MORPHOTAXONOMY AND ALLOMETRY OF *OESTRUS OVIS* (LINNÉ, 1758) LARVAE (DIPTERA: OESTRIDAE: OESTRINAE)

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ABSTRACT

The larvae of *Oestrus ovis* Linné, 1758 are well-known parasites of goats and sheep. Larval instars of *O. ovis* are described based on specimens collected from nasal cavities, frontal sinuses and base of the horns of goats from Karachi, Pakistan. Three instars of larvae are described in detail, including body coloration, dorsal and ventral body spines and D- shaped spiracles. Colored illustrations of various larval instars along with typical characters have been given. We also give a key to the third instar larva with some modifications. The mean length (15.65 ± 4.29), width (6.043 ± 1.70) and weight (356.3 ± 168.4) of *O. ovis* larvae were calculated. The one way ANOVA showed significant differences ($P < 0.05$) in length, width and weight of three larval instars of *O. ovis*. Larvae growth relationship showed negative allometry between weight-length and weight-width, while length-width showed isometry.

Key words: *Oestrus ovis*, Morphometric measurements, Taxonomy, Morphology, Karachi, Pakistan.

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INTRODUCTION

Animals and humans are infected by dipteran larvae; this infection is known as myiasis. Oestridae, Calliphoridae, Muscidae and Sarcophagidae are considered the most important families that can cause myiasis in different hosts (Zumpt, 1965; Pezzi *et al.*, 2019). The Oestridae family has 28 genera which consist of 151 myiasis causing species. Morphologically, they are classified into four subfamilies i.e. Oestrinae, Gasterophilinae, Hypodermatinae and Cuterebrinae (Robbins and Khachemoune, 2010; Francesconi and Lupi, 2012). The Oestrinae consists of four genera such as *Oestrus*, which cause myiasis in sheep, goat and man (Ahaduzzaman, 2019; Borade, 2019), *Rhinoestrus* which cause myiasis in donkey, Horse and man (Otranto *et al.*, 2004; Yan *et al.*, 2019), *Cephenemyia* which cause myiasis in Reindeer (Nilssen and Haugerud, 1995) and *Cephalopina* which cause myiasis in Camel (Basu and Charles, 2019). Genus *Oestrus* composed of eleven species, including *O. ovis*, *O. aureoargentatus*, *O. caucasicus*, *O. cervi*, *O. curvicauda*, *O. dubitatus*, *O. elaphi*, *O. fasciculosus*, *O. gozdevi*, *O. macdonaldi* and *O. variolosus* (Myers *et al.*, 2020).

The larvae of *O. ovis* are well-known parasites of the nasal cavities, frontal sinuses, and sometimes also the maxillary sinuses of goats and sheep (Ahaduzzaman, 2019). The incidence of *O. ovis* larvae in goats has attracted attention in almost every country around the globe from the earliest eras. There has been extensive

information on the problem exist scattered across the scientific publications of many countries (Ahaduzzaman, 2019). The pathogenic effects lead to considerably less animal production and significant economic losses. Interference with feeding and rumination reduces weight gain and may reduce milk and wool production which has been roughly calculated as up to 22% in body weight, 16% in wool production and 10 % milk per animal (Dorchies *et al.*, 1993; Ipek, 2018). Oestrosis is additionally regarded as a zoonotic disease. *O. ovis* larvae cause ophthalmomyiasis in man. Ophthalmic and nasopharyngeal myiasis were reported in humans in many parts of the world (Al-Antary *et al.*, 2018; Velev and Mikov, 2018; Jenkins and Layton, 2018; D'assumpcao *et al.*, 2019).

In Pakistan no research work has carried out on the incidence of *O. ovis* larvae in animals. However, only three cases of ophthalmomyiasis were reported in humans (Ali *et al.*, 2006; Fasih *et al.*, 2014; Abbas and Amla, 2016). Due to its zoonotic importance and disease-causing ability in goats and sheep, we described the morphology and morphometric features of 1st instar (L1), 2nd instar (L2) and 3rd instar (L3) larvae.

