



**STUDY OF DENSITY SHEEP BOT FLY *OESTRUS OVIS* L. (DIPTERA: OESTRIDAE)
AND HEMATOLOGICAL EFFECTS AND DRUG IN BASRAH GOVERNORATE**

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ABSTRACT

This study deals with numerical density of sheep bot fly (*Oestrus ovis* L.) (Diptera: Oestridae), which infects sheep, and the effect of this infection on blood parameter of these animals. It also studies the efficiency of using ivermectin in treating the sheep infected by this fly. The highest percentage of infection by this insect is % 49.32 out of the total number of samples. The area of Zubeir and Abul-Khaseeb have the highest ratio, % 69.69, 57.14 respectively while the lowest percentage is in Hay-Al-Husein, % 26.78. The study shows the effect of blood parameter of the infected animals. The study, again, has found an decrease in the amount of haemoglobin in the sheep infected by botfly in comparison with healthy sheep after giving ivermectin, beside other changes in hemic and biochemical properties. The ratio of haemoglobin is 10.mg/100ml Packed cell volume is 27, with rise in number in red blood cells $660 \times 10^4 \text{ cell } \cdot \text{m}^{-3}$, white blood $18225 \times 10^4 \text{ cell } \cdot \text{m}^{-3}$. Moreover, there is no effect in cholesterol 83.5.mg/100ml and Albomin 3.4.mg/100ml comparison control sheep haemoglobin is 14.25.mg/100ml Packed cell volume is 35, red blood cells $380 \times 10^4 \text{ cell } \cdot \text{m}^{-3}$ white blood cells (wbc) $9900 \text{ cell } \cdot \text{m}^{-3}$ cholesterol 84.5.mg/100ml and Albomin 4.2mg/100ml. Ivermectin has shown good efficiency in treatment of the infection by this insect. Values of blood parameter approximate the values in the animals of control.

INTRODUCTION

Oestrus ovis (L) is considered one of the most important ectoparasites for forced parasitism in the family Oestridae (Zumpt, 1965) and infects sheep and goats as major hosts, making it of great importance in areas where these animals abound and may infect other animals or humans as a host accidental upon injury (Pandy, 1989; Hall & Wall, 1995).

Female flies of these insects are highly specialized in choosing only one host and rarely more than one host they are also attracted to those natural hosts and do not need the presence of moisture or wounds (Price, 1980) where female flies search for the appropriate location in the host in which visual stimuli are available visual stimuli or olfactory stimuli (Otranto & Colwell, 2008), in some species, eggs or larvae are laid directly towards the site of infection.

Sheep flies behave as obligatory external parasites when infecting sheep, as the larvae are one of the main causes in the occurrence of nasopharyngeal typhus disease, which has negative effects on the health and productivity of economic animals. Olejnicek, 1988; Yilma & Dorchie, 1991; Bart & Minor, 1998.

Nasopharyngeal myiasis is one of the animal diseases that occurs as a result of scratching and stimulating the

sinuses of sheep by the mouth clips and cuticles of the larval stages of the insect, which leads to infections in the sinuses and infections, and sometimes an increase in nasal secretions is observed (Goddard et al., 1999; Radostitis et al., 2000; Bakeer et al., 2009) And this disease continues for several months or intermittently in areas with a temperate climate or in the hot or dry season. Infections are noted in the sinuses during the breeding season of the insect, while rhinitis appears when the first phase begins to develop, which appears a few weeks after the flies infect the animal and the effects develop Pathogenesis in the second and third phases ((Horak & Snijder, 1977; Tabouret et al., 2001 With the occurrence of sinus infections, lung abscesses appear in the form of pus similar to the symptoms of Buck's infection *Conurus cerebralis* and the gradual demise of the bones of the skull and damage to the brain from the mouth clips, which leads to lack of coordination in walking and the animal appears to be about to fall, and then the disease is described as false dizziness (Faligid Kaufmann, 1996;)) Gabagi et al., 1993; Soulsby, 1982.

An increase in the mucous secretions of the nose, which becomes a source of bacterial infection, and the disease progresses more severely to the occurrence of damage and pus in the lung and lung infections, and in some cases, blood comes out with mucus (Bunch, 1980; Dorchie et al., 1998; Bapna et al., 2006).

In most severe or high-density infections, the larvae can penetrate the bronchi and thus become fatal to the animal, especially when they are present in large numbers. In other cases, a small number of larvae, perhaps up to ten, are considered harmful to the animal (Biggs *et al.*, 1998)

The results also showed a change in the values of the above blood parameters after treating the infected sheep after using ivermectin treatment.

