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Molecular identification of *Linognathus* spp. lice infesting sheep and goats in Mosul city, Iraq

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Abstract

Lice infestation is prevalent worldwide and it is one of the most significant veterinary parasitic diseases. This study was applied from August to December, 2022, a total of 25 sheep lice and 25 goat lice were collected. The study based on microscopic and molecular identification by traditional polymerase chain reaction (PCR) technique and sequencing to confirm the *Linognathus* species infesting sheep and goats reared together. Microscopically, the morphological results identified the species infesting sheep and goats were sucking lice belonging to the genus *Linognathus* spp. The morphological characteristics of the adult lice were somehow identical for the same genus and was difficult to identify species, thus using polymerase chain reaction (PCR) and gene sequencing on five samples of sheep and goats, the results confirmed that the species are belonging to *Linognathus africanus* by amplification of mitochondrial cytochrome c oxidase subunit I (COI) gene with reaction product of 379 bp that were isolated from the city of Mosul, with the accession numbers 598894PP, PP598895, PP598896, PP598897 and PP598898, genetic tree results revealed similarities with results of other countries recorded in the Global Genbank, 100% with Hungary, 99.44% Mexico, China and the United Kingdom while 99.17% in Mexico, but had a significant distance other strains for different species recorded in the United Kingdom, China, Canada and Australia with percentages 77.81%, 77.78%, 77.5% and 76.94 respectively.

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Introduction

Small ruminants (sheep and goats) are infected with different parasitic diseases either internal infections like GIT helminths (1-3) protozoal disease (4,5) or external infestations with flies ticks and lice (6). Pediculosis is an ectoparasitic disease threatening both human and animals either through their feeding habits (blood sucking and chewing) or by their ability in transmitting diseases. The majority of the 5000 identified species of lice are infesting wild birds and mammals (7). Lice affect the productivity and growth of sheep, goats, cattle and buffalo herds (8). Phthirapterosis is an important veterinary problem and has a vital role in threatening the animal industry leading to serious economic losses (9). The direct effects are anemia due to blood loss, skin irritation and inflammation as well as allergic response due to toxic effects resulting in self-mutilation of pruritic animal (9,10); whereas the indirect effects are anxiety, restlessness and loss of appetite, reduce in weight, decrease in milk production, changes in normal behavior such scratching and the discolored, greasy wool and hair. Heavy lice infestations cause alopecia, especially on young or older poorly-health animals or those kept in unhygienic places (11). Lice identification is depended on the gross morphological characteristics by dissecting microscope accompanied with standard economic keys (12). The genus *Linognathus* (blood sucking lice) infests sheep and goats which is mainly caused by the species *Linognathus africanus* (African sheep louse); while the two species *Linognathus ovillus* (long-nosed sheep louse) and

Linognathus pedalis (sheep foot louse) infest sheep whereas *Linognathus stenopsis* infests goats. Chewing (biting) lice infesting sheep and goats is *Bovicola* (*Damalinia*), the species *Damalinia ovis* infests sheep while the two species *Damalinia caprae* and *Damalinia limbata* infest goats (8,13,14).

The aims of the study were to identify *Linognathus* species of lice infecting sheep and goats reared together based on morphological characteristics using dissecting microscope and confirming species of lice using polymerase chain reaction technique and sequencing.

Materials and methods

Ethical approve

The institutional animal care and use committee in the College of Veterinary Medicine, University of Mosul, ethically permitted this study (UM.VET.2022.088) on 15/7/2022.

Sample collection and microscopical identification of lice

A total number of 50 lice (25 sheep, 25 goats) were collected from August to December 2022 by brushing using lice-comb and kept in 70% ethyl Alcohol in plastic containers until examined in the lab (15). Lice were carefully examined using dissecting microscope to determine the major characteristics of length, width, number of antennae segments, paratergal plates and color depending on (8).

Extraction of DNA

A commercial kit (AddPrep Genomic DNA Extraction Kit, Addbio, Korea) was used to extract lice DNA according to the manufacturer's instructions. Briefly, 20 mg of lice was placed in 1.5ml Eppendorf tube containing 200µl of lysis buffer. A twenty microliter of proteinase K solution (20 mg/ml) was added to each tube and then incubated at 56◦C with mixing by vortexing until the tissue was completely lysed. Finally, the DNA was eluted into 1.5 ml microcentrifuge tube by adding 100 µl of elution buffer and centrifuged at 13,000 rpm for 1 min. The obtained DNA was kept at -20ºC until extra assay.

Measurement of DNA purity

The purity of DNA was assessed using NanoDrop (ThermoFisher, USA). A ratio between 1.7 and 2.0 indicates the pure DNA in the preparation (16).

