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Morphological and Molecular Detection of *Oestrus ovis* Isolated from Goats in Babylon Province, Iraq

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Oestrosis caused by *Oestrus ovis* is a very impactful illness for the small ruminant industry in Iraq, resulting in substantial economic losses. The present study was conducted to characterize *Oestrus ovis* of Larvae morphologically and molecularly from goat at a slaughter house in Babylon province, Iraq. The study relied on the collection of 50 larvae at various developmental stages between November 2023, and March 2024. The morphological analysis was conducted with a stereomicroscope depend on distinct larval traits such as the dorsal and ventral position, spiracles, hooks, and cephalopharyngeal skeleton. The molecular characterization involved PCR and PGS techniques on the COX1 gene, focusing on a 330-bp section. In terms of morphology, the L1 larvae had unique mouth hooks, while the L2 larvae showed noticeable terminal stigmas. The most important traits of the L3 were the colour of their body parts and the existence of the spines. PCR analysis showed successful amplification at 330bp regions in 12 isolates. The PGS analysis found 12 unique local isolates that have genetic similarities with isolates from Spain and Czech Republic. In conclusion, this study highlights the significant important of *Oestrus ovis* in goats in Babylon province, Iraq.

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Keywords: Goat; Oestrus ovis; PCR; larvae; babylon; COX1 gene.

1. INTRODUCTION

Oestrus ovis parasites are extensively researched larvae that are widely distributed across the globe. The nasal cavities and sinuses was impact by the larvae [1] causing myiasis in goats and sheep, namely the frontal and maxillary sinuses of sheep, goats, and a variety of wild ruminants. This results in the development of oestrosis, a disease caused by these larvae [2,3]. The oviparous insect females form large swarms and deposit their many eggs around the nostrils and in the eye sockets of the animal's head [4,5]. The eggs of *Oestrus ovis* are fertilized, developed, and hatched within a female and the larvae traverse the nasal cavity and sinuses [6-9] the larvae finish their maturation stage, return to the nasal cavity, and are expelled through sneezing [10,11] persistent sneezing, and nasal discharge (seromucous or purulent) are symptoms that infected goat may exhibit [12,13]. Rapidly after the L1 deposition, the migration of *O. ovis* larvae in the nasal cavities of the host induces an inflammatory response due to the parasite hooks and spines [10,14,15]. oestrosis is a parasite disease caused by the *O. ovis*. It affects humans in rural areas who live near infected ruminants and causes ophthalmomyiasis, respiratory infections, and other complications [16,17,18]. Reports of oestrosis have come in from all over the globe, including many Asian nations as well as European and African nations bordering the Mediterranean [19]. From an epidemiological perspective, sheep and goats worldwide are infested by the disease has a substantial impact on the Iraqi small ruminant industry, resulting in enormous economic losses [20-22] the control of this condition depends on treatment with macrocyclic lactones and nitroxinil Reinfections are common, and controlling nasal bots is not so simple [23-25]. The present study aimed to conduct morphological and molecular characterization of *O. ovis* larvae obtained from goats at a slaughterhouse in Babylon city, Iraq.

2. METHODOLOGY

Sample collection and study area.

50 larvae samples obtained from goat in slaughterhouse in Babylon Governorate throughout the period from November 2023 to March 2024, in Babylon city. with different age (<one years, 1-3years, > three years).

2.1 DNA Extraction

Initially, about 20mg of larval tissue was placed in individual Eppendorf tubes for lysis and extraction with a DNA extraction kit from Macrogen in Korea. Based on the kit's guidelines, 200µl of lysis solution and 20µl of proteinase k (20 mg/ml) were included in every tube. The tubes' tissues were disrupted through pestle-based homogenization, followed by an incubation at 56°C for 3 hours to ensure complete lysis. After that, the tubes were spun in a centrifuge at 5000 revolutions per minute for 2 minutes. Afterwards, the remaining processes of the kit were finished. The NanoDrop spectrophotometer was used to measure the concentration and purity of the extracted DNA.

2.2 PCR Processing

A Semi-nested PCR technique was employed for molecular characterization, specifically to examine the COX1 gene at a segment of 300-bp. The approach used for the semi-nested PCR was based on the method by Ipek and Altan [26], with a forward oligo (UEA7: 5' TACAGTTGGAATAGACGTTGATAC 3') and a reverse oligo (UEA10: 5' TCCAATGCACTAATCTGCCATATTA 3') used at 0.5pmol/20µl concentrations. The master mix from ADDBIO in Korea was mixed with the DNA sample. The thermocycler parameters included an initial denaturation stage at 95ºC for 5 minutes, followed by 34 cycles of denaturation at 95ºC for 40 seconds, annealing at 57.7ºC for 30 seconds, and extension at 72ºC for 40 seconds, with a final extension at 72ºC for 5 minutes. In the second amplification round, the same conditions as round-1 were used, with an internal forward primer (UEA9) (5' GTA AACCTAACATTTTTTCCTCAA CA 3') at a 60ºC annealing step for a 1µl DNA sample from round-1 amplicons.

