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Epidemiology of *Rhinoestrus* Spp. Larvae Infesting Donkeys in Egypt

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Abstract

The aim of this study was updating the information about the prevalence of infestation with *Rhinoestrus* spp. larvae in Egypt and as a result determining the correct time for chemotherapy. 303 Egyptian donkeys (*Equus asinus*) of different ages and sex slaughtered at Giza Zoo slaughter house from June 2014 to May 2015 were examined for the presence of *Rhinoestrus* spp. larvae. A total of 8388 *Rhinoestrus* spp. larvae [7221 L1 (86.08%), 545 L2 (6.49%), 622 L3 (7.41%)] were recovered from 74.91% of donkeys with an overall mean of 27.68 ± 2.43 larvae per head. Heavier infestations were recorded in winter and summer. There were more than two generations of *Rhinoestrus* spp. occurred in the year. Two yearly treatments during June and November are recommended for eradication of such infestation from donkeys.

Keywords: Rhinoestrus spp, Prevalence, Donkeys, Egypt.

1. Introduction

Donkeys are widely utilized in both rural and urban areas of Egypt because its inclination to work, facile to train and can utilize poor aliment [5]. Precedent studies on *Rhinoestrus* spp. of donkeys from Egypt are constrained to few reports [15],[26-28].

Rhinoestrosis is a parasitic diseases caused by larvae of *Rhinoestrus* spp. (Diptera, Oestridae), which localize in the nasal cavities, sinuses and pharynx of horses, donkeys and zebras [30]. The presence of larvae of *Rhinoestrus* spp. induces local inflammation and the infection is characterized by clinical designation of varying intensity and astringency, ranging from inflammation to dyspnea, sneezing and cough [11]. Moreover, lesions of the upper respiratory tract, lungs and damage of the olfactory nerves were reported [10]. Restlessness and reduction in sport yield in integration, death may occur due to encephalomyelitis caused by the perforation of the larvae of *Rhinoestrus* spp. to the ethamoid and meninges [3]. The first stage larvae (L1) ejected by the adult females near the nostrils of the animals, migrate through the upper respiratory tract and reach nasal cavities where they develop into second (L2) and third stage (L3) larvae [10]. Third larval stage leaves the host through the nasal cavities becoming pupa in the soil and emerging as adult insects in the environment [30].

Precedent information on *Rhinoestrus* spp. prevalence, seasonal abundance and life cycle in general are crucial for understanding its chronobiology which will avail in orchestrating the critical period for its treatment and control. This study was carried out for updating the information concerning the epidemiology and chronobiology of *Rhinoestrus* spp. in Egypt.

2. Materials and methods

2.1 Collection of *rhinoestrus* Spp. larvae from donkeys

Regular weekly visits were done to donkey's slaughter house in the Zoo for updating the information concerning the prevalence of the different larval stages of *Rhinoestrus* spp. and to study the effect of age and season on the prevalence of these larval infestations in donkeys.

A. Study design

The study was carried out on 303 Egyptian donkeys (*Equus asinus*) of different ages and sex slaughtered at Giza Zoo slaughter house from June 2014 to May 2015. On average, 6-8 donkeys' heads were examined weekly.

The donkeys of both sexes were grouped into 5 age groups based on the eruption of the teeth and the teeth wear pattern.

The groups of donkeys were: group1 (<1-4 years), group 2 (>4 – 8 years), group 3 (>8 – 10 years), group 4 (>10 – 15 years) and group 5 (> 15 years).

Information on the locality (Province) from which the donkeys were admitted were recorded.

B. Post-mortem examination

The heads of the slaughtered animals were separated from the rest of the body, the skin was dissected and the skull was sagittally incised. All the detected larval stages were collected with blunt forceps and transferred to plastic containers capped with a perforated plastic lid, containing thin layer of PBS (PH 7.2) and labeled with the number of animal, age, sex and date of collection. Moreover, all other nasal mucosae and turbinates were kept in labeled plastic bags. The specimen were transferred to the laboratory and freshly examined by naked eye.

C. Samples collection

C.1 Collection of different larval stages

C.1.1 Collection of 1st stage larvae

The nasal mucosae and turbinate bones were thoroughly washed and scraped several times in Petri-dish containing warm (37°C) water. After removing the nasal mucosae and turbinates the washing solution was examined using stereoscopic microscope (X75). All the larvae were counted, collected and identified [8].