MATERIALS AND METHODS

Collection: *O. ovis* Larvae infecting different goats were collected from the slaughtered goat heads from various slaughterhouses of different areas of Karachi. The heads of slaughtered goats were separated from the rest of the

carcass, cut along the sagittal axis and examined for larvae in the main larvae sites according to the method described by Dorchie *et al.* (2000). Larvae present in the nasal cavity, frontal sinuses and base of the horns were collected in small glass bottles, labeled accordingly, immediately stored in the icebox and transferred to the laboratory.

Laboratory Analysis: The Larvae were studied under a stereomicroscope (OLYMPUS SZ61, Model-SZ2-ILST) and pictures were taken using the OLYMPUS DP22 color camera. All three instars (1st instar, 2nd instar and 3rd instar) of larvae were separated according to their main morphological characters described by Zumpt (1965). Each larva was weighed on a digital balance (resolution of 0.001g), and then morphometric measurements (length and width) of each larva were carried out with the help of divider and centimeter scale. The relationship between total larvae weight, length and width were found out with regression and allometric calculations.

Allometric growth analysis: The relationship between the dependent and independent variable was studied based on the allometric function (Huxley, 1950):

$$Y = a X^b$$

Where: a= y-intercept, b= allometric growth constant or Slope, x=independent variable, y= dependent variable
This equation must be log-transformed (Kamaruddin *et al.*, 2011; Le Cren, 1951)
 $\text{Log}_{10} y = \text{Log}_{10} a + b \text{Log}_{10} x$

The allometric growth constant (b) shows the relationship between x and y, it was used for a statistical test for the morphometric calculation. When b>1 shows positive allometry, b=1 shows isometry and b<1 Shows negative allometry (Klingenberg, 2016; Shingleton *et al.*, 2009; Castiglioni and Negreiros-Franozo, 2004). While comparing weight with the other measurements, weight was always taken as 'y'. In conditions like this; isometric growth occurs when b=3 (Mayrat, 1970; Quinn II and Deriso, 1999). When b>3 indicates positive allometric growth and b<3 indicates negative allometric growth, (Farias-Tafolla *et al.*, 2015; Qureshi and Saher, 2011).

Statistical Analysis: All the statistical calculation [descriptive statistics, chi-square test, allometric growth analysis and one-way analysis of variance (ANOVA)] was done using Minitab (Version 17). One way analysis of variance (ANOVA) was performed to find out the difference between length, width and weight of three larvae instars. The test of significance was accepted significant at $\alpha=0.05$.

RESULTS

Taxonomy: *O. ovis* Linné, 1758: *O. ovis* Linné, 1758: 584; Rodhain and Bequaert, 1916: 85; Zumpt, 1965: 175-

177, Fig. 246-252; Giannetto *et al.*, 1999: 75-76, Fig. 1-12.

Material examined: 661 spec, 56 L1, 228 L2, 377 L3, Pakistan: Sindh Province: Karachi. (Larvae collected from slaughtered goats head)

Taxonomic Key of *O. ovis* 3rd instar larvae according to Zumpt (1965) with some modifications

1. Ventral side of segments 6th to 8th with five to six rows of spines.

Oestrus macdonaldi Geddoelst

- Ventral side of segments 6th to 8th with three to four rows of spines (Fig. 2B).

Oestrus ovis Linnaeus

Color: Changes in coloration occur according to growth and maturation. 1st instar larvae are transparent or white in color, 2nd and immature 3rd instar larvae are white whereas the mature 3rd instar larvae are dark black color (Fig. 1).

