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Epidemiological studies on screwworm infestation in sheep flocks in Sulaimani province

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Abstract---This study was conducted in different region in Sulaimani province, Kurdistan Region-Iraq, from August 2021 to April 2022. Isolated randomly, 527 (3.4%) out of 15360 sheep examined had myiasis, identified three genus and sex species of flies in all locations based on morphological characteristics. Chrysomya Bezziana is found in all regions instead of Qardagh and Sharbazher, Lucilia Sericata, Calliphora Vomitoria, and Calliphora vicina were found in all regions. The prevalence rate of myiasis was found in Dukan region at 5.5% (95/1715) and the lowest rate was revealed in the Garmiyan region 2.6% (81/3160). During the seasonal distribution, the highest rate of myiasis was found in Spring (4.8%), followed by (3.8%) in the Summer and (3.4%) in the Autumn, while shows the lowest distribution in Winter (0.8%). In this study, to accurately detect flies, fresh meat was placed in several areas, observed the heavy flies landing spring season. The highest myiasis was noted in the neck at 24% and the lowest was shown in the ear at 1.9%. the Study's results demonstrate a highly significant between the body sites, location, and season when using chi-square test at (P< 0.01), the distribution rate of myiasis was 3% (151/5020) at less than 2 years, and 3.6% (376/10340) at more than 2 years, also a highly infestation distribution of myiasis was 3.66% in female and 2.95% in male. notice an ineffective difference between infested sheep when compared according to the Chi-square test (P< 0.01) with the age and sex, The aims of this study are to investigate the prevalence rate of screwworm in sheep in different region (district and sub-district) in Sulaimani province and identify the species of fly and larva screwworm that infect the sheep.

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Introduction

There are two economically and medically significant screwworm species found around the world, new word Screwworm (NWS) *Cochliomyia hominivorax* (Coquerel, 1858), which is only found in the Western Hemisphere, notably in tropical areas (Ali *et al.*, 2021). *Chrysomya bezziana* (Villeneuve, 1914), the Old-World screwworm (OWS), is found in Sub-Saharan Africa, the Indian subcontinent, Southeast Asia, and the Arabian Peninsula's Gulf region, including Iraq (Hosni et al., 2022). Any open wound can become infected, and the infection is frequently accompanied by a foul-smelling discharge and the presence of larvae within the tissue (Singh and Singh, 2022). C. bezziana is the most significant fly that causes myiasis out of the numerous species in the genus *Chrysomya*, only around 10 species of which are known to cause myiasis in human. (Ahmadi, *et al.*, 2009). Screwworm infections in small ruminants such as those in sheep, cause serious economic losses to the livestock industry in both developed and developing nations through death, miscarriage, decreased milk supply, weight loss, infertility, and poor hide quality (Rafinejad, *et al.*, 2014). The General Veterinary Commission/Ministry of Agriculture recorded the first case of old-world screwworm fly myiasis in Iraq in September 1996, as the case was a cow injury from Sheikh Hamad- Altagi village/ Baghdad, and it was diagnosed by the Iraqi Natural History Museum, and confirmed by the British Museum (Abdul-Rassul, *et al.,* 1996). The weather in Iraq is suitable of distribution to this fly, with temperatures ranging from 22 to 26 degrees Celsius and humidity levels ranging from 40 to 60%. (Al-Ani, 2004). The ectoparasites; mange mites, lice and ticks causes more problems such as; tissue damage due to reduction in wool and itching and becomes a source of skin diseases and loss of wool quality (Mustafa, 2019).

Materials and Methods

This study was caret out in Sulaimani province in different region (districts and sub-districts), Kurdistan Region. It is located between 35°04 – 36°30 latitude and 44°50 – 46°16 longitude. The district and sub-districts include Bakrajo, Tanjaro, Sharbazher, Qaradagh, Dukan, and Garmiyan, from August 2021 to April 2022.

