

Molecular Diagnosis of Ocularmyiasis Causative Agent in Diwaniya City and Study of Epidemiological Data about infection Distribution in The City

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Abstract

This study has aimed to diagnose the etiological agent of ophthalmomyiasis in Iraq in diwaniya city exactly as so as was aim to obtain epidemiological data about cases ratio and distribution in the city and affiliated areas, so that 30 samples of first instar larva from infected cases have been collected from the eye by mechanical removal with ophthalmologist aid, then kept in ethanol 70% until used for DNA extraction and then the extracted DNA used in PCR-RFLP. The results give only 20 samples were positive for *osterousovis* which also known as sheep botfly or nasal botfly, for epidemiological study have used the recorded data of cases in diwaniya educating hospital and hospitals of districts and sub-districts within the period from March to October 2020, the results showed that there is no significant difference in the number of cases among different ages, the results also showed that male cases are more than female cases. The study also has noted that most of the cases were recorded in villages more than city center due to shepherds and farmers presence. In addition to cases in spring were higher than summer and autumn seasons.

Keywords: Ophthalmomyiasis, Mitochondrial cytochrome oxidase, *Chrysomya bezziana*, *Dermatobia hominis*, *oestrusovis*

Introduction

Myiasis is a term given by fly maggots for infection. As in other types of parasitism¹, there are many categories of myiasis, including eyes, ears, bowels, and urogenital tract². Ophthalmomyiasis is a term used when the larva infects the eye³, it can be further classified into ophthalmomyiasis externa when the invasion occurred in the conjunctival sac and ophthalmomyiasis interna if the organism enters the globe of the eye⁴, in general, this disorder is rare in developed countries, but it is popular in the world's underdeveloped area where hygiene is bad. The clinical picture of the infection is allergic conjunctivitis can vary to Keratitis, corneal ulcer, uveitis, retinal subcorpus endophthalmitis, and hemorrhages⁵. These complications might occur in immunosuppressed patients, Knowledge, and relatively quick access to ophthalmic medicine. Despite the low number of injuries in most countries of the world, but in Iraq, there are about six ophthalmomyiasis cases every month from spring until the end of autumn in Diwaniyah governorate only. Most of the injuries happen to shepherds, farmers, and people living in rural areas. and with all this rise ratio of injuries little known about the causative agent and how to prevent infection where the recent study about ocular myiasis was done in 2004 in the north of Iraq and included just one case⁶ .i.e. without statistic data .according to most studies the etiological agent of ocular myiasis in human which consider an accidental host it might be one of three species *Chrysomya bezziana*⁷, *Dermatobia hominis*, or *oestrus ovis*⁸, the two last species are the most common in external ophthalmomyiasis cases, they also known as human and sheep botfly respectively⁹. molecularly these species can be diagnosed depending on their specific Mitochondrial cytochrome oxidase subunit I gene *mtCOI* gene which can be considered a beneficial mean for molecular identification of the parasite^{6,7,8}.

The present study aimed to diagnose the species responsible for ocular myiasis, in addition to statistics of casualties in Al-Diwaniyah governorate during the spring, summer, and fall seasons.

Material and methods

1- Diagnosis

According to the symptoms which include itching, burning, lacrimation, rhinorrhea, foreign body sensation, and swelling, the history of the patient is very beneficial in diagnosis as the patient might recently be in close contact with animal or not, Also, while the patient had suffered an insect strike in his eye in the previous days.

Pictures 1, 2, 3below are from patients who have visited the hospital due to infection with ocular myiasis



Pic1 patient with ocular myiasis 32 years old , pic2 patient with ocular myiasis 21 years old, pic3 patient with ocular myiasis at 8 years old

Sample collection

The samples used in the study were larvae that were isolated from the infected eye with aid of a specialist ophthalmologist in Al-Diwanyia teaching hospital, in addition to hospitals in the affiliated areas such as Al-Hamza, Al-Shamiya, Al-Daghara, and Afak Hospitals. The method of isolation was as the following:

- Adding a xylocaine 4% as a topical anesthetic to the infected eye ;
- After about 5-10 minutes at slit lamp and by using forceps the larvae were removed.
- The removed larvae were put in plane tubes contained ethanol 70% and then send to the lab to be diagnosed.