Diagnostic Characteristics: *O. ovis* larvae have an oval-shaped segmented body, ventrally each segment lined by multiple rows of small spines; spine absent on the posterior side. Dorsal broad transverse blackish bands are present in the mature instars. A pair of sharp, dark brown oral hooks joined to a cephalopharyngeal skeleton on the anterior side. Pair of D shaped spiracles present in the posterior end (Fig. 1, 2).

1st instar larvae (L1): 1st instar larvae were fusiform in shape, near about 1-5mm in length, comparatively large cephalo-skeleton and a pair of strongly curved mouth-hooks. Two to three complete rows of spines were observed on the ventral side present on anterior segments. There are 22-25 terminal hooks arranged in two scallops. Rows of small spines on the dorsal side of the third segment and a pair of dark mustard yellow spiracles were present on the posterior side (Fig. 2C, 2D, 2E).

2nd instar larvae (L2): The 2nd instar larvae were white, about 9-15mm long with few weak spines on the dorsal side of the second segment, the median part of the post-anal bulge having small spines. Ventrally the segments were with multiple rows of curly comb-like spines. The posterior spiracles were D-shaped with a brown central button. The mustard-yellow channels were indicated by a distinct brown suture (Fig. 2F, 2G, 2H).

3rd instar larvae (L3): The 3rd instar larvae were 14-27mm in length, white or yellowish (earlier stage) change into light brown with dorsal transverse blackish band in mature stage. Number of irregular small denticles were found in the dorsal side of the second segment, while the following segment has no denticles and rough leather-like skin pattern. Ventrally, all the segments consist of regular multiple rows of strong spines that were irregularly

placed on the third segment. The pre-anal bulge has no spines while the post-anal bulge was with a small number of spines. Posterior spiracles were circular and black with central spot and without a distinct suture (Fig. 2I, 2J).

Morphometric Characteristics: A total of 661 larvae morphometric variable, i.e. length, width and weight were measured. The mean length (15.65 ± 4.29), width (6.043 ± 1.70) and weight (356.3 ± 168.4) were calculated. The mean length was 3.230 ± 1.724 mm for L1, 13.908 ± 2.319 mm for L2 and 18.249 ± 1.755 mm for L3 respectively. The Mean weight was 61.43 ± 13.41 mg for L1, 232.72 ± 94.31 mg for L2, 474.30 ± 89.25 mg for L3. The mean length, width and weight of L1 were smaller than that of L2 and L3 (Table 1). The one way ANOVA showed significant differences ($P < 0.05$) in length, width and weight of three larval instars of *O. ovis* (Fig. 3).

Larvae Growth Rate Relationship: Weight-length relationship; the regression coefficient for the weight-length relationship was high ($r=0.80$) and showed a strong positive correlation. The value of b is equal to 1.504 which shown negative allometry as $b < 3$. This implies that the weight of the larvae had slower relative growth related to the length (Table 2). Weight-width Relationship; the value of slope b is equal to 1.843 which shows negative allometry as $b < 3$. This implies that the weight of larvae increases slower as compared to the width (Table 2).

Length-width Relationship; the regression coefficient showed a strong correlation ($r=0.74$) between width and length. The value of b is equal to 1.057 whereas comparing the value of allometric growth constant b with 1 it showed isometry as $b=1$ and a constant rate of relative growth (Table 2).

Table 1. Comparative morphometry and descriptive Statistics of Length, width and weight of three larval instars of *O. ovis*.

Variable	Larval Instar	Total Count	Mean $\pm$ StDev	Minimum	Median	Maximum
Length (mm)	L1	56	3.230 ± 1.724	2.0	3.53	5.00
	L2	228	13.908 ± 2.319	9.00	14.0	15.0
	L3	377	18.249 ± 1.755	14.0	18.0	27.0
Mean		661	15.65 ± 4.290	2.0	17.0	27.0
Width (mm)	L1	56	2.696 ± 0.600	2.00	3.00	4.00
	L2	228	5.101 ± 0.981	3.0	5.00	7.00
	L3	377	7.11 ± 1.043	4.0	7.00	9.00
Mean		661	6.043 ± 1.700	2.0	6.0	9.0
Weight (mg)	L1	56	61.43 ± 13.41	40.0	60.0	80.0
	L2	228	232.72 ± 94.31	90.0	230.0	450.0
	L3	377	474.30 ± 89.25	210.0	480.0	710.0
Mean		661	356.3 ± 168.4	40.0	390.0	710.0

Table 2. Relative Growth analysis of *O. ovis* Larvae based on weight-length, weight-width and length-width relationship.

