Polymorphism larval stages of *Oestrus ovis* isolated from frontal sinuses and horn cavities of sheep's head Slay in Basrah abattoir

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Abstract

The study include isolated larvae of *Oestrus ovis* of diptera from frontal sinuses and horn cavities of sloughed sheep heads. It could be differentiation from another species of Diptera which cause myiasis by some characters. Three stages of the larvae were isolated. In generally the larva stages contain eleven segment, cephalopharyngeal skeleton connect with two hooks. The characteristic of L1 stage were white in color, measured L1(3-4mm) in length ,have not spines and structure of posterior spiracular have D shape & yellow in color whereas internally small structure like D shape was present with brown color and the pits or pores occur in whole structure . The L2 stages are slightly grey in color ,measurement was (6-12mm). In contrast the L3(20-19 mm), with grey to brown in color and have plate dark brown to black color on dorsal surface .posterior spiracular of L2 & L3 stages have D shape and brown in colors with many spread pores, the ventral surface tegument contain three arrows of spines. Third instar larvae are developed and become pupate during 6- 12 hours out of animal body .

Introduction

The myiasis has been define as infestation of live vertebrate animals with dipterous larvae for certain period and feed on dead or living tissue, liquid body substance, or ingested food of the host (1) .Larvae of *oestrus ovis* infestation had taken many terms as synonym, sheep nasal fly, sheep bot fly and oestriosis which belonging to family oestridae , infestation of *Oestrus ovis* was considers specific myiasis (2) .The life cycle began when female flies deposited their tiny larvae around the nostril (3), the larvae beginning pray on tissue in order to grow than creeping up ward to penetrate the nasal barrier to the frontal sinuses and horn cavities to moult from first stage larvae L1 to second stage larvae L2 and than L3 or (maggots), these stages cause upset of animals to cause what called false gid (4), thoroughly the sneezing of animal lead to throwing the L3 instar to ground and pupate during 24 hours is developed stays 3-6 week before to hatch to the adult fly (5).Myiasis as generally are dangerous disease on human

and animals health . The larvae were caused myiasis which assure harmful to the animals and cause large decay wounds and at spinning season, the larvae move to the frontal sinuses lead loss of growth or may be animal's, body weight loss of 1.1–4.6 kg of weight and up to 10% in milk production in infection by *Oestru ovis* (6).Myiasis were recorded in human,when *Chrysomya bezziana* occurred in two children (4-8 years) in Basrah city (7). Also the infestations of *oestrus ovis* were recorded in human were record in some countries like Spain when ten person suffer from *Oestrus ovis* myiasis (8). (9) in kuwait was observed one patient suffered from the symptoms of acute foreign body sensation, irritation, redness, lacrimation and photophobia and re-examined confirm a ophthalmomyiasis by *Oestrus ovis*, also(10) recorder ophthalmomyiasis in person at 25 age in India The aim of this study was to isolation and identification of larval stages of *Oestrus ovis* in slaughtered sheep in Basrah city.

Material and Methods

A total of 180 larvae from 42 infected sheep were collected in the sloughers houses in basrah city during the period from (April , May- 2010).The samples of different stages of larvae from frontal spaces and horn cavities of sheep after slougher in abattoir were taken, then the larvae were kept in ethyl alcohol 70 % and others in formaline 10% then sent to the laboratory for diagnosis.A procedure by (11) was done depended upon the samples which prepared in laboratory in order to recognize the diagnostic features which included the posterior end spiracles and cephalopharyngeal skeleton, as the following :

1- cut the target part and washed with normal saline and then these parts were kept in solution of sodium hydroxid 10% for 24 hours .

2- washed these parts with normal saline for 10 minutes, repeated wash for three times

3- The samples were passed an ascending concentration of ethanol 35% , 50% ,70 % , 90% , 100 % .

4-Samples were kept in mixed solution of xylol and ethanol (1:1) for 10 minutes.

5- samples were passed in absolute xylol for 5-10 minutes.

