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PREVALENCE AND MOLECULAR ANALYSIS OF CATTLE CHEWING LICE *BOVICOLA BOVIS* IN MOSUL, IRAQ

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ABSTRACT

Lice are small wingless permanent obligate external parasites, which cause different economic losses, such as alopecia, pruritus and weight reduction. This study was done in the period from January - December 2022 by collecting lice samples from different cattle herds located in different areas. Microscopically, the total lice infestation rate of 40.37% (220/545) was from the chewing lice *Bovicola bovis*. According to some risk factors, such as seasons, gender and breeds, a higher infestation rate was recorded in winter 56%, in males' group 66.7%, and the Georgian foreign breed was higher 45.3%, compared to local breeds. Whereas there are no significant differences among age groups. Concerning the area of study, Izhilila 50% and Bazwaya 46.42% showed a higher infestation rate, compared to other areas. Molecular analysis, using the traditional polymerase chain reaction (PCR), confirmed that the species isolated from cattle in Mosul city was the chewing *B. bovis,* by amplification of the Cox1 gene, with an end product of 379 bp. The results of gene sequences confirmed the PCR findings, with the accession number PP599070, which matches 100% of the USA isolate and 94.33 to 98.29100% identity with a significant distance from the isolates of the same species in Australia recorded in the World GenBank. This is the first study at the molecular level to confirm the infestation of cattle with the species *Bovicola bovis* in Mosul, Iraq.

Keywords: Chewing lice, cattle, PCR, Cox1 gene, risk factors.

INTRODUCTION

Pediculosis is an ectoparasitic disease that threatens both humans and animals either through their feeding habits (blood sucking and biting) or by their ability to transmit diseases. The majority of the 5000 identified species of lice infest wild birds and mammals (Shao *et al.,* 2015; Durden, 2019). Lice are apterous insects that host specific skin. They complete their entire life cycle: egg, three nymphal stages, and the adult on the body of the host, feeding chiefly on skin and skin products. Cattle can be affected by both chewing and sucking lice. Chewing lice are small, dorsoventrally flat insects. *Suborder Ischnocera* is one of four important suborders belonging to Order Phthiraptera. Ischnocera known as Mallophaga, chewing or mandibulate lice, which includes the family Bovicolidae includes the genus *Bovicola* (earlier Damalinia) infesting ruminants (Mehlhorn, 2016; Mullen and Durden, 2019; Taylor *et al.,* 2016; Urquhart *et al.,* 2007).

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Mild lice infestation does not have a significant economic loss, whereas heavy infestations may lead to many clinical signs due to the irritation caused by lice, where lousy cattle bite, rub against fences and scratch their skin, leading to hair loss and skin damage. In addition, reduced weight gain, promoting secondary infections and reducing hide value by changing its appearance into rough and scruffy (Cortinas and Jones, 2006; Syamsul *et al.,* 2020). Clinically, chewing lice is easily detected through gross examination. Heavy infestations are more common on older or younger poorly-health animals, especially animals reared in unhygienic conditions (Otter *et al.,* 2003).

Lice species are traditionally identified according to morphological features. Nevertheless, accurate identification of lice species depending on morphological characteristics is hard, because lice are variable and similar in morphology. Thus, researchers have applied molecular markers, including mitochondrial genes and nuclear ribosomal genes. An appropriate genetic marker is important for success in many evolutionary studies, specific characteristics of Cox1 make it an exceptional and suitable marker for evolutionary studies (Lunt *et. al.,* 1996). The current study was organized to investigate the prevalence of lice species associated with some risk factors, such as sex, age, season and area, as well as molecular and phylogenetic tree analysis, using mitochondrial cytochrome c oxidase subunit 1 gene (Cox1) to confirm lice species.

MATERIALS AND METHODS

Sample Collection

This study was performed during the period from Jan. 2020 to Dec. 2022. A total of 545 cattle were inspected. Lice samples are obtained from infested cattle. Data were collected according to sex, age, breed and season from the following areas: Gogjali, Bazwaya, Izhilila and Orta Kharab then preserved in 70% ethyl alcohol and examined under a dissecting microscope. Morphometrical identification keys depended on (Taylor *et al.,* 2016).

Molecular identification of lice

1- DNA Extraction

According to the manufacturer's instructions, DNA of lice was extracted by DNA extraction kit from the tissue mini-kit" (Korea Add bio). The concentrations and purity of extracted DNA were measured using NanoDrop (Thermo Fisher, USA). Finally, DNA was kept at -20 °C until further study.