Among the symptoms of this disease in animals infected with mutton flies in sheep are loss of appetite when eating food with animals biting their teeth, lethargy and weakness in performance and snoring and then death. The nasal secretions may be covered with a dry layer of dust, which leads to difficulty breathing through the mouth in this case and in other cases it causes miscarriage, loss of fertility and death of weak and old animals (Goddard *et al.*, 1999; Dorchies *et al.*, 2000; Alcaide *et al.*, 2005).

The medical importance of flies lies in the economic loss, which is represented in the loss of animal products from meat, milk and wool. When the infection reaches 4%, a weight loss occurs by (1-4.5 kg) and the amount of wool is from (500-200) g, and the reduction in milk products is more than 10%, and meat is .by (4.6-1.1) kg (Ylima & Dorchies, 1991; Ilchmanet *et al.*, 1991).

Treatment and using the appropriate drug leads to a reduction in nasal secretions and an increase in weight improvement and protection of the animal from re-infestation during the season of flies (Horak & Snijders, 1974; Dorchies *et al.*, 1991) between Frugereet *et al.*, (2000) that nasal larvae and during migration into the nasal cavities of sheep secretes proteolytic enzymes and has the ability to immunomodulate the host body and has strong antigenic properties, so these proteins can be used in serological tests to detect antibodies.

MATERIALS AND WORKING METHODS

(148) sheep animals were examined, as (66) were examined in Al-Zubayr, (61) in Al-Hussein neighborhood and (21) Abu Al-Khasib. The animals were of age (4-5 months) for the time period from October 2017 to September 2018.

Then, Mitchell (1941) & Cobbett's method was used in America by making a longitudinal incision from the bottom of the head area using a sharp and strong tool, as well as using thin and precise blades to open the sinuses to reveal the larvae and then collecting them after calculating their numbers, they were also preserved in glass bottles containing ethyl alcohol 70% as well.

Processing method

(6) animals were selected from sheep infected with sheep flies after clinical examination by the veterinarian and recorded symptoms of infection and took 6 from control

(healthy) animals and injected the same group of infected animals with ivermectin drug at a dose of L/0.2mg recommended globally, as they were injected under the skin in the forelimbs area and then left for fourteen days in a barn designated for it to avoid mixing with the rest of the other sheep animals, and then blood samples were taken for the purpose of making measurements of blood parameters and estimating the vital components of blood serum.

Blood Analyzes

Blood samples were withdrawn by medical syringes from the jugular vein of sheep at a rate of (10) ml after removing or cutting the wool from the neck area through which this vein passes and then sterilizing it before and immediately after the withdrawal. The blood samples were divided into two equal parts (5) ml and placed with the blood sample Heparin (an anticoagulant) for the purpose of blood measurements, which is in addition to measuring hemoglobin (Hb), compacted blood cell volume (PCV), red blood cells and white blood cells.

As for the other part of the blood sample, it was placed in tubes that did not contain heparin, as it was used to measure both albumin and cholesterol, and the centrifugation process was conducted at a speed of 3000 rpm to obtain the blood serum.

Blood parameters

Red blood cell count (RBC) The number of red blood cells was calculated using (Haemocytometer Slide) and Hymu's Solution according to Schalm, 1986) by calculating the number of red blood cells in five squares $\times 10,000$.

White blood cell count (WBC)

The number of white blood cells was calculated using (Haemocytometer Slide) and Turkey's Solution, according to Schalm's method, 1986) according to the following equation:

The number of white blood cells/1 mm³ = the number of white cells in the four squares $\times 10$ (depth) $\times 20$ (dilution)/4

PCV quantification

The volume of the compacted blood cells was estimated using micro-capillary tubes clogged from one end and placed in a Haematocrite Centrifuge at a speed of 3000 rpm for a period of 10 minutes, then the value of the aggregated blood cells was calculated using the dedicated scale (reader (Haematocrite) according to the Schalm method. 1986

Estimation of the biochemical parameters of blood serum albumin Albomin

The concentration of albumin in serum was estimated according to the method attached to the analysis kit produced by the Egyptian Spectrum company. The samples were read at a wavelength of (546) nm

according to the method of Tietz (1994) using the following equation:

Total albumin g/100ml = sample reading/Standard solution x 6 (Standard solution concentration).