Samples collection

Samples collected from many locations in Sulaimani province by two ways:

Inspection of sheep

A total of 15360 sheep were visually inspected randomly to detect the myiasis lesion in different seasons and different ages and both sexes, also the site of wounds, and place of sample were also recorded. Adult flies and larvae from livestock wounds were collected by visiting farms, houses, and animal markets, or by collecting from animals brought to veterinary clinics by their owners. Owners of animals were asked about myiasis and encouraged to present active cases. The

adult was collected by using an insect net and placed into small containers for identification in the laboratory. Forceps were used to remove the larvae. Samples were collected in accordance with a standard protocol. At least 3–8 maggots were removed from each wound. Larva were killed by immersion in hot water (> 80 °C) for 15-30 seconds and then stored in 80% analytical grade ethanol at ambient temperature (Hall *et al.*, 2009).

Putting pieces of meat

Several pieces of fresh meat are put in plastic buckets in various regions and different seasons in some districts and sub-districts in Sulaimani province in order to capture the flies by closing the bucket and insect net and larvae that feed on them and comparing them to flies and larvae collected from sheep. As in the previous method, all the information such as the season and place of collection of the samples is recorded and then the samples are preserved in the same previous way.

Identification in a laboratory

Flies and larvae were collected from the skin lesions on sheep, and the fresh meat was sent to a laboratory for identification. The larvae and adults were prepared and kept in a petri dish and the identification was carried out under the binocular microscope (X50 magnification) according to the characters described morphological method. Larvae were identified according to (Zumpt, 1965) and (Spradbery, 1991), and adults according to (Verves 1986) and (Hall, et al., 2009).

Statistically Analysis

Statistical analysis was performed to test the hypothesis which confirmed that the positive cases of infections by Screw Worm in sheep within different locations, seasons of the year, sit of body, are equally distributed, using Chi-square as a test of goodness of fit and Chi-square test for independence used for the distribution between different ages and gender, ($P< 0.01$).

Results

In this study, we isolated randomly 527 (3.4%) out of 15360 sheep and examined them with myiasis. Collected from some districts and sub-districts in Sulaimani province, Kurdistan Region, from August 2021 to April 2022. Obtained data in (table 1) revealed that there was a highly significant occurrence of the positive cases of the affections ($P<0.001$) between the locations. Screwworms were reported in some locations of Sulaimani province 2021-2022. Out of 15,360 sheep examined, 527 sheep (3.4%) were infected with myiasis from six visited locations. The highest rate of infested sheep was found in Dukan (95 cases from 1715 sheep, 5.5%), whereas a lower number of infested sheep was found in Garmiyan (81 cases from 3160 sheep, 2.6%).

Table 1
Prevalence rate of sheep myiasis in different regions (district and sub-district) in Sulaimani province during the year

Location	No of Examination	No of infected	Infestation rate %	x^2 Stat	x^2 Cal
Dukan	1715	95	5.5		
Tanjaro	1350	58	4.3		
Sharbazher	1160	48	4.1		
Qaradagh	4345	147	3.4	70.347**	15.086
Bakrajo	3630	98	2.7		
Garmiyan	3160	81	2.6		
Total	15360	527	3.4		

significant differences between locations according to chi-square (< 0.001)

Screwworm distribution varies according to season in all Regions (table 2), in such a way that in Dukan it was 3% (12/400) in Summer, 8.9% (31/350) in Autumn, 0.7% (3/420) in Winter and 9% (49/545) in Spring. In Tanjaro, it was 5.6% (14/250) in Summer, 4.3% (13/300) in Autumn, 0.8% (3/350) in Winter and 6.2% (28/450) in Spring. In Sharbazher, it was 4.4% (11/250) in Summer, 3.5% (8/230) in Autumn, 0.5% in Winter (1/210), and 5.9% (28/470) in Spring. In Qaradagh, it was 3.9% (41/1050) in Summer, 2.7% (25/950) in Autumn, 0.8% (8/1000) in Winter and 5.4% (73/1345) in Spring. In Bakrajo it was 4.1% (33/800) in Summer, 2.2% (19/850) in Autumn, 0.8% (7/880) in Winter and 3.5% (39/1100) in Spring. In Garmiyan, it was 2.6% (13/500) in Summer, 3% (17/560) in Autumn, 1% (6/600) in Winter and 3% (45/1500) in Spring.

