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Microscopic and molecular detection of *Cephalopina titillator* in camels in Al-Diwaniyah province, Iraq

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Abstract

Parasitic infestations (myiasis) in camels are essential and can decrease the camel performance. The current work was conducted to identify the presence of *Cephalopina titillator* in camels in Al-Diwaniyah Province, Iraq. The study collected 150 direct and fresh samples from the head of both sexes (27 males and 123 females). The larvae were restricted to the nasopharyngeal cavity and turbinates, while a few larvae were found in the turbinate bones and ethmoid area. The colors of the first and second larvae were white or grey. The length of the first larvae ranged from 0.6 to 1.2cm (0.8 ± 0.2), and its width ranged from 0.2 to 0.4cm (0.2 ± 0.1). The length of the second larvae ranged from 1.4 to 1.7cm (1.4 ± 0.1), and its width was 0.2 to 0.5cm (0.1 ± 0.4). The color of the third larvae was yellowish, with a dark brown line on the ventral surface, its length ranged from 1.8 to 2.9cm (2.40 ± 0.3), and its maximum width ranged from 0.5 to 1.2cm (0.80 ± 0.1). There were significant differences ($P<0.05$) in the number of larvae among camels of different body condition. These samples were entered into a series of identification and studies, which started with regular macroscopy and microscopy, *16S rRNA* gene-based PCR, and partial gene sequencing. The results showed that 90(60%) of the samples revealed the presence of the larvae. However, the remaining larvae were assigned to other types of parasites. The PCR further confirmed the parasite's identity. In addition, the sequencing demonstrated that the current (12) isolates were closely similar in their nucleotide sequencing to an isolate from China. The present investigation unveils that the *Cephalopina titillator* is an important larva highly frequent in infected camels.

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Introduction

C. titillator, commonly known as the camel nasal bot fly, is a parasitic insect that infests the nasal passages of camels. *C. titillator* belongs to the Oestridae family comprising various bot flies that infest the nasal cavities of mammals. This species specifically targets camels, which causes significant discomfort and health issues. Adult female flies lay their first larvae (L1) in the nasal passages of camels, where the larvae molt twice and cause irritation, inflammation, and potential secondary infections. The larvae undergo two stages of development before eventually being expelled from the camel's nostrils to pupate in the soil (1-8).

The life cycle of *C. titillator* involves four distinct stages: larva1, larva2, larva3, pupa, and adult. The female bot fly deposits its larva1 on the nostrils of the camel, and upon hatching, the larvae penetrate the nasal passages. The larvae then migrate through the nasal cavity, causing damage to the nasal tissues. Once fully developed, the larva3 are expelled through sneezing or nasal discharge, falling to the ground to pupate. The pupa stage lasts several weeks, after which the adult flies emerge and repeat the cycle (9-14). *C. titillator* larvae in camel nasal passages pose various health risks. The larvae cause irritation and inflammation, leading to nasal discharge, sneezing, and nasal bleeding. The constant irritation may also result in secondary bacterial infections,

compromising camel health. Moreover, the excessive presence of larvae can obstruct the nasal airway, causing breathing difficulties and reduced performance in camels. These detrimental effects can significantly impact camel population's overall well-being and productivity (15-23). Efficient management and control of *C. titillator* infestations are crucial to safeguard camel health. Several strategies have been proposed to mitigate the impact of this parasite. Regular veterinary check-ups and monitoring of camel herds are essential for early detection and intervention. Insecticides such as ivermectin have been used to control the larvae within the nasal passages. Environmental management, including the removal of manure and proper sanitation practices, can also reduce the risk of larvae infestation (24-29).

The current work was conducted to identify the presence of *Cephalopina titillator* in camels in Al-Diwaniyah Province, Iraq.

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 1314 on 18/10/2022).