2- Larvae diagnosis

In the lab, the larvae were examined under a microscope to be diagnosed according to size, color, number, and site of spikes.

3- Extraction of DNA

DNA extracted from larva by using DNA extraction kit provided from

Bio-Rad company and by following the method of extracting DNA from the tissues.

PCR amplification

The primers explained in table 2 below are used to amplify fragments of *mtCOI* gene from the previously extracted DNA, under conditions included

- 40 cycle of denaturation for 30 sec at 94 C.
- 30 sec at 52C for annealing.

- 30 sec at 72 for an extension. after adding

Then the amplified DNA fragments are electrophoresed in TBE buffer in 1.5 percent agarose gel and visualized using UV Transilluminator after ethidium bromide staining.

Table 1 primers provided for PCR amplification

Primers	Sequence		Amplicon
<i>Oestrusovis</i> CO1 gene	F	GAGCTTGATCCGGCCTTGTA	-
	R	TTGCTCCTGCTAAAACGGGG	
<i>Dermatobiahominis</i> cox1 gene	F	CTTCATTCTTTGACCCAGCAGGWGG	-
	R	TGAGTATGARTGTTCTGCWGGNGG	
<i>Chrysomyabeziziana</i> CO1 gene	F	ACAGTTGGAATAGACGTTGATAC	-
	R	TCCAATGCACTAATCTGCCATATTA	

Results and Discussion

a- Epidemiological data

About 83ophthalmomyiasiscases have been recorded during spring, summer, and early autumn (from March till 15 of October 2020). These cases were obtained

From the five above-mentioned hospitals. Table 2 showed the number of samples and the date of collecting from each hospital

Table 2Number of ocular myiasis cases distributed as site and date

Date	Diwaniya teaching Hospitals	Al-Hamza Hospital	Al ShamiyaHospital	Al Daghara Hospital	Afak Hospital
March	6	1	1	2	1
April	5	5	5	5	4
May	8	4	4	4	3
June	4	1	1	1	2
July	1	0	0	1	1
August	0	1	1	2	3
September	0	1	1	1	0
October	2	1	1	0	1
Total number	26	14	13	16	14

As obvious the highest number was in AL Diwaniya teaching Hospitals but that not mean all patients were living in the city because many of them were living in a rural area where no hospitals near to their houses. It was also noted that not all of the injured were farmers or shepherds, but the injuries included some people living in the city center. The reasons for this may be due to the cases of random grazing spread between the alleys and the presence of butchers near their areas of residence, We also notice the disappearance of infections in September and October in some hospitals, and here the reason can be attributed to the fact that during that period of the year the number of new infections with the Coronavirus increased and the eye

centers closed in some hospitals, so it may be difficult for patients to review the hospital, especially when referring to the year's records. The previous cases were found to be not negligible in the same period (September and October), in general, it can be said that this number of injuries cannot be underestimated. Concerning only one governorate in Iraq, considering that ocular myiasis cases have become rare in most countries of the world.

The study also includes the age of patients which are explained in table 3 below

Table 3 the number of cases as the age of patients

Age group	7-14 year	15-25 year	26- 35 year	36- 50 year
Total number	19	21	22	20

Through the above table, it can be easily seen that there are no significant differences among different age groups. By depending on myiasis definition which is "the invasion of human and animal organs and tissues by dipteran fly larvae or maggots which optionally or necessarily feed on living or necrotic tissues for at least a period"¹¹. I.e. The likelihood of infecting adults is the same as in children since the parasite can feed on soft and old tissues as well.

As regarding gender, tables 4 below show the number of infections according to gender

Table 4 the number of cases as gender

Male cases	Female cases
60	21

It is very clear that there is a high difference between male and female and male cases form the higher than female due to their close contact with the animal and most shepherds are male. The phenomenon of sheep graze women is a common occurrence in Iraq in the countryside, but what is noticed is wearing the veil, which can help avoid exposure to botfly strike.