Table 2
Prevalence rate of the screwworm during the seasons in different regions in Sulaimani province

Location	Season							
	Summer		Autumn		Winter		Spring	
	No of Exam	No of Infected %	No of Exam	No of Infected %	No of Exam	No of Infected %	No of Exam	No of Infected %
Dukan	400	12 3%	350	31 8.9%	420	3 0.7%	545	49 9%
Tanjaro	250	14 5.6%	300	13 4.3%	350	3 0.85%	450	28 6.2%
Sharbazher	250	11 4.4%	230	8 3.5%	210	1 0.5%	470	28 5.9%
Qaradagh	1050	41 3.9%	950	25 2.7%	1000	8 0.8%	1345	73 5.4%
Bakrajo	800	33 4.1%	850	19 2.2%	880	7 0.8%	1100	39 3.5%
Garmiyan	500	13 2.6%	560	17 3%	600	6 1%	1500	45 3%
Total	3250	124	3240	113	3460	28	5410	262

		3.8%		3.4%		0.8%		4.8%

The seasonal prevalence rate of screwworm in sheep varied between 0.5% in Winter and 9% in Spring in all locations. According to the data Obtained and displayed in (table 3) there were highly significant occurrences of emotion-positive cases ($P<0.001$) among the seasonal prevalence during the study period (table 3). In both years of the study, the seasonally of screwworm case incidence peaked in the period of Spring (4.8%) and decreased to (3.8%) in the Summer and to (3.4%) in the Autumn, reaching its lowest level in the long, cold winter (0.8%).

Table 3
Distribution of the screwworm during the seasons of the year in Sulaimani province

Season	No of Exam	No of Infected%	x^2 Stat	x^2 Cal
Summer	3250	124 3.8%		
Autumn	3240	113 3.4%		
Winter	3460	28 0.8%	213.592**	11.344
Spring	5410	262 4.8%		
Total	15360	527 3.4%		

Significant differences between the seasonal according to chi-square (< 0.001)

Flesh meat was placed in some regions of Sulaimani province in all four seasons of the year and the landing rate of flies was monitored according to the seasons. Heavy fly landing was observed during the spring season in all areas. In the Autumn season, a highly number was observed except in Garmiyan region (table 4).

Table 4
Experimentally detection (naked eye) of Screwworm fly infested on flesh meat putted in some regions in Sulaimani province during the year

Location	Seasons			
	Summer	Autumn	winter	Spring
Bakrajo	+	++	-	+++
Tanjaro	+	++	+	+++
Dukan	+	++	-	+++
Qaradagh	+	++	-	+++
Sharbazher	+	++	-	+++
Garmiyan	+	+	+	+++

{-} = Negative {+} = Low infested {++} = highly {+++} = highly infested

Larvae and flies were collected on the body of sheep and meat placed in all areas and were detected by morphological characteristics (table 5), *Chrysomya Bezziana* was found in Bakrajo, Tanjaro, Dukan, and Garmiyan but was not observed in Sharbazher and Qaradagh. Lucilia Sericata, Calliphora Vomitoria, and Calliphora vicina were found in all locations. Chrysomya megacephala were found in, Tanjaro, Sharbazher, Qaradagh, and Garmiyan but were not observed in Bakrajo and Dukan. Chrysomya albiceps was found only in both Tanjaro and Dukan regions and was not seen in all other locations.

Table 5
Detection of screwworm fly species morphological during the study

Species	Region/ Frequent screwworm fly					
	Bakrajo	Tanjaro	Dukan	Qaradagh	Sharbazher	Garmiyan
Old world screwworm (*Chrysomya Bezziana*)	+	+	+	-	-	+
Green bottle fly (*Lucilia Sericata*)	+	+	+	+	+	+
tropical African latrine blowfly (*Calliphora Vomitoria*)	+	+	+	+	+	+
Blue bottle fly (*Calliphora vicina*)	+	+	+	+	+	+
oriental latrine flies or oriental blue fly *Chrysomya megacephala*	-	+	-	+	+	+
Chrysomya albiceps	-	+	+	-	-	-

{+} = Positive frequent {-} = Negative frequent

The infestation rate differs between the body sites of the sheep, acquired data in (table 6) revealed that there was a highly significant occurrence of the positive cases using Chi-square test ($P<0.001$) among the body sits. The highly infestation rate was in neck 24% (126/527) 2-5 larvae were collected, and the lowest infestation was in ear 1.9% (10/527) 2 larvae were collected.