Amplification and gel electrophoresis

The PCR was applied to amplify cytochrome c oxidase I (COX1) of lice. A 25 µl PCR reaction consisted of 2µl of DNA, 12.5µl of 2x AddTaq Master Mix (AddBio Inc., South Korea), one microliter of forward (5'-CCG GAT CCT TYT GRT TYT TYG GNC AYC C-3') and reverse (5'-CCG GAT CCA CAN CRT ART ANG TRT CRT G-3') primers (17), and 8.5 µl of PCR grade water. Amplification was carried out using Bio-Rad thermocycler (Bio-Rad, USA) under the following conditions: one cycle at 95°C for 10 minutes, followed by 35 cycles at 95°C for 45 seconds, 58°C for 45 seconds, and 72°C for 45 seconds. Then, for final extension, one cycle at 72°C for 5 minutes was set. Finally, the reactions were cooled at 4°C until proceeding to the gel electrophoresis. In a 1.5 % agarose gel prepared with 1x Tris-Borate-EDTA buffer and stained with a red safe DNA staining solution (GeNetBio, South Korea), the amplified products were verified. The results were visualized using UV transilluminator and digital camera (Bio-Rad, USA). In all electrophoresis performed, DNA molecular weight marker 100bp (AddBio Inc., South Korea) was introduced. The presence of specific amplified DNA fragment with 379bp indicating a positive result for lice.

DNA Sequencing

The PCR products of positive samples were sent to Macrogen, Korea for sequencing using Sanger sequencing method. Briefly, 20 µl of PCR product of the target gene was sent with the corresponding primer. The results of sequencing were obtained as FASTA format text files.

Results

The microscopical results showed species of lice infesting sheep and goats are sucking lice *Linognathus* spp. the morphological features of adults are 0.75 mm width and 2.2 mm length, bluish-black with a prolonged narrow head with slender body, first pair of legs is smaller than the second and third pair (Figure 1). The results also showed that lice species found in goat is the sucking lice *Linognathus* spp. according to the following morphological features: adults are 0.7 width and 2.14 mm length, blue to black, head narrow, long and elongated body has five segmented antennae and one claw in each leg as shown in (Figure 2). The extracted DNA purity of lice was found to be ~1.83 and the amplification results of COI gene revealed that lanes 1-9 represent positive samples for lice from sheep, 10 negative control while lanes 11-19 are lice for goats and bp being 379 as in (Figure 3). The extracted DNA purity of lice was found to be ~1.83 and the amplification results of COI gene revealed that lanes 1-9 represent positive samples for lice from sheep, 10 negative control while lanes 11-19 are lice for goats and bp being 379 as in (Figure 3). The results of the sequences COI gene showed that the species of lice are *Linognathus africanus* with the accession numbers PP598894, PP598895, PP598896, PP598897 and PP598898 had a significant distance to other species recorded in different countries when matched with the World Gene Bank, gave 100% match with species of Hungary and 99.44% of Mexico, China and United Kingdom and 99.17% of Mexico (Table 1 and Figure 4).

Figure 1: Sheep sucking lice *Linognathus* spp., male, 25X.

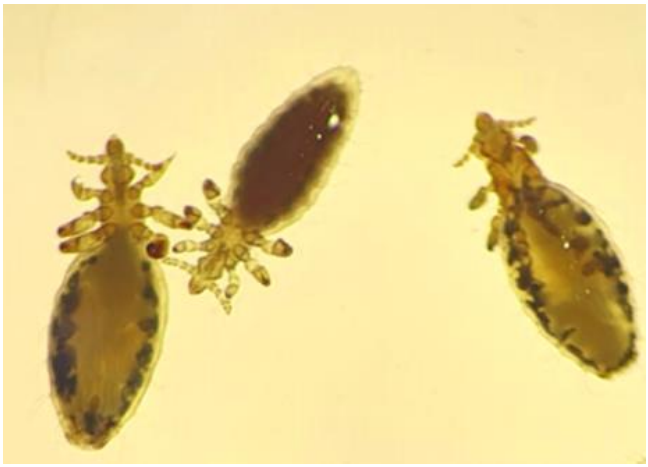

Figure 2: *Linognathus* spp. (sucking lice in goat), males, 25X.

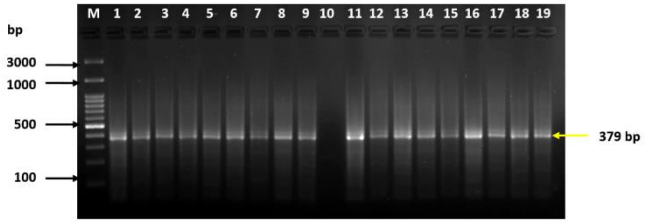

Figure 3: Polymerase chain reaction (PCR) of COI gene for lice. Lane M represents 100 bp DNA marker. Lanes 1-9 are positive samples for lice from sheep, Lane 10 negative control, and Lanes 11-19 are positive samples for lice from goats.