2.3 Sequencing of COX1 Gene

For analysis of COX1 gene, the PGS method was used for this area. The agarose gel 1.5% prior to analysis were purified from the.

3. RESULTS

3.1 Morphological Examination

The L1 larvae had unique mouth hooks morphologically, as shown in Fig. 1. The change

in coloration of larvae at various stages is also depicted in the same illustration, with second-stage larvae (L2) having unique terminal stigmas. The main features that set the third-stage larvae (L3) apart were the body segment color and the spines as shown in Fig. 2.

3.2 PCR Amplification in Goat

By T100 Thermal cycler PCR (Bio rad) USA The PCR analysis revealed amplification at a specific DNA fragment size of 320 base pairs in samples 1-12, which were isolates. However, the NS sample served as a negative control. as depicted in Fig. 3.

3.3 Gene Sequencing

The results of phylogenetic tree showed that the local isolates were depicted as red circles, whilst blue circles were used to indicate global isolates (Fig. 4). All isolated strains genetically aligned with those recorded in Spain and Czech respectively.

Fig. 1. Color changes happen based on development and aging. First stage larvae are transparent or white with mouth hooks, second and young third stage larvae are white, while the older third stage larvae are dark black in color

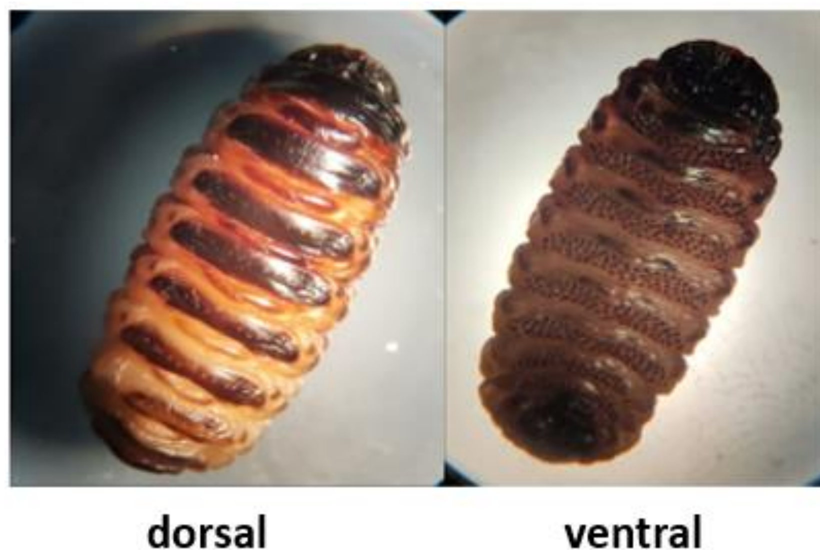

Fig. 2. Larvae3 (L3): The 3 rd instar larvae dorsal and ventral, White or yellowish color turns light brown with a blackish transverse band on the back as it matures

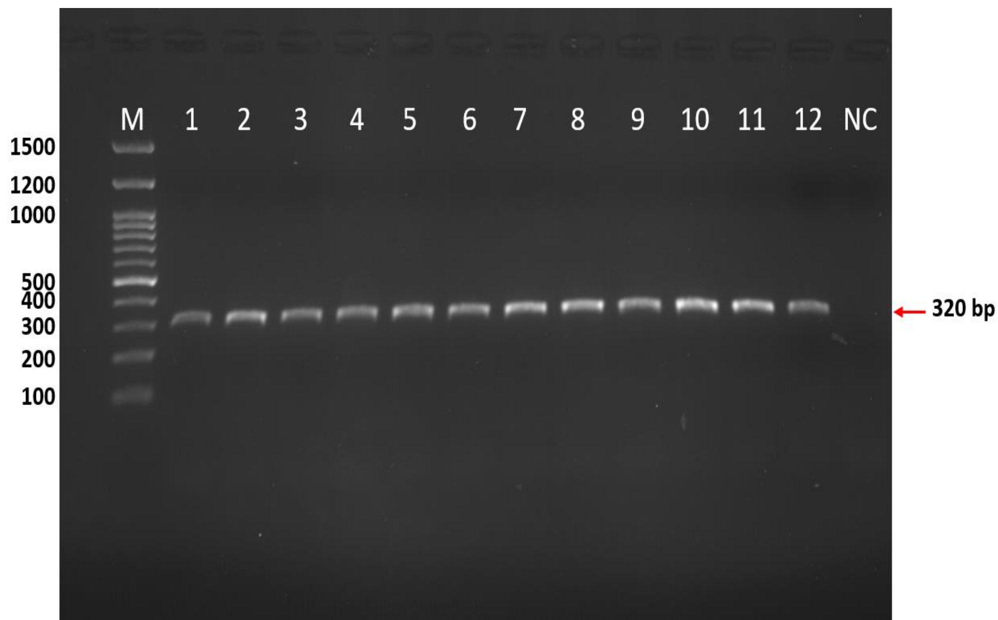

Fig. 3. Agarose gel electrophoresis image (1.5 % agarose) shows positive PCR products (1-12) of *Oestrus ovis* larva isolated from Goat targeting partial region within COX1 gene. M is molecular marker from Genedirex (South Korea). NC is negative control in which similar PCR conditions were used except H2O was added instead of template DNA