Table 6
Infestation rat according to the body site of the sheep

Body site	No. Infected animal	Percentage rate %	No. of larva collected	x^2 Stat	x^2 Cal
Neck	126	24	2-5	203.981**	20.090
Back	97	18.4	4-5		
Coccygeal	82	15.6	9-13		
Abdomen	62	11.8	2-4		
Fat tall	61	11.6	7-11		
Thigh	44	8.3	3-5		
Head	32	6.1	5-8		

Udder	13	2.5	5-7		
Ear	10	1.9	2		
Total	527	%100	-		

Significant differences according to chi-square (< 0.001)

There was no significant difference between animal ages using Chi-square test for independence (P<0. 001) (table 7). Rate infestation was 3% (151/5020) at less than 2 years, and 3.6% (376/10340) at more than 2 years.

Table 7
Distribution rate of infestation sheep with myiasis according to age

Age	No. of sheep infested	No. of sheep infested	%	x^2 Stat	x^2 Cal
≤ 2years	5020	151	3.00	4.027$^{n.s}$	6.634
≥ 2years	10340	376	3.63		
Total	15360	527	3.43		

No Significantly differences between age according to chi-square at level (P< 0.001).

The data in (table 8) revealed that there was no significant difference in the occurrence of positive cases using Chi-square test for independence (P<0. 001) between males and females, in males was 2.95% (150/5069) and in females were 3.66 % (377/10291).

Table 8
Infestation rat according to animal sex

Sex	No. of sheep infested	No. of sheep infested	%	x^2 Stat	x^2 Cal
Male	5069	150	2.95	5.083$^{n.s}$	6.634
Female	10291	377	3.66		
Total	15360	527	3.43		

No Significantly differences between sex according to chi-square at level (P< 0.001).

Discussion

During 2021–2022, the prevalence of screwworms was reported in some districts and sub-districts of Sulaimani province. Our results, as illustrated in (table1), out of 15,360 sheep examined, 527 sheep (3.4%) were infected with myiasis during the year (2021-2022) from six visited locations. The prevalence rate of myasis in this study shows a high compared with previous studies in some countries, such as: In Riyadh region of Saudi Arabia, the prevalence of myiasis infestation was estimated to be 2% in sheep (Alahmed, 2004), and in Kashan-Iran County, the prevalence of myiasis infestation was estimated to be 2.3% in sheep (Dehghani, et al., 2012) and 2.91% in several farms in Ecuador (Rodrguez, et al., 2019). Also, (Giangaspero, et al., 2011) who reported that the overall prevalence of myiasis was 3% in Italy. As well as (Jahan, et al., 2019) recorded the prevalence of

myiasis in sheep was 2.68% in Bangladesh. And the findings are thought to be close to those of Juli et al., (2015) and Rodrguez et al., (2019), who found that the overall prevalence of myiasis was 4.11% in Dinajpur, Bangladesh, and 3.36% in Ecuador, respectively. The results we have obtained are lower than the other researchers' results by (Alhakima, et al., 2020) in Babel/Iraq and (Osman and Omer, 2017) Saudi Arabia, as well as (Imtiaz, et al., 2014) in Bangladesh, while in the same country record the highly prevalence of myiasis was 7.52% (Jahan, et al., 2019 The differences might be attributed to regional climatic differences and the prevalence of screwworm which is determined by a variety of factors like fecal sailing, animal rearing and husbandry.

In Spring, the prevalence of myiasis is higher than in other seasons in all regions except Bakrajo, where the highest prevalence of myiasis was in Summer. These results were closely similar to those of (Eesa and El-Sibae, 1993) who recorded a maximum abundance of Chrysomia flies in Spring and a minimum in Winter. Also, (Madeira, et al., 1998) found that the infestation reaches its peak during Summer and Spring and is lowest in the fall and Winter. Our results were similar to those of (Alhayali and Mohammed 2021; Broughan and Wall, 2007), who stated that the highest prevalence rate in sheep was recorded during Summer and Spring. However, our results are contrasting to the results of (Abass and Abdull, 1997) study, which found that the highest screwworm infection was in the early Winter season in Al-Qadisya and Babylon/Iraq, then decreased gradually until the hot months. It reached zero during July and August.