Samples

In Al-Diwanyia province, camels were slaughtered. Fresh samples were taken immediately from the head of the animals; 150 samples were collected from both sexes (27 male and 123 female), ranging in age from 3 to 22 years. Information about the slaughtered animals was documented to identify the animal's sex, age, and infection period from September 2021 to February 2022. The specimens are

transferred in a cold Box to the College of Veterinary Medicine at the University of Al-Qadisiyah, Parasitology laboratory. The reach-maturity larvae were carefully removed with forceps to avoid destruction and allow for the diagnosis of the organism based on the body characteristics identified by Soulsby (30). The specimens are then rinsed with distilled water. It is split into two sections: the first is kept for PCR in 70% ethyl alcohol, and the second is submerged in water for two hours before being dried with filter paper and prepared with lactophenol to set up the slides for microscopic inspection (31).

Macroscopic and microscopic examination

Soulsby (30) stated that the adult larvae were found by microscopic analysis using diagnostic characteristics. They were positioned on a slide to visually determine the larvae's length and width for both sexes. A glass piece containing a drop of lactophenol is then placed, and pressure is applied to determine which model is suitable for microscopical recognition (power 40x), ocular micrometer recognition (10x or 40x), 10=10cm, and 40=2.5 (31).

DNA extraction

This procedure was produced using the genomic DNA purification kit the manufacturer Geneaid (Korea) provided. In a brief, a single larva was used as a starting material for the extraction, in which the sample was homogenized and was ready for the completion of the kit protocol steps. The primers were ordered from Macrogen (Korea). Primer of *16S rRNA* and *COX1* RNA gene *C. titillator* (Table 1).

From the positive PCR tests, 12 samples were chosen for phylogenetic analysis and DNA sequencing of the pathogens in Macrogen Company in Korea. These were placed in the gene bank along with accession numbers. The phylogenetic tree was carried out using Mega X.

Table 1: Primers used in the current study

Gene name	Primer sequence (5'- 3')	Size (bp)	Target gene	Reference
<i>LCO1490-L</i>	GGTCWACWAATCATAAAGATATTGG	650	<i>COX1</i>	(7)
<i>HCO2198-LR</i>	RAAACTTCWGGRTGWCCAAARAATCA			
16Sbr-F	CCGGTCTGAACTCAGATCACGT	548	<i>16S rRNA</i>	(32)
16Sar-R	GCCTGTTTAACAAAAACAT			

Results

The results showed that 90(60%) of the samples revealed the presence of the larvae (Figure 1). The count of *C. titillator* first larvae were found in 2(2.3%), second larvae were found in 35(38.8%), and third larvae were found in 53(58.9%). The larvae were restricted to the nasopharyngeal cavity and turbinates, while a few larvae were found in the turbinate bones and ethmoid area. The colors of the first and second larvae were white or grey. The length of the first

larvae ranged from 0.6 to 1.2cm (0.8±0.2), and its width ranged from 0.2 to 0.4cm (0.2±0.1). The length of the second larvae ranged from 1.4 to 1.7cm (1.4±0.1), and its width was 0.2 to 0.5cm (0.1±0.4). The color of the third larvae was yellowish, with a dark brown line on the ventral surface. Its length ranged from 1.8 to 2.9cm (2.40±0.3), and its maximum width ranged from 0.5 to 1.2cm (0.80±0.1), there were significant differences (P<0.05) in the number of larvae among camels of different body condition (Table 2 and Figure 2).

The third stage larvae were photographed by microscope for morphological description of the surface ultrastructure the anterior end or pseudocephalon had two long curved maxillae with the absence of mandibles. The two antennary lobes were large and supported by sensory papillae; the 1st and 2nd thoracic segments were supported by small spines ventrally. At the same time, the 3rd segments and abdominal segments were supported by large fleshy spines with tapering ends and small spines. The last abdominal segment contains two peritremes in the bottomless pit; this previous one is formed from dorsal and ventral lips. The lips contain several sensory papillae at their surface and small spines (Figure 3). The PCR further confirmed the parasite's identity. In addition, the sequencing demonstrated that the current 12 isolates were closely similar in their nucleotide sequencing to an isolate from China (Table 3 and Figure 4).

