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Use molecular method to detect the screw worm fly in Al-Diwaniyah province

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Abstract*---Chrysomya bezziana* this study was carried out between October, 2021 to March 31, 2022 in six region from Al-Diwaniyah province. Observe the influence of the month and type of animal on the region's. The total number of animals infected was 33 (1) cattle, (7) lambs, and (35) sheep. Al-muhnawa had the highest rate of infestation, while Al-hamza had the lowest rate of infestation. The highest rate of infestation recorded in March ()% and the lowest in February ()%.Conventional PCR (Polymerase Chain Reaction) was used for molecular identification. A DNA extraction was performed on 50 randomly chosen larvae from the total population. The size of the PCR product (548 pb) For *Chrysomya bezziana*, from the infected animals larvae were taken to DNA extracted and used following targeting 16S rRNA gene product size (548 bp). There follows sequence analysis to obtain the nucleotide (16S rRNA) gene, and the sequence was recoded in the National Center for Biotechnology (NCBI) with accession numbers (ON059979, ON059980, ON059981, ON059982, ON059983, ON059984, ON059985)Iraq. This gene's homology sequence identity percentage was calculated by comparing it to another globally registered gene (NCBI). The compared this gene with another global registered in (NCBI) showed the homology sequence identity percentage between them (98.22,98.67)% identity with Australia isolates and (99.06, 98.44, 97.33)% with Brazil isolates.

***Keywords*---**Chrysomya bezziana, PCR, 16S rRNA gene.

Introduction

Myiasis, a noun derived from Greek (mya, or fly), has been defined variously by numerous different authors over the years (Colwell et al., 2021). When Frederick

William Hope coined the term "myiasis" in 1840, he referred to dipterous larval diseases, as opposed to those caused by other insect larvae, called scholechiasis (Rawat, 2020). There are two screw-worm fly species found around the world: one from the *Ch. bezziana* and one from *Chrysomya hominivorax* (Spradbery, 1994). The larvae parasitize warm-blooded animals such as livestock and humans. Professor Mario Bezzi of Turin described *Ch. bezziana* based on material collected from cattle in the Belgian Congo (Villeneuve, 1914). Scientific Classification: Domain:Eukaryota, Kingdom:Metazoa, Phylum: Arthropoda, Subphylum: Uniramia, Class: Insecta ,Order: Diptera, Family: Calliphoridae, Genus: Chrysomya, Species:*Ch. bezziana* and *Ch. hominivorax* (Villeneuve, 1914). *Ch. bezziana* adults, lay their eggs near the edges of wounds or body orifices, with their mouth hooks, they tear the capillaries as they begin to excavate the tissue with their first move: up to 245 within 24 hours, deep subcutaneous cavities appear (Scholl *et al.*, 2019). The Old World screw-worm fly *Ch. bezziana* causes traumatic myiasis in many warm-blooded animals throughout the tropical regions of the Old World; there is seasonal variation in the incidence of screw-worm infection, most cases in early winter, in Al-Qadissya 36% of cases in October and increased gradually in November 40.2%. Highest present was in December 54% (Abass & Abdull 1997). It the first discovered in Iraq on September 9th, 1999, in Baghdad, where it had never been found before, this disease then spread throughout Iraq's central region (Alexander,2006). In Diala, for example, on December 9, 1996, northern and southern Iraq have seen nothing, in the Arabian Gulf region, which was first documented in 1997 when sheep were brought from Australia to Bahrain (Siddig *et al.*, 2005). Sultan Oman and the United Arab Emirate both imported sheep others Saudi Arabia, focused on the prevalence of the old word screw-worm infection they can be found in the eastern and southern parts of Asia, In January, February, and March, high infection rates were observed (Spradbery *et al.*, 2019). The larvae only the final segment of the smooth body of the third instar larva shows any signs of body processes (Thyssen, 2009). Because the posterior spiracles are not concealed within the body cavity, their peritreme is visible (Szpila, 2009). The dorsal tracheal trunks distinguish this species from its New World cousin, *C. hominivorax* (Mastrangelo & Welch, 2012). Unlike *C. hominivorax*, which is heavily pigmented from the 12th to the 10th or even the 9th segment, *Ch. bezziana* is only heavily pigmented from the last half of the 12th segment (Dogra & Mahajan, 2010). The anterior spiracle can have as many as seven lobes (Dutra *et al.*, 2018).