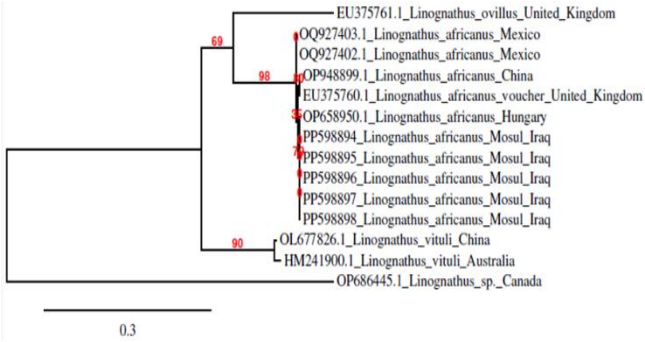

Figure 4: Phylogenic tree of *Linognathus* lice from Iraq. The phylogenic tree was constructed using the Maximum Likelihood method based on the Tamura-Nei model in MEGA11 software and bootstrap analysis with 1000 re-samplings. Partial DNA sequences of partial COI gene were used as input data.

Table 1: Sequence identity between local *Linognathus africanus* louse species and others have recorded in the GeneBank

Species	Gene name	Accession number	Country	Identity
Linognathus africanus isolate MAFLY24	COI	OP658950.1	Hungary	100%
Linognathus africanus isolate LACH002	COI	OQ927403.1	Mexico	99.44%
Linognathus africanus	COI	OP948899.1	China	99.44%
Linognathus africanus voucher GLA.M06	COI	EU375760.1	UK	99.44%
Linognathus africanus isolate LACH001	COI	OQ927402.1	Mexico	99.17%
Linognathus ovillus voucher GLA.M09	COI	EU375761.1	UK	77.81%
Linognathus vituli	Chromosome 4 (M)	OL677826.1	China	77.78%
Linognathus sp. n. LPS-2023a voucher Nunavut-4	COI	OP686445.1	Canada	77.5%
Linognathus vituli clone	COI	HM241900.1	Australia	76.94%

COI: Cytochrome c oxidase subunit I. M: mitochondrion.

Discussion

The current study was conducted, for the first time, to present and discuss the reason for increasing problem of Pediculosis in sheep and goats. Sucking lice in the suborder Anaplora have hematophagous feeding and the genus *Linognathus* is considered the most important one infesting sheep and goats (18). They cause damage through their bites as well as their ability to suck blood from the host in heavy infestation leading to anemia, weight loss, reduce in meat and milk production. Veterinary authorities, veterinarians and farmers pay more attention to diseases with high morbidity and lethality such as foot and mouth disease, brucellosis or tuberculosis (19,20). On the other hand,

endemic parasitic diseases such as external parasites, GIT and blood parasite infections, which induce lower losses but may concern a high percentage of the population and may last for several years sometimes the whole life of the animal are often neglected (21). However, lice burdens differences differ according to many reasons like immunity, nutrition and the condition health of individual animals, breed, rearing system, and having a healthier condition are the major risks affecting the incidence and spreading of lice among goats (22-24).

The current study also concluded identifying the lice species infesting small ruminants. Microscopically, the study identified the sucking lice *Linognathus* spp. in sheep and goats on morphological identification between species is very difficult and complicated at least on morphology on genus level only due to similarities between members of this species, thus more accurate methods were needed for identifying species depending on the PCR and sequence level to determine the specific species for this infestation to apply the appropriate treatment strategy. A study in Mosul city was conducted on *Linoganthus stenopsis* in goats (21). Researchers in Kurdistan- Iraq reported the blue lice in sheep infested with *Linognathus africanus* 35%, and 24.11 with *Damalina ovis* (25). In goats, *Damalinia caprae* 80% and *Linognathus africanus* 30%. Other studies in Iraq determined five species of sheep lice in Mosul, *Linognathus africanus* 0.2% and *Damalina ovis* 6.4% (26), whereas Zangana *et al.* (27) described sheep infestation with 75% with *Damalina ovis* and 33% with *linognathus stenopsis*, while goats' infestation with the same parasites was 80% and 19%, respectively. Mustafa (26) reported two lice species in sheep namely *Damalina ovis* 17.7%, *Linognathus stenopsis* 13.6% and two species of goat infestation, *Damalina caprae* 10.9% and *Linognathus stenopsis* 6.2%. A study of goat external parasites in Erbil, Iraq. Pols and Mawlood (28) diagnosed *Damalina caprae* and *Linognathus africanus* from goats. Lice identification is mainly based on animals from which lice were isolated (29).