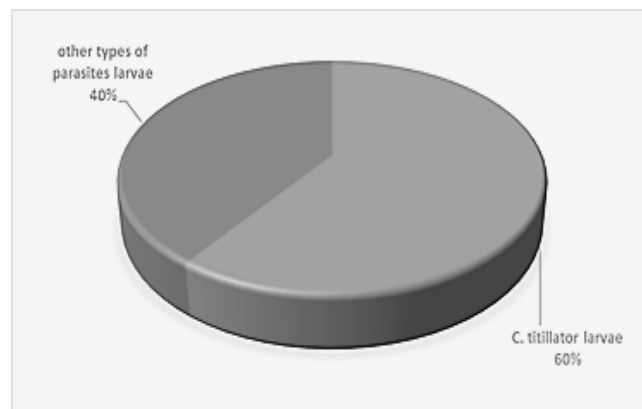


Figure 1: The larvae *Cephalopina titillator* infection rate of camels.

Table 2: Numbers, length, and width of three phases of instar larvae of *Cephalopina titillator* from infested camels

No.	Larvae phase	Isolates n (%)	Length (mean±SD)	Width (mean±SD)	color	P value
1	L1	2(2.3%)	0.6-1.2 0.8±0.2	0.2-0.4 0.2±0.1	white or grey	P<0.05*
2	L2	35(38.8%)	1.4-1.7 1.4±0.1	0.2-0.5 0.1±0.4	white or grey	P<0.05*
3	L3	53(58.9%)	1.8-2.9 2.40±0.3	0.5-1.2 0.80±0.1	yellowish	P<0.05*

P<0.05*: were significant differences.



Figure 2: The three phases of instar larvae of *Cephalopina titillator* from infested camels.

Discussion

Research on the prevalence of *C. titillator* larvae in camels has yielded varying results worldwide. A study conducted by Spratt *et al.* (33) in Australia found that 60% of the analyzed camel samples were positive for *C. titillator* larvae. This

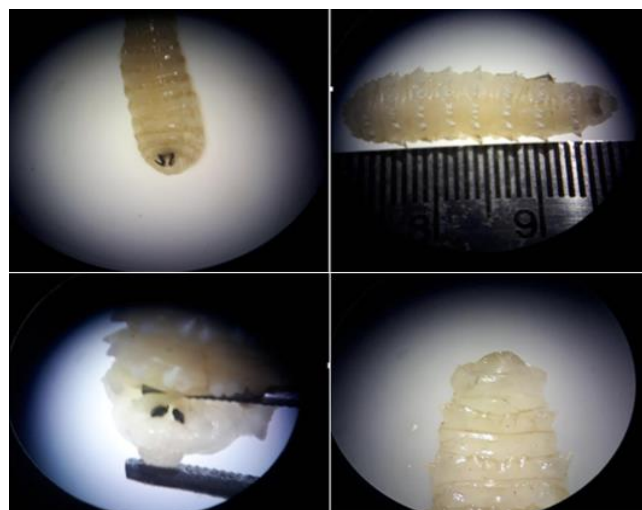


Figure 3: Microscopic examination of third *Cephalopina titillator* larvae.

finding aligns with the results of Yao *et al.* (34) in China, where 55% of the camel samples were infested with *C. titillator* larvae. In contrast, a study conducted by Al-ani *et al.* (35) in Pakistan reported a higher % prevalence rate of 75% in their camel samples. Similarly, a survey conducted by Shamsi *et al.* (36) in Iran found that the analyzed camel

samples were positive for *C. titillator* larvae. These higher prevalence rates in Pakistan and Brazil suggest a potentially higher infestation rate in these regions compared to Australia and China.