Aim of Study

1. Detection of *Chrysomya bezziana* in ruminant.
2. Study the effect of months and region on the infestation rate of the parasite.
3. Molecular diagnosis of *Chrysomya bezziana* larvae and phylogenetic analysis.

Materials and Methods

This study was design for traditional and molecular detection of *Chrysomya bezziana* in ruminants. The morphological characteristics of the larvae were studied by measuring the length of each larva using a ruler under the dissecting microscope, the larvae were identified as described by (Abul-hab, 1970) and

through its colors and the form of respiratory spiracles in the last segment of larvae (Hanan, 2018).

Collection of sample

Forty three head were examined ,one cattle ,seven lamb and thirty five head infected from sheep were collected by weekly visit twice a week at Al-Diwaniyah province throughout the period from the 1/ 10/2021 to the 31/3/2022, in Ghamass, ,Nfer, ,Al-Shaamia, Salahia, Al-muhnawa, and Al-Hamza regions of Al-Diwaniyah province where it relied on gross observation and determined of infestation site in the animal's head and number of larvae. These samples for both sex. All samples collected directly from animal, and putted in clean plastic containers with alcohol and marked with sample's numbers; Then these samples were transported in refrigerator bag to the parasitology laboratory which belongs to the College of Veterinary Medicine, University of Al-Qadisiyah for laboratory examination.

Molecular examination

Molecular technique for detection of larvae. The examined larvae of the various evolutionary stage are placed in ethanol an alcohol concentration of 70% later, in deep freeze under -20 C° for DNA extraction (Hendawy *et al.*, 2012).

Results

Macroscopic and Microscopic examination

The larvae appear to the naked eye in a creamy white color. By using an optical microscope, note the circular rows of spin dark brown tend to black in color ,darkened and tapered tips re-curved toward the body, with a pointed end. The larvae are segmented into eleven rows. The posterior end is wider than the anterior end and contains a pair of sharp pointed protuberances and is characterized by being larger than the spin that covers the body. The anterior end has pointed hocks called "mouth hook" that are used for feeding on live tissue. The larvae appear to the naked eye in a creamy white color. By using an optical microscope, note the circular rows of spin dark brown tend to black in color ,darkened and tapered tips re-curved toward the body, with a pointed end. The larvae are segmented into eleven rows. The posterior end is wider than the anterior end and contains a pair of sharp pointed protuberances and is characterized by being larger than the spin that covers the body. The anterior end has pointed hocks called "mouth hook" that are used for feeding on live tissue. Fig(1)

A total of 33 samples were collected and send to the laboratory of veterinary medicine, Al-Diwanyia University, from both sexes during October (2021) to the March (2022).

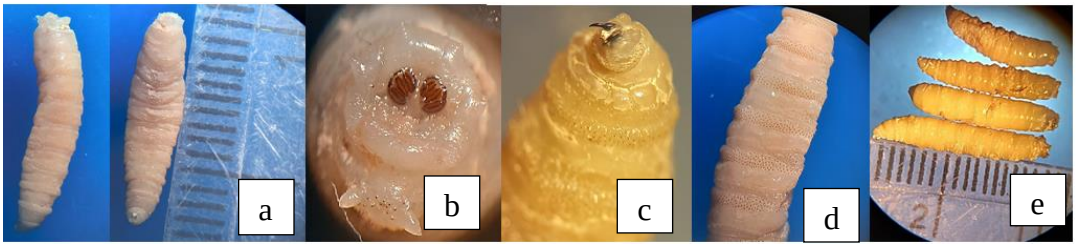


Figure (1): Microscopic examination for *Ch. bezziana* (a)ventral and lateral surface,(b) anterior end,(c)posterior end, (d)middle piece (e)morphological shape

DNA Extraction

According to Geneaid, the manufacturer of the genomic DNA purification kit, this method was developed and implemented (Korea).