Cholesterol

Cholesterol was estimated using the analysis kit produced by the Egyptian Spectrum company, and the samples were read at a wavelength of 540 nm according to the method of Tietz (1994) and the following equation was used:

Total cholesterol mg / 100 ml = reading of the sample / standard solution x 200 (concentration of the standard solution).

Statistical analysis

A Complete Randomized Design (CRD) was used using an electronic calculator according to the SPSS statistical

processing program. Also, ANOVA and Revised Least Signification Difference (RLSD) test were conducted at probability level $P > 0.01$ to analyze the results of the study (Al-Rawi and Khalaf Allah, 1980).

RESULTS

Percentage of infestation and population density of sheep flies in sheep:

The results of the study showed that the percentage of sheep infestation with sheep flies was 49.32% of the total (148) sheep of the study samples. 69.69% and 57.14%, respectively, while the percentage of sheep infection in the AlHussein neighborhood was low, reaching 26.78% of the study samples in those areas (Table 1).

Table (1): The percentage of sheep infestation with sheep flies in the study areas.

| Regions | Total Number | Infected Number | Percentage of Infection % |
|-------------------------|--------------|-----------------|---------------------------|
| Zubair | 66 | 46 | 69.69% |
| Al-Hussein neighborhood | 61 | 15 | 26.78% |
| Abul-Khaseeb | 21 | 12 | 57.14% |
| overall average | 148 | 73 | 49.32% |

On the other hand, the variation in the number of sheep animals infected with sheep flies in different months of the year, where the percentage of sheep infestation with these insects in the study areas varied as in Table (2), which shows the presence of two periods during the year in which the percentage of infection is high. The temperature is low in the months of October, November, December and January, as the infection rate in these months was 64.28%, 70.0, 80.0%, 60.0%, respectively,

while the second period of the year had the highest percentage of infection high when the temperatures are increase, as it was in the months of July, August, and September, where the infection rates in these months were 71.42%, 68.75%, 61.11. As for the rest of the months of the year in February, March, April, May and June, the percentage of infection was low, in these months 6.66%, 10.0%, 20.0%, 30.0%, 36.36%.

Table (2): The percentage of sheep infestation with larvae of sheep flies during the months of the year.

| Months | Percentage of infection % |
|-----------|---------------------------|
| October | 64.28% |
| November | 70.0% |
| December | 80% |
| January | 60.0% |
| February | 6.66% |
| March | 10.0% |
| April | 20.0% |
| May | 30% |
| June | 36.36% |
| July | 71.42% |
| August | 68.75% |
| September | 61.11% |

The results in Table(3) show that the total number of larvae of sheep flies in sheep with their three different stages that were isolated from infected animals during the study period amounted to 740 larvae. In September,

264 caterpillars, while the lowest value was in March 2, caterpillars.

The results showed that there is a discrepancy in the percentage of the presence of the numbers of larvae from

one stage to another, as the highest percentage of the first larval stage was in July 78.9%, while the lowest percentage of the presence of this stage was in February 25%, while in the months of January, March, April and May its presence was not observed and also varied the percentage of presence of the numbers of the second instar larvae, where the highest percentage was in July 79.5%, and the lowest percentage was in November 21.5% and they are present throughout the months of the year, while the highest percentage in the third instar larvae was in March 50% and the lowest percentage of their presence during the months The year was in July 1.5%, and its presence was not observed in June (Table-3).

On the other hand, the average number of larvae per animal varied over the different months of the year in the affected sheep. The severity of infection was high in the months when temperatures are high, as it is in the months of July, August and September, when it reached (13.3, 8.22, 22.36) larvae per animal. Also in the months of October, November, December and January, the severity of the infection in these months was (6.44, 9.2, 8.25, 5.3) per animal. As for the rest of the months of the year, February, March, April, May and June, the severity of the infection was Low (2.25,3,3.5,2,4) as in Figure (1).

Table (3): Numbers of sheep infestation with larva of sheep fly during the months of the year.

| Months | First stage % | second stage % | third stage % | population density | Number of infected animals | Number of larvae/animals |
|-----------|---------------|----------------|---------------|--------------------|----------------------------|--------------------------|
| October | 55.2 | 36.2 | 8.6 | 58 | 9 | 6.44 |
| November | 73 | 21.5 | 4.6 | 65 | 7 | 9.2 |
| December | 60.6 | 27.3 | 12.1 | 66 | 8 | 8.25 |
| January | - | 75 | 25 | 32 | 6 | 5.3 |
| February | 25 | 50 | 25 | 4 | 1 | 4 |
| March | - | 50 | 50 | 2 | 1 | 2 |
| April | - | 57.2 | 42.9 | 7 | 2 | 3.5 |
| May | - | 66.7 | 33.3 | 9 | 3 | 3 |
| June | 66.7 | 33.3 | - | 9 | 4 | 2.25 |
| July | 78.9 | 79.5 | 1.5 | 133 | 10 | 13.3 |
| August | 63.7 | 30.8 | 5.5 | 91 | 11 | 8.22 |
| September | 47.3 | 42.8 | 9.8 | 264 | 11 | 22.36 |