The low infection rate in Winter may also be due to the fact that screwworms cannot tolerate the winter cold and stop growing, and sheep are less susceptible to injury due to longer stays in the field and more monitoring and control by their owners. Fly population changes are largely driven by the weather. However, screwworm fly species had two population peaks in Spring and Autumn. Similar results were obtained in other studies in the Middle East/Iraq (Alahmed, et al., 2006; Siddig, et al., 2005) these authors concluded that the screwworm fly and other fly pupae did not develop into adults during the cold winters and that adult flies had low survival rates during the hot and dry Summers. Our findings support the findings of (Al-Taweel et al., 2014; Siddig et al., 2005; and Osman and Omar 2017), who found that pupal development conditions are optimal in the Spring.

Our results disagree with (Spencer, et al., 1981) who reported that the seasonal population lows during Spring 1978 and 1979 and the seasonal population highs during Winter in the tropical area and (Koller, et al., 2011) have recorded that screwworm populations reach their peak in late Autumn and early Winter. Oliveira, et al., (1982) also reported greater abundance of this species during the Winter and Spring in Rio de Janeiro. However, (Ribeiro, et al., 1993) described a single population growth in early summer in studies in Rio Grande do Sul. Furthermore, large numbers of fly were caught during the summer months in six sites in the southern United Kingdom in the year 2005 (Goulson, et al., 2005). Variations in the seasonality of screwworm may result from climatic conditions found in different Sulaimani province regions, and this might be due to the increased activity of adult screwworm flies and favorable climatic conditions for

them. This result suggests that low temperatures may restrict flies' development during the winter, also vegetation cover played a role in screwworm distribution.

Investigation three genus and sex species are observed in all regions and determined by morphological characteristics, Chrysomya Bezziana is found in all regions instead of Qardagh and Sharbazher, this species is responsible in most cases of myiasis, this is in agreement with the results of the study (Raad, 1999) which found that 97.01% of the larvae of Chrysomya Bezziana were responsible for myiasis in Baghdad province. Lucilia Sericata, Calliphora Vomitoria, and Calliphora vicina were found in all regions, our results are roughly consistent with the results found by (DN and EC, 2009), Also (Şaki and Özer 1999; Özdal, 2004 and Sevgili, et al., 2004) reported that C. vicina, C. vomitoria, and Ch. Albiceps, one of the flies that cause myiasis in Turkey. In this study indicated the main site of infestation in sheep was the neck (24%) and the lower infestation site was the ear (1.9%). Our result was in agreement with (Rodríguez, et al., 2019). However, our results also contrasting with (Alhayali and Mohammed, 2021) who recorded the number of animals infected with myiasis of the breech region was higher than the infection level in other parts of the body.

The results showed that the sheep older than 2 years were more infested than sheep younger than 2 years. The present result was in agreement with (Osman and Omar, 2017; Dehghani, et al.,2012; Islam, et al., 2015 and Rahman, et al., 2009) who investigated that the higher infestation was in the old age group than the young. But our result doesn't agreement to the result obtained by some authors, including (Imtiaz, et al., 2014; Juli, et al., 2015; Hasan, et al., 2008 and Alahmed, 2004) stated that the occurrence of myiasis was higher in young animals than the adult animals, this difference may be due to an adult's ability to defend themselves more than a young.

The results presented in (table 8) shows infestation higher in females compared to males. Our study was in agreement with (Jahan, et al., 2019; Osman and Omar, 2017; Imtiaz, et al., 2014 and Alhakima, et al., 2020) who reported that the infestation in females was more than in males, while the results was disagreement with those described by some authors (Alhayali and Mohammed, 2021; Taleb, et al., 2015 and Tenquist and Wright, 1976) who identified that the infection rate was high in males compared to female, The physiological variations between the sexes or the higher density of females in comparison to males may be the cause of this difference and the sex-related pattern outcome may be due to hormonal changes

Conclusions

Three genus and sex species are observed in all locations and determined by morphological characteristics. Chrysomya Bezziana is found in all regions instead of Qardagh and Sharbazher, Lucilia Sericata, Calliphora Vomitoria, and Calliphora vicina were found in all regions. The highest distribution is shown in the Dukan region. The highest rate of myiasis was found in the spring. In this study, to accurately detect flies, fresh meat was placed in several areas, observed the heavy fly landing during the spring season. The highest myiasis was noticed in the neck, which shows the high infestation of myiasis in females.

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