2- PCR and gel electrophoresis

PCR was performed by amplification of the Cox1 gene of *B. Bovis*. The primers were chosen according to (Hafner *et al.,* 1994) and obtained from Macrogen Co (Korea). A 25 µl total PCR reaction mixture included 8.5 µl grade water, 2 µl DNA, 12.5 µl 2x Taq Master Mix (Korea Addbio), one µl of each forward (5'-CCGGATCCTTYTGR-TTYTTYGGNCAYCC-3') and reverse (5'-CCGGATCCACNACRTARTANGTRTCR TG-3') primer (Hafner *et al.,* 1994). PCR amplification was done with the following conditions: 1 cycle at 95°C for 10 minutes, followed by 35 cycles for 45 seconds at 95°C, 45 sec. at 53°C, 1 min at 72°C. Final extension was then set for 1 cycle of 72°C for 5 minutes. At 4°C, the processes were finally cooled. About 1.5% agarose gel was set with 1x Tris-Borate-EDTA "buffer with a red safe DNA staining solution" was used to verify the amplified products. Then PCR product was separated through gel electrophoresis by 1.5% agarose gel mixture with Gel Red stain 3 µl containing a 1X TBE buffer. Then, electrophoresis was performed. The DNA ladder of 100 bp, 5 µl is used. Then we examined the gel under UV light using (USA, Gel Doc EZ Image, Bio-Rad) for verification and determination of the probable band.

DNA Sequencing

PCR product for the positive samples were sent to Macrogen, Korea to be sequenced through Sanger method. A 20µl of PCR product belonging to the Cox1 gene of B. Bovis was sent with the primer. Results were obtained in the form of FASTA t text files.

Statistical analysis

The Chi-square test was carried out using the SPSS V25 software, considering all the data had been analyzed, and setting the significance at P≤ 0.05 (Petrie and Watson, 2013).

RESULTS

Microscopical examination under dissecting microscope has identified the lice species found in cattle, which was the chewing lice *B. bovis* (Figure 1).

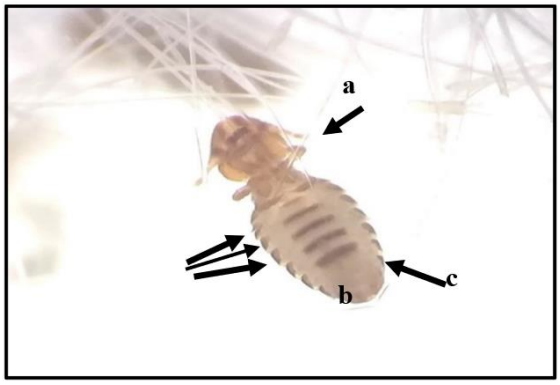

Figure 1. Cattle chewing lice *B. bovis*, (a) antennae, (b) Transverse band, (c) Paratergal plates, 25X.

The total recorded infestation rate was 40.37 % (545/220). Highest infestation rate was recorded in the winter months: 56% in January, February and March, 55% in the autumn months: October, November, and December, 39.29% in spring months April, May, and June, and the lowest rate was in summer: Juli, August, and September 22.2% at P≤ 0.05. According to age, the study documented a 42% rate in less than 3 years, while 38.9% in more than 3 years, with no significant difference. Concerning sex, the study indicated that the infestation rate in males was 66.6% higher than in females 14.5% at P≤ 0.05. Breed risk factor was the highest in Georgian 45.3%, compared with local and Turkish breeds 35.2% and 36.36%, respectively. Area risk factor was higher in Izhilila 50% as well as in Bazwaya 46.4%, while the lowest was recorded in Orta Kharab "25% as shown in Table (1). Molecular analysis results showed that 20 of 220 lice samples were positive for the Cox1 gene amplification. The amplified product was 379 bp. The lanes (1-9) contained different samples, and lane (10) was the negative control (Figure 2).