PCR Primers

The following primers targeting the 16S rRNA gene for *Ch. bezziana* was recruited (Hendawy *et al.*, 2012). Table (1)

Table (1): Primer of *16S rRNA* gene *Chrysomya bezziana*

Primers	Sequence	Product size	Target gene
For ward	CCGGTCTGAACTCAGATCACGT	548bp	16S rRNA
Reverse	CGCCTGTTTAACAAAAACAT		

Sequencing DNA and phylogenetic analysis

By selecting ten larvae from fifty positive PCR samples, DNA sequencing and phylogenetic analysis were used to identify *Chrysomya bezziana* larvae from Al-Diwaniyah province. The sequencing of the (16S rRNA) gene by the Korean company macrogen was then compared to that of other world strains. The Basic Local Alignment (BLAST) program and the Bio Edit program were used to direct the homology search at the (NCBI) online (http://www.ncbi.nlm.nih.gov). After that, all of the identified samples of *Chrysomya bezziana* was sent to NCBI-Gen Bank in order to get an accession number and use that number to build a phylogenetic tree.

A total of 33 sheep and cattle were positive to infestation by screw worm fly in different area of Al- Diwaniyah province. Total(2,3) show the high number of infestation Ghamass (8) in sheep and (1)cattle while the lower in Al-hamza (1) sheep .

Table (2): Distribution of screw worm in Al- Diwaniyah according to region with type of animal

Region	Type of animal	Num. 2021-2022	Percentage (%)
Ghamass	Sheep	8	27.3

	cattle	1	
Nfer	sheep	2	6.1
Salahia	sheep	2	6.1
Al muhnawa	sheep	6	39.4
	lamb	7	
Al shaamia	sheep	6	18.2
Al hamza	Sheep	1	3
Total		33	100

Distribution according to months

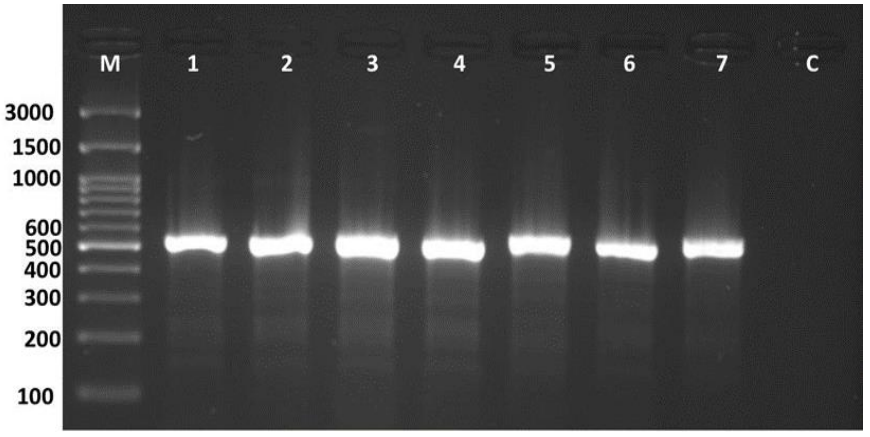
A total of 33 animals were positive to infestation by screw worm fly in different area of Al- Diwaniyah province. Total(3) show the higher percentage of infestation Al muhnawa (39.4)% in while the lower percentage in Al-hamza .

Table (3): Distribution of screw worm in Al- Diwaniyah according to region with month.

Month / Region	Oct	Nov	Des	Jan	Feb	Mar	%
	2021			2022			
Ghamass	1	3	1	1	1	2	27.3
Nfer	1	1	0	0	0	0	6.1
Salahia	0	2	0	0	0	0	6.1
Al shaamia	0	2	1	0	1	2	18.2
Al muhnawa	1	4	4	2	0	2	39.4
Al hamza	0	0	0	1	0	0	3
Total	3	12	6	4	2	6	100

Molecular Study

The larvae of *Ch. bezziana* was amplicons with (16S rRNA) in 548 pb Fig (2).