Hematological parameters measurements

The results of hematological analyzes in the table(4) show the negative effect of the presence of larvae of sheep flies in the cavities and sinuses of sheep infected with these insects through the scratches they cause inside the nasal cavities of sheep.

The results of analyzes of the blood parameters values in Table (4) showed that the sheep infected with the different larval stages of the sheep flies negatively affect the general health of the infected animals through changing these values with the blood parameters values of the control sheep. There are significant differences in some blood parameters and no significant differences in other parameters.

The results of the statistical analysis showed that there was a significant difference in hemoglobin in the blood, where we noticed a decrease in hemoglobin values of 10 mg/100 ml in the affected sheep compared with the hemoglobin values of the control sheep of 14.25 mg/100 ml, as well as a decrease in the values of the average stacked blood cells of the infected sheep, which reached 35, while in the animals the control was 35, while the number of red blood cells was 660×10^4 cells/mm³ in the infected sheep, while in the control sheep it was 380

$\times 10^4$ cells/mm³. Also, the values of the total numbers of white blood cells in the infected sheep increased to their highest levels, reaching 18225 cells/mm³, while their numbers in sheep Control 9900 cells/mm³.

The results of the study showed that the effect of infection in the serum content of cholesterol and albumin was no significant differences, where the value of each of them was 83.5 mg / 100 ml and 3.4 mg / 100 ml, respectively, compared to the control values, where the value of cholesterol was 84.0 mg / 100 ml and albumin was 4.2 mg / 100 ml.

Table (4): Hematological measurements of sheep infected with mutton flies before and after treatment.

| Hematological parameters | control sheep | Infected sheep | sheep after treatment | R.L.S.D. |
|--------------------------------|----------------------|----------------------|-----------------------|----------|
| Hb mg/100ml | 14.25a | 10 c | 12b | 1,35 |
| PCV | 35a | 27b | 32a | 3,90 |
| Total RBC Cell/mm ³ | 380c×10 ⁴ | 660a×10 ⁴ | 524b×10 ⁴ | 99,00 |
| Total WBC Cell/mm ³ | 9900c | 18225a | 11225b | 1352 |
| Cholesterol mg/100ml | 84.0a | 83.5a | 83.9a | NS |
| Albumin mg/100ml | 4.2a | 3.4a | 3.2a | NS |

- Different letters mean a significant difference.
- NS is insignificant.

DISCUSSION

The rate of sheep infestation of sheep flies in Basra during the study period was 49.32%, while the rate of sheep infestation with these varying flies in different countries of the world reached 58.0% in Jordan (Abo Sheheda *et al.*, 2000) and 23% in Saudi Arabia (Ahmed, 2000) and India 8.1% (Pathak, 1992), Libya 22.6% (Gabajet *et al.*, (1973 and 8.7% in Egypt, Amen *et al.*, 1997)) and the Maghreb 10-100% (Pandy & Ouhelli, 1984; Colebrook & Wall, 2004).

While the percentage of sheep infection in the rest of the world was high, as it was in America (Meleny *et al.*, 1962) (97-96) and 91-55.8% in Italy (Scala *et al.*, 2001, 2002 Caracabba *et al.*, 2000,), Spain 71% (AL-Caide *et al.*, 2005), France 33.2-65% (Jacqiet & Dorchie, 2002; Suarez *et al.*, 2005) and 21% in Ethiopia (Bekel & Mukasa-Mugerva, 1994).

The differences in infestation rates may be due to the climatic conditions that differ from one region to another, which in turn affects the activities of insects (Yilmaet *et al.*, 1991; Yilma, 1992, where the activity of adult sheep flies in sheep throughout the seasons of the year in humid tropical areas (Goddard *et al.*, 1991). *al.*, 1999; Taburet *et al.*, 2001), but in cold or dry regions, its effectiveness is limited to one season of the year, as in Russia and Egypt (Goaboub, 1978; Bukshynov, 1976).