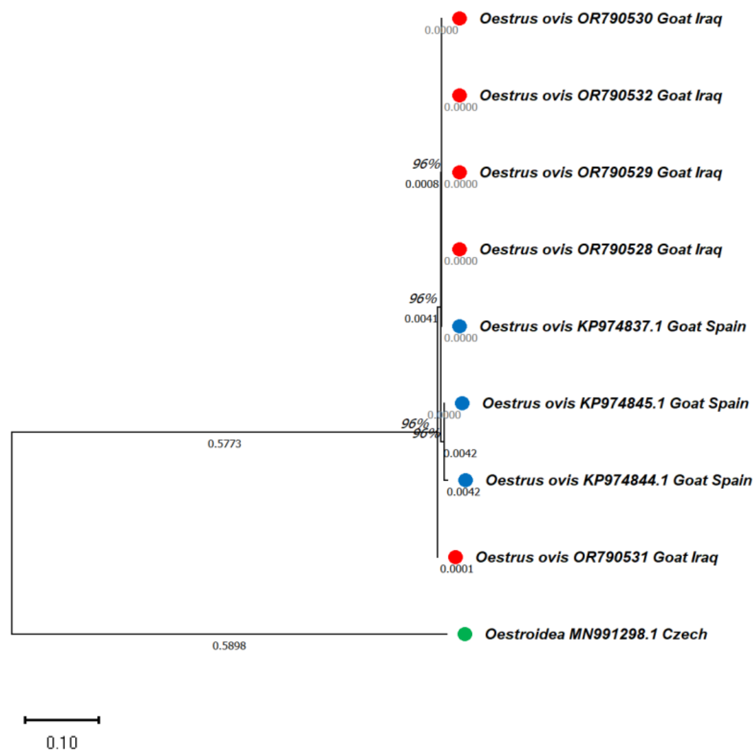

Fig. 4. Phylogenetic evolutionary analysis by Maximum Likelihood method of the identified sequences (COX1) of *Oestrus ovis* in goat

4. DISCUSSION

Oestrosis is widespread in numerous geographic areas. When conducting a survey to determine the actual occurrence rate of this disease, it is crucial to highlight the factors that influence its prevalence. This will enhance the well-being of goats and sheep and lessen the impact of estrus cycle. Vaccination or utilizing other preventive methods is vital in order to reach these objectives [27,28]. Due to scanty knowledge regarding about the occurrence and developmental changes of larvae in Iraq, specifically in Babylon city we performed the present study. A total of 50 larvae of *O. ovis* were isolated from goat heads in Babylon slaughterhouse. By using the microscope and ruler the larvae were observed and measured. The length of these larvae exhibited variability in millimeters. These results exhibit similarities to Al-Ubeid [29]. The number of ventral spines increased as the larvae matured, and the oral hooks became more curved and connected to the cephalo-pharyngeal skeleton. These findings align with the results reported by Moya et al. [30]. The stigma plates are dark brown or black in color and have breathing holes arranged in a radial pattern. These findings align with the *O. ovis* identification described in many studies performed in Iraq [31,28,32,33] found that nasal discharge, nasal obstruction, muco-purulent myasis in frontal sinuses, reduced appetite, and difficulty breathing are some clinical symptoms of *O. ovis* in sheep in Misan city, Iraq. For PCR methods to identify and confirm the species of the larvae. In particular, their focus was on a 330bp segment of the COX 1 gene. Alhayali and colleagues conducted partial gene sequencing in Al-Qadisiya province, Iraq and found a 97% similarity to an isolate in the NCBI-GeneBank. Knowing about goat infestation, especially at the beginning of winter, is crucial to avoid infestation. Furthermore, it is advised to implement a seasonal treatment in the month of April for complete prevention according to Hanan [34]. Meanwhile, Ipek and Altan [26] discovered larvae in 38.5% of goats and 84.2% of sheep in Turkey by examining their morphology. Additionally, semi-nested PCR was used to amplify segments of the COX1 gene that were around 700bp and 300bp in size. Additionally, it was found that the sequences from their sample larvae closely resembled Italian NCBI isolates of the *O. ovis*, with a similarity of 99%. It was determined that the 2-step PCR test outlined in this study displayed a diagnostic sensitivity and specificity of 95% for oestrosis.

5. CONCLUSIONS

This study is considered as a first recorded in Babylon city, Iraq by molecular diagnosis of *Oestrus ovis* by the sequencing of the 28S rRNA gene and registered in Gene Bank. Many epidemiological studies of the parasite in different parts of Iraq specially the middle and south province were needed. Moreover, control the spread of the infection by use effective treatment such as macrocyclic lactones, Endectocide and Fasciolicide against domesticated animals twice a year.

ETHICAL APPROVAL

The locally ethics committee in Al-Qasim Green University reviewed and approved of the study protocol, experimental animals, and consent form according to the document number (Ref. 313, 3.6.2023).

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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