Figure(2): Gel electrophoresis image (1.5 % agarose) shows the amplicons of *Chrysomya bezziana* (16S rRNA gene) (Lane: 1-7) (size= 548 bp). M is a molecular marker (Genedirex, Korea)

NCBI-BLAST Homology Sequence identity (percent) between local *Chrysomya bezziana* sequences deposited in the gene bank under accession numbers ON059979, ON059980, ON059981, ON059982, ON059983, ON059984, ON059985, and NCBI-BLAST deposited sequences from other countries is shown in Table (4).

Table (4): In the gene bank, researchers deposited the NCBI-BLAST Homology Sequence Identity between local *Chrysomya bezziana* sequences

Sequence name	Accession number	NCBI-BLAST Homology Sequence identity (%)			
		Identical to	Gen bank Accession number	Country	Identity (%)
1	ON059979	*Chrysomya bezziana*	JX913737	Australia	98.67
2	ON059980		JX913737		98.22
3	ON059981		JX913737		98.67
4	ON059982		JQ246710	Brazil	99.06
5	ON059983		JX913737		98.44
6	ON059984		AF352790		97.33
7	ON059985		JQ246710		99.06

As for the results for *Ch. Bezziana* from (cattle and sheep) isolated number (1,2,3) close relationship with NCBI-BLAST for Australia country in (98.67, 98.22, 98.67)% successive while the no(5,6,7) isolates, identical with Brazil is isolated in (99.06, 98.44, 97.33, 99.06)% in NCBI-BLAST as homology sequence identity.

In Figure (3) shows a phylogenetic tree constructed using the currently known sequences of the *Chrysomya bezziana* (16S rRNA) gene, denoted by the yellow circles. There is a global gene bank, and these accession numbers are used to identify them. Previous global sequences, which were known as "red circles," were compared with these newly discovered ones. Mega X analyzed these.

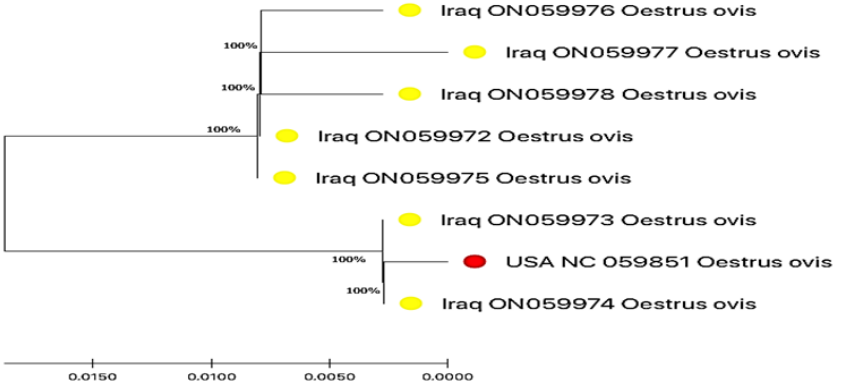


Figure (3): Phylogenetic tree analysis of *Chrysomya bezziana* (*16S rRNA*) gene

Multiple sequence alignment with homologous sequences for the newly discovered *Chromomyia bezziana* (16S rRNA) is shown in Fig (4). Four colors are used to emphasize the similarity. Mega X analyzed this data.

Figure (4): A comparative multiple sequence alignment of the newly discovered *Chrysomya bezziana* with known global homologs

Discussion

This the first study use 16S rRNA gene to diagnosis *Ch. Bezziana* in Iraq and sequencing with registered in Gen Bank.*Ch. Bezziana* recommend in high number in Al-muhnawa in December. The rate of infestation by screw worm was in sheep than cattle.

Conclusions

This the first study use 16S rRNA gene to diagnosis *Ch. Bezziana* in Iraq and sequencing with registered in Gen Bank.*Ch. Bezziana* recommend in high number in Al-muhnawa in December. The rate of infestation by screw worm was in sheep than cattle.

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