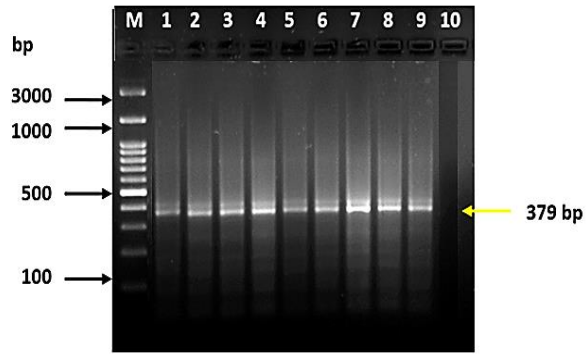

Figure 2: Polymerase chain reaction of Cox1 gene. Lane M stands for100 bp DNA marker. Lanes 1-9 are positive samples for chewing lice from cattle, Lane 10 negative control.

The local isolate of *B. Bovis* with the accession number (PP599070) identified by sequence analysis of the Cox1 gene matched 100% with USA isolate and between 94.33-98.29% with a significant distance, with the isolates of the same species in Australia recorded in the World GenBank and as shown in Table (2).

Table 1: Infestation rates of cattle's chewing lice according to risk factors.

Risk factors		No. of cattle examined	No. of cattle infested	Percentage
seasons	Winter	125	70	56% [a]
	Spring	140	55	39.29% [b]
	Summer	180	40	22.2% [c]
	Autumn	100	55	55% [a]
age	Less than 3 years	250	105	42%
	More than 3 years	295	115	38.9%
sex	Males	270	180	66.6% *
	Females	275	40	14.54%
breed	Local	170	60	35.2%
	Turkish	110“	40	36.36%
	Georgian	265	120	45.3%*
area	Gogjalii	305	115	37.7% [b]
	Orta Kharaab	40	10	25% [c]
	Bazwayaa	140	65	46.4% [a]
	Izhiilila	60	30	50% [a]
Total		**545**	**220**	**40.37%**

Different letters between the percentages of infestation indicate significant differences at P≤0.05.

Table 2: Sequence identity between local *Bovicola bovis* isolate MN-B-M24 louse species (PP599070) and others recorded in the GenBank.

No.	***Bovicola bovis* isolate MN-B-M24 louse species**	Gene name	GenBank Accession No.	Country	Sequence identity
1	*B. bovis* voucher Bobov Botau	Cytochrome oxidase subunit I	AF545680.1	USA	100
2	*Bovicola bovis* isolate minichromosome 9 mit.	Complete sequence	MH001197.1	Australia	98.29
3	*Bovicola bovis* isolate minichromosome 4 mit.	Complete sequence	MH001192.1	Australia	96.29
4	*Bovicola bovis* isolate minichromosome 10 mit.	Complete sequence	MH001198.1	Australia	95.77
5	*Bovicola bovis* isolate minichromosome 7 mit.	Complete sequence	MH001195.1	Australia	95.48
6	*Bovicola bovis* isolate minichromosome 2 mit.	Complete sequence	MH001190.1	Australia	95.47
7	*Bovicola bovis* isolate minichromosome 6 mit.	Complete sequence	MH001194.1	Australia	94.93
8	*Bovicola bovis* isolate minichromosome 8 mit.	Complete sequence	MH001196.1	Australia	94.92
9	*Bovicola bovis* isolate minichromosome 5 mit.	Complete sequence	MH001193.1	Australia	94.92
10	*Bovicola bovis* isolate minichromosome 11 mit.	Complete sequence	MH001199.1	Australia	94.57
11	*Bovicola bovis* isolate minichromosome 12 mit.	Complete sequence	MH001200.1	Australia	94.37
12	*Bovicola bovis* isolate minichromosome 1 mit.	Complete sequence	MH001189.1	Australia	94.33

Mit, mitochondrion

Furthermore, twelve Cox1 gene sequences from different *B. Bovis* isolates were combined into a neighbor-joining phylogenetic tree. These sequences and phylogenetic analysis generally identified clades among the Cox1 gene sequence members (Figure 3). Based on the generated phylogenetic tree, the Cox1 gene is preserved in all *B. Bovis* isolates and is closer to voucher AF545680.1 from USA and MH001195.1 from Australia (Figure 3).

