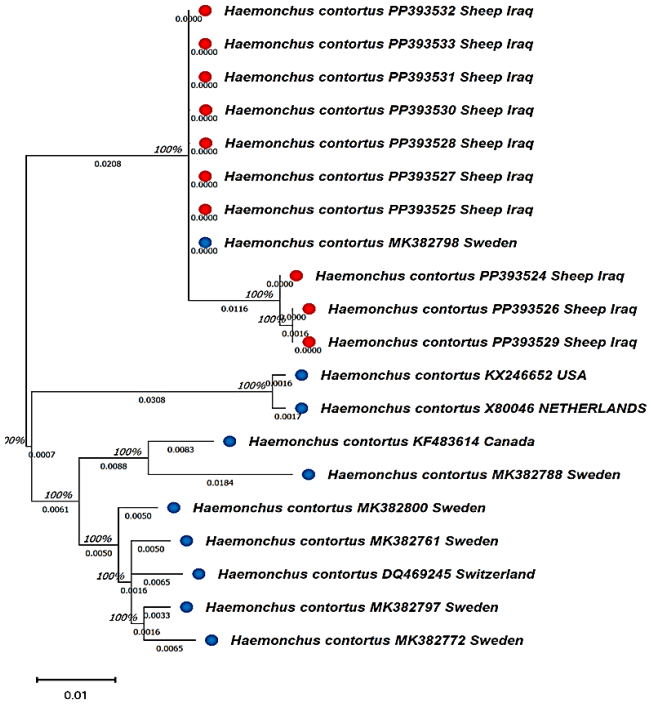

Figure 2: Phylogenetic tree comparing the current study isolates and world isolates from GeneBank based on the sequencing of the β-tubulin gene of *Haemonchus contortus* in sheep.

Discussion

The current study was focused on detecting the resistance gene β-tubulin using PCR and sequencing. The results detected a wide genetic diversity and a high degree of population with demographic implications. For the phylogenetic analyses, identical and evolutionary relationships demonstrated a close similarity between the current study isolates and some isolates deposited in the GeneBank, such as those from Sweden (22-26).

These results suggest the parasite is active in transmission between countries and continents. The higher genetic similarity between spatially distinct populations may indicate shared ancestry or recent genetic exchange between Iraqi and Swedish isolates. In addition, livestock trade and the movement of migratory wild ruminants may play a major role in such transmission. The findings of the current study revealed that molecular methods are potential tools for better understanding the epidemiology and evolutionary dynamics of parasitic infections (26-31).

These results reinforce the need for international collaboration in monitoring and controlling parasitic infections since the genetic relationships of *H. contortus* populations can inform targeted interventions to limit the spread of this economically important parasite. Additionally, comparative genomic approaches might identify conserved genomic regions or common selective pressures shaping the evolution of *H. contortus* populations globally (32-37). The PCR results agreed with Roeber *et al.* (38), who reported detecting gastrointestinal nematodes in small ruminants (39,40).

Alubadi and Alfatlawi (41) reported the presence of *H. contortus* in 49/63 (77.8%) in Al-Diwaniyah City. Amana and Alkhaled (42) found that the parasite has diverse chromosome characterization of heterozygous at 31.11%, homozygous at 57.77%, and homozygous, which is a resistant genotype, at 11.11%. Moreover, Kandil *et al.* (43) found that condensed tannins, a *Medicago sativa* seed extract on *H. contortus*, were effective against the parasite *in vitro* and *in vivo* by fecal egg count reduction test (FECRT). In addition, Shehab and Hassan (44) found that albendazole, Levamisole, Oxyclozanide, and Ivermectin were effective against GIT nematodes at 84, 87, and 95%, respectively, as detected using FECRT. Furthermore, Hade *et al.* (45) found that their isolates were closely similar to isolates from Germany, New Zealand, and Austria at 94, 94, and 93%, respectively. Hadree *et al.* (46) investigated GIT parasites in buffalos and found that Nematodes, Cestode, and Trematode were at 85%, 10%, and 5%, respectively. Duly *et al.* (47) mentioned that microtubules are essential in different cell machinery, such as transport, motility, and mitosis.

Conclusion

The data reveals essential information about this gene's genetic evolution and presents critical data about drug resistance development in the current study isolates of *Haemonchus contortus*.

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Conflict of interests

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

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الكشف الجزيئي عن الجين المشفر لبروتين بيتا توبولين في طفيلي قطب الحلاق في الأغنام في محافظة القادسية، العراق

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الخلاصة

يعد بروتين بيتا تيوبيولين عنصرا مهما في مقاومة الطفيليات ضد أدوية الديدان المستخدمة على نطاق واسع. تواجه العديد من دول العالم تحديات كبيرة بسبب فشل الأدوية المضادة للديدان بسبب المقاومة في القضاء على العدوى الطفيلية في الحيوانات المختلفة وخاصة الأغنام. لذلك، هناك حاجة كبيرة لزيادة مستويات الأبحاث التي تركز على مكونات المقاومة في الطفيليات، وخاصة بيتا توبولين. بناء على ذلك، أجريت الدراسة الحالية للتعرف على الجين بيتا تيوبولين ودراسة التطور الجيني لهذا الجين المسؤول عن تخليق بروتين بيتا تيوبولين في طفيلي قطب الحلاق في الأغنام. هنا، تم استكشاف ٢٥٠ خروفا مذبوحا، وتم جمع ٢١ دودة خيطية واستخدامها في الفحص المجهري (١٠ دودة) عن طريق وضع كل دودة على شريحة زجاجية واستكشافها تحت المجهر الضوئي بتكبير ١٠٠. علاوة على ذلك، ١١ دودة أعدت للفحص بالطرق الجزيئية، وفحص تسلسل القواعد النيتروجينية. أظهر الكشف المجهري تحديدا إيجابيا للدودة. وكشفت النتائج عن تضخيم المنطقة الجينية في ١١ دودة. وأظهر فحص تسلسل القواعد النيتروجينية التعرف على ١٠ عزلات متشابهة إلى حد كبير مع عزلات من السويد بنسبة تشابه وصلت إلى ٩٨%. تشير بيانات الدراسة إلى وجود كبير لجين بيتا توبولين، والذي قد يكون مسؤولا عن مقاومة الدواء والكشف عن وجود معلومات مهمة حول التطور الوراثي لهذا الجين الذي يقدم بيانات مهمة حول تطور مقاومة الأدوية في عزلات الدراسة الحالية من طفيلي قطب الحلاق.