6- The samples were mounted on slides and fixed by canada blism and left 24 hours on hot plate and inspect under microscope .

Results

The infected sheep by *Oestrus ovis* in many barns had suffer clinically from nasal discharge and in sever cases combination with bleeding , sneezing and at sometime expelled out, head shaking, fever, pneumonia.In the abattoir the postmortum showed different larvae which are settle in frontal sinuses of the head horns' cavities, also observed inflammation fluid & congested area figure (1-2).Larval stages: The general structure of larvae consist of eleven segment or rings,Different posterior spiracular (Figure 3), three arrow of spines in ventral surface of rings, and cephalopharyngeal skeleton connect with two hooks. Also showed different stages of larvae with different measurement (figure 4).

1- First stages larvae (L1): white in color, the measurement was 3-4 mm in length, posterior spiracular were small and like D shape the whole structure have spread pores

or pits which are separated by fine tubercles , and loss of spines, (figure 5,6).

2- Second stage larvae(L2): slightly grey in colour, the measurement was 6-12 mm in length, have clearly three arrows of minute spines, the posterior spiracular brown in colour and have D shape the pores separated by fine tubercles (figure 7,8,9).

3- Third stage larvae (L3): brown to grey in colour, the measurement 19-20 mm in length three lines of large clearly spines, dark brown plates in dorsal surface of larvae , the posterior spiracular brown in colour with D in shape and the pores separated by fine tubercles (figure 10 , 11, 12).

-Third stage larvae were transformed to pupate during 12-24 hours (the turgument began to shrink and solidation, (figure 13 and 14).

-Cephalopharyngeal skeleton connect with two hooks like sickle present in anterior end, turgument with three arrow of spines (figure 15,16, 17,18).

Figure (1, 2): Larva in front-sinuses and horn cavities (↙), inflammtion fluid in infection sites (↘)

Figure (3): Posterior spiracles before prepare to slides

Figure (4): different stage larvae

Figure (5) : first stage larvae (L1) (4mm)

Figure (6) Small Posterior spiracular of L1

Figure (7) : second stage larvae (L1)
(9mm)

Figure (8): (A) tubercles, B) pores ,
(C)extended active spiracular

Figure (9) : second stage larvae (L2)
(12mm)

Figure (10) : complete extended
posterior spiracular especially in L3

Figure (11) : Third stage larvae
(20mm)

Figure (12) : third stage larvae (9mm)
With spiracular plate dorsally (↙)

Figure (13) : Third stage larvae began transform to pupate during 12-24 hours (the tegument began to shrinkag)

Figure (14) : pupate after 24 hours

Figure (15) : Cephalopharngéal skeleton in larvae of *Oestrus ovis*

Figure (16) : Cephalopharngéal skeleton in larvae of *Oestrus ovis*

Figure (17) : show three arrow of spines in tegument of L2 ,L3 stages of *Oestrus ovis*

Figure (18) : L1 stage loss spines in tegument

Discussion

The *Oestrus ovis* is very important flies of Dipetra that cause myiasis in head cavities of sheep and rarely in goat. It is also have a risk in human health with wide spread or become outbreak (12) . (13) was identified the dipetra depend on infection site such as *Hypoderma bovis* which parasitized the cattle skin and *Oestrus ovis* in nostile, while the accurate diagnosis involve the isolation of larvae from deepest part of infection sites inorder to examination of different stages of the larvae (14).The infectious animals were suffered from nasal discharge with staining by blood , also internally the inflammation fluid were found around larvae in sinuses that confirm the response of immunity system against the larvae, nevertheless (15) refer to hypersensitive reaction at the site of tissue invasion with a massive infiltration of eosinophils and mast cells in the nasal mucosa beneath the epithelium.The collection 4-16 *Oestrus ovis* larvae from frontal space and horns cavities of one to two infected animals confirm the intensity of infection, this may be due to the present of predisposing factors to spread of *Oestrus ovis* like temperature, wind and solar irradiance (16).(1) and (17) were identified a key to classification the larvae belong to dipetera, its precious characters of *Oestrus ovis* than others larvae of dipetera are present numbers of segment, number of lines spines and especially the form and structure of posterior spiracles.In generally the maggots of *oestrus ovis* have eleven ring also considers one diagnostic feature(18) remained the *Oestrus ovis* have 12 segment but the one and two segment are connected .whatever it contrast with other species such as *Musca domestica*.The larvae of diptera have different characters in posterior spiracles in shape or structure , Larvae of *Oestrus ovis* have spiracles D shape whereas in *Chrysoma bezziana* the as plates and in *Lucilia sericata* as pyiform (19).A new