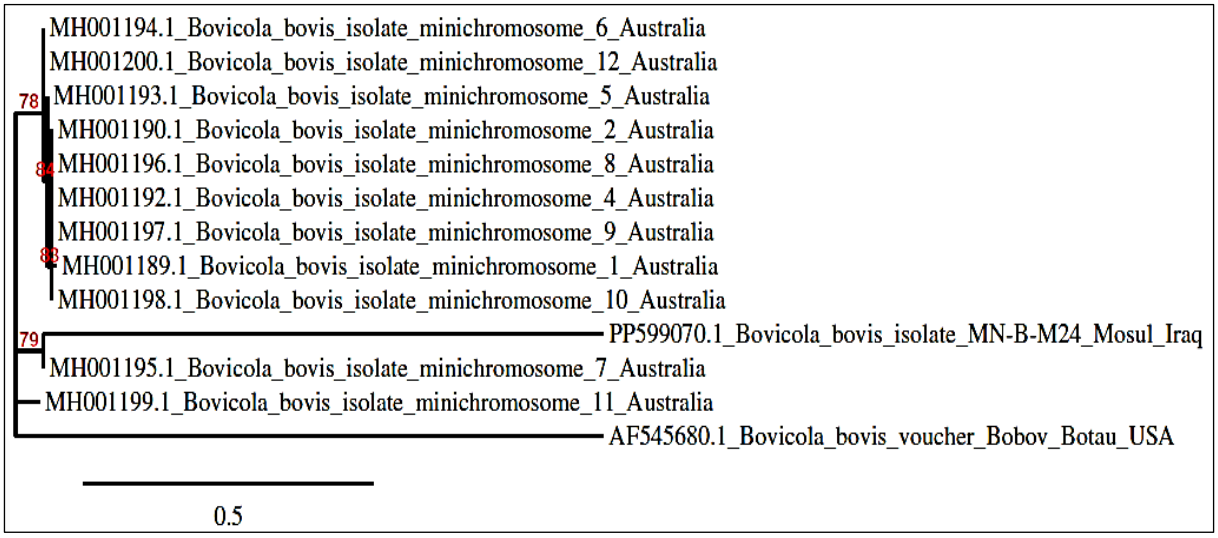

Figure 3: Neighbor-joining phylogenetic tree between local *B. Bovis* isolates PP599070 and other isolates registered inGenBankk.

DISCUSSION

The current study recorded microscopically that the cattle were infested only with chewing lice *Bovicola bovis,* contrary to another study by Gharbi *et al.* (2013), who recorded that cattle were infested with both chewing and sucking lice. Results of other researchers showed higher infection with biting lice from 26.7%-97%, respectively (Colwell *et al.,* 2001; Milnes *et al.,* 2003). The total infestation rate with the chewing lice *B. bovis* was significantly higher 40.37% (220/545) and indicating a real problem in cattle herds, compared with the studies of Meguini *et al.* (2018) and Ouarti *et al.* (2020), who recorded lower rates 27%, 14.3% in cattle in Algeria and Tunisia, respectively (Gharbi *et. al.* 2013; Meguini *et al.,* 2018). However, the findings of this study are lower than another study, where the infestation rate recorded 71.41% in cattle (Ouarti *et al.,* 2020).

Infestation rate differences could be due to different factors, like animals health, feeding system, or breeding of animals (Gustafsson *et al.,* 2018). As well as poor management, malnutrition and coherent infection i.e., viral, bacterial, and parasitic infections (Taylor et al., 2016) and environmental factors, such as climate changes (Yakhchali and Hosseine, 2006).

Rates of lice infestation recorded in this research were higher in the winter season (56%). Infestations are heavier in winter, where reproduction of lice occurs due to heavy coats, different types of malnutrition, stress (Franc, 1994; Taylor *et al.,* 2016), low hygiene practices, abiotic factors, and overcrowding of animals with lice, which prefer cooler skin temperatures. High infestations occur in cattle with poor state, especially when infected with chronic diseases (Gharbi *et al.,* 2020; Eydal and Richter, 2010). In addition, the nature of hair greasiness, depending on the breeds of cattle, is also another important factor that increases susceptibility to infestation. However, lice burdens differ according to different reasons, such as immunity, level of

nutrition, the hygienic condition and rearing system (Gharbi *et al.,* 2020, Dejene *et al.,* 2020).

The study showed no significant differences among age groups less than three years and more than three years, which agrees with (Dejene *et al.,* 2020), who recorded an infestation rate of 40.8% in adult sheep and the lowest percentage of 22.6% in younger ages. Contrary to the results achieved, Dejene *et al.* (2020) recorded the highest infestation rates in small ruminants (50.5%), while in adults realized as 39% and these were attributed to the age of examined animals and the type of animal management (Mullen and Durden, 2019).

In this study, a higher infestation rate in cattle was recorded in males than in females. This may be attributed to importing males more than females for meat production. Concerning breed, the infestation rate was higher in the Georgian breed, 45.3%, due to differences in number of examined animals and that farmers prefer certain breeds (Wall and Shearer, 2001).