For lice and other Arthropoda, it may be limited by the safety of the samples during transport and collection due to their fragility and absence of immature stage distinctive morphological criteria such as in tick (30,31). Molecular technique that considered as an alternative and confirmation method has been developed in identifying lice based on analyses of gene sequence 18s rRNA or COI gene commonly used for lice identification (32). The morphological identification low-cost tool but difficult because the species are morphologically similar (31). The information of the database of NCBI GenBank is scarce and less comprehensive concerning animal lice gene sequences (33). At the molecular level by using PCR technique for the detection and confirmations of lice infecting small ruminant by using COI gene, the amplification results using Cox1 gene was positive for lice from sheep and goat; and bp was 379. The results of the current study revealed that the lice species detected by PCR technique is *Linognathus africanus* in sheep and goat. PCR technique is more specific and sensitive than the microscopical study since the complexity of last one at the level of species identification (34).

Sequencing of COI gene determined the species of lice detecting *Linognathus africanus* in Mosul city significantly different from other species determined in different countries by matching with the World Gene Bank. The species *Linognathus africanus* accession numbers PP598894, PP598895, PP598896, PP598897 and PP598898 match 100% Hungary accession number (OP658950) and 99.44% Mexico accession number (OQ927403), China and United Kingdom accession number (OP948899 and EU375760) and 99.17% Mexico accession number (OQ927402), while had a significant for different species recorded in the United Kingdom, China, Canada and Australia with percentages 77.81, 77.78, 77.5 and 76.94, respectively, these high rates of gene similarities or differences between local and foreign isolates recorded in the GenBank are due to many variables such as host immune condition and type of nourishment (35,36), sex, age, breed (37) geographical variability in different regions of the world, climate and environmental changes affecting hygiene (38) and parasitic biology have a significant impact (39). The current study revealed that the molecular tools are very important in uncovering the real reason of lice infestation in sheep and goat with *Linognathus africanus* which is originally named sheep louse but became capable to infect goat due to keeping both animals in the same place and could be the main reason of heavy pediculosis in sheep and goat.

Conclusion

Microscopically, the identification at the species level is very difficult and complex due to morphological similarities for the same genus, therefore molecular tools were needed to confirm the species of infesting lice. The current study confirmed that the blood sucking lice species is *Linognathus africanus* by PCR by amplification of COI gene and sequencing. This species exists in mixed reared sheep and goats and has the ability to infest both animals.

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Conflict of interests

Researchers declare that they have no conflicts of interest regarding the publication of this research.

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التشخيص الجزيئي لقملة الضأن الزرقاء الأفريقية الأغنام والماعز في مدينة الموصل، العراق

مصطفى سالم النعمة و نادية سلطان الحيالي

فرع الأحياء المجهرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

ينتشر التقمل في جميع أنحاء العالم وهي واحدة من أهم الأمراض الطفيلية البيطرية. تم إجراء هذه الدراسة خلال الأشهر من آب حتى ديسمبر ٢٠٢٢. تم جمع ٢٥ عينة من الأغنام و٢٥ من الماعز. اعتمدت الدراسة على الفحص المجهري والجزيئي باستخدام تقنية تفاعل البلمرة المتسلسل وتتابع الجينات لتأكيد النوع التابع لجنس لينوكنيثوس الذي يصيب الأغنام والماعز التي تربى في نفس الحضائر. أظهرت نتائج الدراسة الشكلية المجهرية أن الأنواع التي تصيب الأغنام والماعز كانت من القمل الماص جنس لينوكنيثوس. حيث كانت الصفات الشكلية والقياسية للبالغة متشابهة الى حد ما بحيث يصعب تحديد الأنواع لنفس الجنس وباستخدام تفاعل البلمرة المتسلسل وتعاقب الجينات لخمس عينات من الأغنام والماعز أكدت النتائج أن النوع التابع لهذا الجنس الذي يصيب الأغنام و الماعز هو قملة الضأن الزرقاء الأفريقية بتضخيم جين (COI) وبناتج تفاعل ٣٧٩ زوج قاعدي والتي تم عزلها من مدينة الموصل، وبأرقام تسلسلية PP598894 وPP598895 وPP598896 وPP598897 وPP598898 ، حيث بينت نتائج الشجرة الجينية تقارب مع نفس النوع المسجلة في بلدان مختلفة عند مطابقتها مع بنك الجينات العالمي حيث تتطابق بنسبة ١٠٠٪ مع المجر و٩٩,٤٤٪ مع المكسيك والصين والمملكة المتحدة و٩٩,١٧٪ مع المكسيك في حين أظهرت تباعدا مع الأنواع الأخرى المسجلة في المملكة المتحدة و الصين و كندا و أستراليا وبنسب ٧٧,٨١ و ٧٧,٧٨ و ٧٧,٥ و ٧٦,٩٤% على التوالي.