The results also have included the number of cases according to date, which is shown in table 5 below.

Table 5 Number of cases according to Date

Date	N.O .of cases
March	11
April	24
May	23
June	9
July	3
August	7
September	3
October	2

We can easily note that the higher ratio of cases was in April followed by May and is very accepted if we know that most infections occur during springtime due to breeding and activity season of the parasite¹¹, it is important to know that the number of recorded cases might be affected by Corona pandemic disease And the closure conditions that may have caused them to prevent going to the hospital when an injury with ocular myiasis occurred Especially if we know that most of the injuries

are limited, meaning that they are not contagious and may heal automatically. Therefore, the injuries may be more than what is obtained from the records.

b- Microscopically diagnosis results

Under the microscope, the first instar larva was seen as about 19 mm segmented contain spikes with brown filaments on the body and black hooks.

As shown in figure 1 below.



Fig 1 first instar larva under a light microscope (Olympus)

c- PCR amplification results

PCR -RFLP has been done to investigate about three different species *Chrysomya bezziana*, *Dermatobia hominis*, *Oestrus ovis*. Since they are the most common etiological agent of ophthalmomyiasis, these species can be diagnosed molecularly by mitochondrial cytochrome oxidase gene I *mtCOI* this gene is very beneficial for molecular studies because "its rate of mutation so fast is to distinguish closely related species and also because its sequence is conserved among conspecifics"¹². beside its rate of evolutions is very slow. Of the 81 infected cases, we were able to obtain larvae only from 31 cases, while the ophthalmologist was unable to isolate the larvae from the remaining 50 cases due to the speed movement of the larva away from the light during removal. The numbers of larvae isolated from each case ranged between 3-4. All these 31 cases were used in DNA extraction and then PCR amplification and 20 of them give results after electrophoresis while the other 11 gave negative results, it important to know that all 20 positive samples were successfully amplified 549bp fragments of *mtCOI* from *Oestrus ovis* only while nor one of the other species in the field of the study was amplified, so that we can conclude that the etiological agent of ocular myiasis in Diwanya city is sheep botfly, It is an acceptable result to some extent. According to the information of most cases, they are exposed to an insect strike while grazing their sheep or there are sheep near them. Figure 2 below show PCR amplification results.

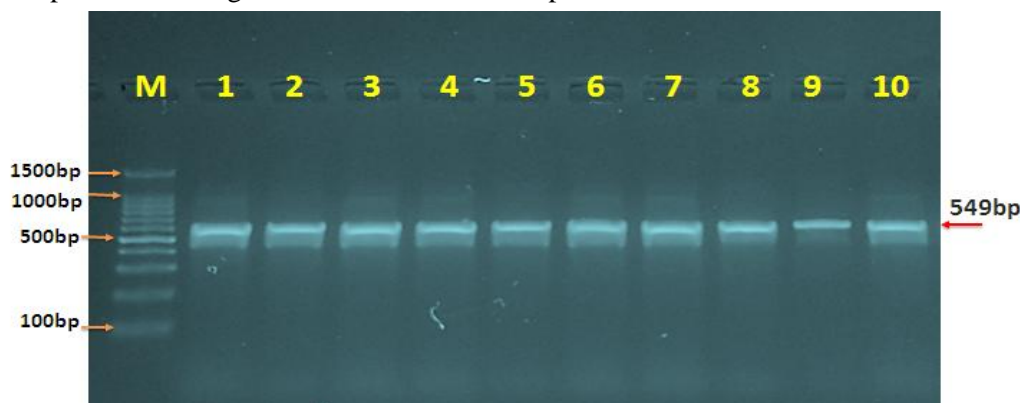


Fig 2 Agarose gel of amplified PCR products of *mtCOI* gene after staining with ethidium bromide

This is the first study since 2004 in Iraq that diagnoses the causative agent of ophthalmomyiasis and the epidemiological distribution of the cases in the city, more future studies include the country should be done. And the phylogenetic tree of the causative agent should be studied to discover if there is a relationship between the diagnosed species in Iraq and neighboring countries.

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