According to the area risk factor, the current study recorded a significant difference inside and outside Mosul governorate. High infestation rates have been recorded in Izhilila 50% and Bazwaya 46.4%, while lower in Orta Kharab 25%. These different infestation rates within areas may be for many factors: management, health status, environmental conditions, type of feeding system, change in climate in areas of study, and susceptibility and immunity of animals (Taylor *et al.,* 2016).

The PCR technique for detection and confirmation of lice infesting ruminants is an alternative method to confirm and identify lice species, relying on analysis of the gene sequences Cox1 or 18S rRNA, widely used for lice identification (Light *et al.,* 2010). Another study reported that the Cox1 gene is a good indicator to describe lice of many species, like: *B. ovis*, *M. stramineus*, *B. caprae*, *B. bovis*, *L. africanus*, *C. meleagridis* and *S. capillatus* (Ouarti *et al.,* 2020). The results of the Cox1 gene amplification were positive with 379 bp. High sensitivity and specificity of PCR is more accurate than traditional techniques, which require time and experience at the species level of lice (Kokas and Al-Hasnawy, 2024).

Sequencing results of Cox1 gene proved that the detected species of lice in cattle was *Bovicola bovis* isolated with the accession number PP599070 in Mosul, with significant distance to the same species recorded in other countries when matched, which 100% matches with USA isolate and between 94.33-98.29 and100% with a significant distance with the isolates of the same species in Australia recorded in the World GenBank.

CONCLUSION

Lice are a prevalent issue in cattle in Mosul city, with varying infestation rates depending on sex, age, breed, season, and geographic location. Molecular analysis by amplification of Cox1 gene is considered a good tool to confirm microscopical results at species level identification. This study is considered the first in Mosul and Iraq at molecular level. Lice infestations have negative economic impacts on the livestock industry.

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دراسة انتشار وتحليل جزيئي للقمل العاض *Bovicola bovis* في الأبقار في مدينة الموصل-العراق

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يعتبر القمل من الطفيليات الخارجية الدائمة بدون أجنحة صغيرة تسبب خسائر اقتصادية مختلفة مثل تساقط الشعر والحكة ونقص الوزن. تم اجراء الدراسة خلال الفترة من كانون الثاني ٢٠٢٢ حتى كانون الاول ٢٠٢٢. وكانت نسبة الإصابة الكلية ٤٠,٣٧٪ (٥٤٥/٢٢٠)، تم جمع عينات القمل من قطعان مختلفة ومناطق مختلفة. مجهريا بلغت نسبة الاصابة الكلية ٤٠,٣٧٪ (٥٤٥/٢٢٠). تم تشخيص القمل العاض نوع *Bovicola bovis*. وطبقا لعوامل الخطورة فقد بلغت اعلى نسبة في فصل الشتاء ٥٦٪ ولم تسجل الدراسة فروق معنوية في الاعمار اقل وأكثر من ٣ سنوات. في حين سجلت الدراسة اعلى نسبة للإصابة في الذكور ٦٦,٦٦٪ وكانت اعلى نسبة اصابة في السلالة الجورجية مقارنة بالسلالة المحلية وبنسبة ٤٥,٣٪ مع فرق معنوي، وعامل الخطورة للمنطقة اظهرت الدراسة ان اعلى نسبة اصابة سجلت في زهيلية وبازواية ٥٠٪ و٤٦,٤٢٪ على التوالي مقارنة بباقي المناطق. اظهرت نتائج التحليل الجزيئي لتأكيد النتائج بالقمل العاض *B. bovis* بتفاعل البلمرة المتسلسل بتضخيم الجين Cox1 وبناتج تفاعل ٣٧٩ قاعدة. واكدت نتائج تعاقب الجيني بان العزلة PP599070 تعود الى النوع *Bovicola bovis* والمعزولة في الموصل، والتي اظهرت تطابق مع نفس النوع المسجل في استراليا وامريكا عند مطابقتها في بنك الجينات. وتعد هذه الدراسة الاولى من نوعها على المستوى الجزيئي لتأكيد الاصابة بالنوع *Bovicola bovis* للأبقار في مدينة الموصل، العراق.

الكلمات المفتاحية: القمل العاض، الابقار، عوامل الخطورة، تفاعل البلمرة المتسلسل، الجين Cox1