Table 3: Sequence identity of *Cephalopina titillator*

Accession number		Identity (%)
Current isolate	China	
OM980102	NC_046479	94.06
OM980103	NC_046479	82.59
OM980104	NC_046479	79.21
OM980105	NC_046479	94.06
OM980106	NC_046479	93.63
OM980107	NC_046479	93.63
OM980108	NC_046479	91.71
OM980109	NC_046479	82.59
OM980110	NC_046479	83.01
OM980111	NC_046479	82.59
OM980112	NC_046479	94.06
OM980113	NC_046479	83.01

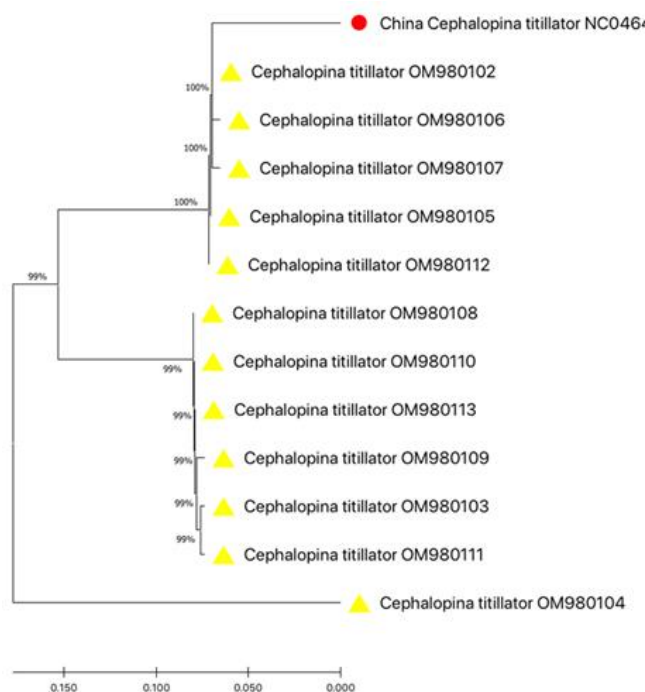


Figure 4: Phylogenetic tree of the 16S rRNA gene of the larvae *Cephalopina titillator* infection rate of camels.

C. titillator larvae in camels can significantly affect their health and productivity. According to Hendawy *et al.* (37), infestation with *C. titillator* can lead to dermatitis and irritation in camels, resulting in decreased feeding efficiency and weight loss. Furthermore, the presence of these larvae

can also lead to secondary bacterial infections, further compromising the health of the camels. When comparing global findings on the prevalence of *C. titillator* in camels, it becomes evident that there is considerable variation among different geographic regions. The study by Spratt *et al.* (33) in Australia and Yao *et al.* (34) in China reported lower prevalence rates compared to the studies conducted by Al-anani *et al.* (35) in Pakistan.

These differences can be attributed to climatic conditions, camel management practices, and suitable breeding sites for *C. titillator*. For instance, Pakistan and Brazil have warmer climates, which may promote the growth and survival of *C. titillator* larvae. Additionally, differences in camel management practices, such as hygiene and grooming, may contribute to the varying prevalence rates observed (37-44).

The study of Khater *et al.* (45) revealed that the three phases of larvae of *C. titillator* of camels infested, the larval phase of *C. titillator* is mainly passed in the third stage. The first larvae stage (L1) is only about 2-4mm long and up to 15 mm in the second stage. Meanwhile, the mature third-stage larvae grow up to 15-23 mm. The study by Elham *et al.* (46) recorded that 0% of L1, 1.5% of L2, and 11.2% of L3 were found out of a total of 151 larvae collected from camels. Al-Rawashdeh *et al.* (47) found that 0% of L1, 1.7% of L2, and 135.4% of L3 were found out of a total of 468 larvae collected from camels. The L1 result was lower than that recorded by Attia and Mahdi (48), while a study by Khater *et al.* (45) reported that L1 larvae prevailed at 90.5%, and another study by Taie *et al.* (49) reported 13.14% L1 larvae. L1 larvae may have passed unseen due to their small size, presence in hidden places such as turbinates and ethmoid bones, or numerous L1 were demolished in the nasal holes during the hypo-biotic period. The morphological characters of L2 and L3 were those stated in the identification key.