record to describe L1 which have posterior spiracular as form like D shape with yellow colour whole size but centrally is brown colour and pits well show extended in all structure of spiracles . In L2, L3 the posterior spiracles are also D shape and brown colour with pits that agree with (20) was refer to found many of pits in spiracles of *Oestrus ovis* when classified the third instar larvae.The L1 length was 3mm in measurement and this was result agree with (21) who refere to L1 length was 1-3 mm and loss of spines in larvae tugumment , After grown of L1 average larval length increased therefore transform to L2 (length 6-12 mm) and than to L3 (length 19-20 mm), however the (22) in study of Weight of *Oestrus ovis* showed that the weight of L1, L2, L3 the weight increased from 0.23 mg in L₁ larvae to 49 mg in late L₂ larvae and L₃ aquired weight 518.The spines of *Oestrus ovis* were minutes in L2 and clearly in L3 stages whoever (23) was described the spines of *Chrysoma albiceps* and *Chysoma rulifacies* like hairy and smoth , Also the L1 loss spines whereas the L2 , L3 have three arrows of spines in ventral surface of tugument that agree with (24) which was remind especially the L2, L3 *Oestrus ovis* as arranged the spines in three lines.The hooks were connected internally to cephalo-pharngal skeleton in *oestrus ovis* so (25) refer the *Cochliomyia hominivorax* and *Chrysomya bezziana* have same structure of cephalo-pharngal skeleton and all genus of Diptera.Larvae need protein to be growth and reach to instar L₃; however, began transform to papuat (papuation process: its soliditary of tegument to began new stage is generation fly) (26) use genetic analysis for isolation DNA (L₁, L₂, L₃) of *Oestrus ovis* (27) using crude protein from second stage larvae as antigen for seroprevalence by ELISA.

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الأطوار اليرقية لذباب نغف الأغنام المعزولة من الجيوب الجبهية للأغنام في مجزرة البصرة

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الخلاصة

تضمنت الدراسة عزل *Oestrus ovis* من الجيوب الجبهية والتجاويف القرنية لرؤوس الأغنام المسلوخة , من الممكن التفريق بين أنواع رتبة ثنائية الأجنحة المسببة للتدويد ببعض الخصائص , عزلت الأطوار اليرقية الثلاث . بصورة عامة الأطوار اليرقية تحوي على إحدى عشر حلقة , بينما الهيكل الراسي البلعومي يرتبط مع كلايين. الطور اليرقي الأول ذي لون ابيض , قياس الطول (3-4mm) , لا تمتلك أشواك , التراكيب التنفسية الخلفية تشبه الحرف D ذات لون اصفر وداخليا تمتلك تركيب تنفسي صغير يشبه الحرف D ذي لون بني بينما الثغرات التنفسية موجودة في التركيب الكلي. الطور اليرقي الثاني ذي لون بني باهت قياس طول (6-12mm) . الطور اليرقي الثالث ذي طول (19-20mm) , ولون بني إلى رمادي وتمتلك صفائح رمادية إلى سوداء على السطح الظهري . التراكيب التنفسية الخلفية للطور الثاني والثالث تشبه الحرف D ذات لون بني مع انتشار الثغور التنفسية . السطح البطني للطور الاول والثاني يحوي على ثلاث صفوف من الأشواك . الطور اليرقي الثالث الناضج يتطور ويتحول إلى شرنقة خلال 12-24 ساعة خارج جسم الحيوان .