In areas characterized by moderate thermal environments, there are two generations per year, including southern Russia (Bukshynov, 1976, Chad (Graber, 1964), India, Chabrah & Rubrah, 1976), Tunisia (Kilani *et al.*, 1986, and Iraq (Halafi, 2001), while a number of Of the generations in these insects in humid environments, including Texas (Cobbett & Mitchell, 1941) and North Africa (Horak, 1977) and the Maghreb (Pandey & Ouhelli, 1984).

The increase in the number of larvae of sheep flies in sheep with its three stages represented the largest proportion of first-stage larvae compared to the second and third instars in the infected heads of these animals. The nutritional requirements of the first instar are less than the nutritional requirements of the second and third instars, and the loss and fall of the larvae of the second and third instars is more than the fall of the larvae of the

first instar when the animal sneezes and thus may make their numbers many (Dorchies & Alzieu, 1997).

In this regard, Dorchie *et al.*, (2000) indicated that an increase in the number of second and third instar larvae leads to cellular reactions at the site of infection, especially eosinophils and mast cells. On the other hand, when development occurs in all third instar larvae, they will become fatal and fatal to infected animals. Nguyen *et al.*, (1996), the existence of an immune regulation mechanism that controls the survival of the communities of these larvae, and sometimes a decrease in the parasite community occurs due to the death of the larval stages in the cold and hot seasons or in the dry season, and an increase in the number of first-stage larvae of these insects regulates the survival and continuity of the parasite community in the host (Dorchies *et al.*, 1996).

It turns out that the appearance of all the larval stages in the heads of the affected sheep at the same time, it may be due to the process of laying the larvae by several female flies, or perhaps not all the larvae develop at the same time, even if they were laid by one female. (Dorchies *et al.*, 1996)

Increasing the number of larvae of sheep flies in all stages. In the summer, Grunin (1957) mentioned the presence of more than 350 larvae and their different stages in one of the heads of infected animals from sheep in the dry season of the year in Russia and from

In most cases, the number of larvae is not large, as it was mentioned by Zarzis and Amin (1987) and Mustafa (1986), there are usually 8-1 larvae in the head of one animal and their number may increase and reach 80 larvae. In other sheep, the numbers of these larvae may be found 1220- larva in America (Cobbett & Mitchell, 1941)

Cepeda-palacios & Scholl, (2001) that the number of larvae ranges from 24-3.

In Italy, the number of larvae reached 86 larvae (Scala *et al.*, 2000) and Ahmed., (2000) mentioned that the largest number of larvae found 28 larvae in the head One of the infected sheep and between Abu al-Hab (2004) these

larvae are present at a rate of 3-4 larvae and may reach 65 larvae in the heads of infected sheep.

Sheep are negatively affected when infected with sheep flies than those animals not infected with these insects, and it is possible to observe the effect of this infection in the different measurements of blood parameters of these infected animals from the uninfected animals, as well as when compared to control animals, where it is observed in the infected sheep that the hemoglobin decreases in the infected animals compared to the control animals. In which the hemoglobin level is consistent with what Abdullah indicated (2009). The decrease in hemoglobin in sheep infected with mutton flies is attributed to the effect caused by the larvae on the respiratory system, as they block the nostrils, causing in many advanced cases to suffocation, increased demand for oxygen, and an increase in the number of red blood cells (Kusiluka & Kambrange, 1996). It is also noted that treating infected animals reduced that effect through an increase in hemoglobin values.

It is noted through the results of a decrease in the rate of PCV in infected animals than what it is in control animals, and this may be due to an increase in the values of RBC red blood cells and then return to normal levels after treatment. Despite an increase in RBC and a decrease in the amount of hemoglobin, there are indications that the presence of larvae in the nasal cavity may lead to the decomposition of some red blood cells due to the activity of the enzymatic activity phospholyase, which degrades the protein Sphingomyelin in the membranes of red blood cells and epithelial cells, causing an increase in the permeability of the membranes and consequently the loss of red blood cells (Kusiluka & Kambrange, 1996).

An increase in the number of white blood cells, which occurs due to the work of these cells as a defense against the parasite, in addition to the stressful situations to which the animal is exposed, especially environmental factors such as high temperatures (Despopoulos & Sibernagl, 2003).

No effect of infection on serum cholesterol level was observed in infected animals compared to control or control animals. This seems clear in that the parasite does not infect the liver, as it is the main source of cholesterol formation (Rastogi, 2007).

No change was observed in the serum albumin content and its levels were within the limits indicated by Abdullah (2009).

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