In the study above, 12 strains of *C. titillator* were analyzed, and their genetic makeup was compared to a Chinese strain. The results revealed a remarkable similarity between the strains, suggesting a close relationship and potential common ancestry. This finding aligns with previous studies conducted in different regions across the globe. Research by Spratt *et al.* (32) investigated the genetic diversity of *C. titillator* strains in African camels. Their study utilized molecular techniques and demonstrated high genetic similarity among the strains, indicating a widespread distribution of a single parasite lineage. This finding supports the notion that *C. titillator* exhibits low genetic variability regardless of geographical location. Furthermore, a study conducted by Simon *et al.* (50) focused on DNA sequencing; they identified a genetic marker that was conserved among all the examined strains; these findings suggest a potential common evolutionary origin of the parasite strains across continents.

A study by Xinghua *et al.* (51) in China examined the prevalence of *C. titillator* in camels across different regions.

The results showed a high prevalence rate, with the parasite detected in most examined camels. In contrast, a study by Shamsi *et al.* (35) in Iran reported a relatively lower prevalence rate of *C. titillator* in their sampled camel population. The authors suggested that differences in management practices, vector control measures, and geographical factors might contribute to the observed variation in prevalence. This highlights the importance of considering regional factors when assessing the distribution and prevalence of *C. titillator*.

Moreover, a study in China indicated a higher prevalence of parasites in captive camels than in the wild population. This distinction could be attributed to factors such as confinement stress and limited access to natural habitats, which may increase the susceptibility of captive camels to parasitic infections (52).

Conclusion

The present investigation unveils that *Cephalopina titillator* is an important larva that occurs frequently in infected camels.

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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 المجهرى والجزيئي لطفيلي نغف الأنف في الإبل في محافظة الديوانية، العراق

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الخلاصة

تعتبر الإصابات الطفيلية (النغف) في الإبل مهمة ويمكن أن تقلل من أداء الإبل. أجري العمل الحالي للتعرف على وجود طفيلي نغف الأنف في الإبل في محافظة الديوانية في العراق. جمعت الدراسة ١٥٠ عينة مباشرة وحديثة من رؤوس كلا الجنسين (٢٧ ذكراً و١٢٣ أنثى). تم إدخال هذه العينات في سلسلة من التحديد والدراسة، والتي بدأت باستخدام الفحص العياني والمجهرى. أظهرت النتائج أن ٩٠ (٦٠%) من العينات

كشفت عن وجود اليرقات، حيث اقتصررت يرقات الطفيلي على التجويف الأنفي البلعومي، بالإضافة إلى عدد قليل من اليرقات في عظام المحارة والمنطقة الغربالية. تراوحت ألوان اليرقات المعزولة (الأولى، الثانية والثالثة) بين الأبيض والرمادي. تراوحت أطوال اليرقات الأولى ٠,٦-١,٢ سم (٠,٢±٠,١) سم وعرضها ٠,٢-٠,٤ سم (٠,٢±٠,١) سم. وتراوحت أطوال اليرقة الثانية من ١,٤-١,٧ سم (١,٤±٠,١) سم وعرضها ٠,٢-٠,٥ سم (٠,٤±٠,١) سم. كان لون اليرقة الثالثة مصفر مع وجود خط بني غامق على السطح البطني، ويتراوح طولها من ١,٨-٢,٩ سم (٢,٤±٠,٣) سم وأقصى عرض لها يتراوح من ٠,٥-١,٢ سم (٠,٨±٠,١) سم، وكانت هناك فروق معنوية ($P<0.05$) في عدد اليرقات بين الإبل بمختلف حالات الجسم. بعد اكتمال فحص اليرقات باستخدام الفحص المجهرى استكملت الدراسة بالفحص الجزيئي والذي اعتمد على جينات الرايبوسومي ودراسة تعاقب القواعد النيتروجينية. تم تخصيص اليرقات المتبقية لأنواع أخرى من الطفيليات. أظهرت دراسة تعاقب القواعد النيتروجينية أن العزلات الحالية (١٢) كانت متشابهة إلى حد كبير في تسلسلها النيوكليوتيدي مع عزلة من الصين. يكشف البحث الحالي عن أن يرقات طفيلي نغف الأنف هي يرقات مهمة تتواجد بكثرة في الإبل المصابة.