Measure	N	Log y = Log a + b Log x	r ²	F	P	Allometry
Weight VS Length	661	= $0.7179 + 1.504$	0.80	2750.29	0.000	-ve Allometry
Weight VS Width	661	= $1.073 + 1.843$	0.80	2783.75	0.000	-ve Allometry
Length VS Width	661	= $0.3647 + 1.057$	0.74	1925.80	0.000	Isometry

Significance level $\alpha = 0.05$

Fig. 1. Dorsal view of different stages of *Oestrus ovis* larvae; A-B= Mature 3rd stage larvae, C-H= 3rd stage larvae, I-K= 2nd stage larvae, L=1st stage larvae.

Fig. 2. A= Dorsal side of *O. ovis* larvae, B= Ventral side of larvae showing multiple rows of spines on each segment, C= L1 anterior side showing a pair of hooks connected with cephaloskeleton, D= L1 posterior side showing a pair of D-shaped mustard-yellow colour spiracles, E= L1 posterior spiracles, F= L2 anterior side showing a pair of strong hooks, G= L2 posterior side showing a pair of D-shaped spiracles, H= L2 posterior spiracle with channels indicated by brown distinct sutures, I= L3 anterior side showing a pair of strong hooks, J= L3 posterior side showing a pair of black colour spiracles with no distinct sutures.

Fig. 3. The mean weight, length and width difference through one-way ANOVA in three larvae instars of *O. ovis*.

DISCUSSION

O. ovis larvae cause nasal-sinusal myiasis in goats and sheep, known as oestrosis. This widely distributed disease badly affects the health of the infecting animal, causing breathing hard when the discharge from the nasal cavity becomes solidified with dust. It can cause acute clinical manifestations, which include breathing problems, anorexia, weakness, difficult snoring respiration and breathlessness (Dorchies *et al.*,

1992; Dorchies and Alzieu, 1997). Furthermore, this disease can be complicated by sensual tumors and lung abscesses, and sometimes this infection leads to starvation, which might cause death (Dorchies *et al.*, 1993; Özdal *et al.*, 2016). Occasionally, the larvae from frontal sinuses and nasal cavity may move into the brain leading to false gids (Patra *et al.*, 2018).

Microscopic study of *O. ovis* larvae provided the evidence and better understands their morphological adaptability to the host. 1st instar larvae need the strongly curved mouth-hooks, and the three pointed spines to

move towards the frontal cavities and anchor the mucous surface that the strong expirations or the sneezing of the host caused by their irritating action do not expel them. The 2nd instar larvae have a relatively small number of spines because it doesn't need to anchor their self; it is due to that it is located in the paranasal and frontal cavities of the host where there are no air currents of the nasal passage. Their function is limited to move little and to produce exudates. The 3rd instar larvae have large hooks, strong spines and dorsal plates which are used to slowly move out to the outside environment from the nasal passage and possibly to its successive sinking into the soil for a few centimeters to transform into a pupa.

The morphometric study showed that the length, width and weight of 1st instar, 2nd instar and 3rd instar larvae were significantly different ($P < 0.05$), these findings are similar with the results previously recorded by Cepeda-Palacios *et al.* (1999). The length, width and weight of 3rd instar larvae was higher from that of 2nd and 1st instar larvae. The allometric growth analysis showed that the weight increases slower as compared to length and width, while the length and width increases constantly as the larvae increases in size.

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