C.1.2 Collection of 2nd and 3rd stage larvae

Macroscopical inspection of 2nd and 3rd stage larvae was done in the abattoir from the nasal, sinusal and pharyngeal cavities of the head. The collected larvae were identified at the lab [30].

3. Result

3.1 Prevalence of *rhinoestrus* spp. infestations among donkeys

The overall prevalence of infestation with *Rhinoestrus* spp. larvae among 303 examined Egyptian donkey's heads slaughtered at Giza zoo slaughter house in the period extended from June 2014 to May 2015 was 74.91%. Donkeys at all ages were susceptible to *Rhinoestrus* spp. infestation especially those more than four to eight years old (81.82%). The mean number of larvae per donkey (L/D) was insignificantly different ($p<0.05$) except that of 1st larval instars infesting donkeys more than four to eight years old (46.45±20.12) that was significantly increased than that of donkeys more than 15 years old (18.79±3.15), Table (1), Graph 1. Although Female donkeys showed higher incidence of infestation with *Rhinoestrus* spp. larvae (76.59%) than males (73.45%), there was no significance ($p<0.05$) between number of larvae in both sex except there was significant increase in number of 3rd larval instars in males (2.42±0.52) than that of females (1.65±0.36), Table (2), Graph 2.

Regarding to the monthly prevalence of *Rhinoestrus* spp. larvae among donkeys Table (5), Graph 3. , our records reflected that the infestation rate had 2 peaks throughout the year, the first was from July to September (91.89-85.29%) and the second was in December (86.36%) and in January (85%) while the lowest prevalence was in May (60. 86%) and June (51.72%).

In relation to the seasonal prevalence of *Rhinoestrus* spp. larvae among donkeys Table (5), Graph 4. , the highest infestation rate was recorded in winter (81.36%) while the lowest was in spring (69.01%).

Prevalence of *rhinoestrus* spp. larvae

L/D of *Rhinoestrus* spp. larvae (1st, 2nd and 3rd instars) during each month of the study period represented two peaks of significant difference in abundance, the first was during January (43.95±10.34) and the second during July (46.54±10.04) than those detected during April (13.08±2.87) and September (17.50±5.33), Table (5)., Graph 6. On the other hand, L/D of *Rhinoestrus* spp. larvae during each season of the study period was significantly decreased during spring (15.83 ± 3.80) ($p<0.05$) than was during winter (36.14 ± 5.21) and summer (35.25 ± 5.15), Table (5)., Graph 8. and Fig (1).

A. Prevalence of first larval instars

With respect to the monthly prevalence, the highest infestation rate with first larval instars was reported in June and November (100%) while the lowest was in April (27%) and March (44%), Table (5), Graph 5. L/D of L1 in July (46.19±10.01) and January (43.60±10.38) was significantly increased than that of March (7.57±2.41), April (3.60±1.96) and September (11.71±3.83) ($p<0.05$), Table (5)., Graph 6. and Fig (1)

With regard to the seasonal prevalence, the highest infestation rate with first larval instars was recorded in winter (94.47) while the lowest was in spring (57.6), Table (2) , Graph 7. L/D of L1 in winter (34.14 ± 4.87) and summer (31.63 ± 5.11) was significantly increased than that in spring (9.13 ± 3.42) and autumn (20.21 ± 4.15) ($p<0.05$), Table (5)., Graph 8..

B. Prevalence of second larval instars

Regarding to the monthly prevalence, the highest infestation rate with second larval instars was reported during March (31%) while the lowest one was during May and July (0.4%). The rate of infestation with second larval instars was 0% during both June and November, Table (5), Graph 5. L/D of L2 in August (6.48±2.04) was significantly increased ($p<0.05$) than that of other months except February (4.00±2.47) and March (5.35±2.08), Table (5)., Graph 6. and Fig (1).

Concerning to the seasonal prevalence, the highest infestation rate with second larval instars was recorded in spring (16.53%) while the lowest one was in winter (4.17), Table (5)., Graph 7.. There was no significance ($p<0.05$) in L/D of L2 in different seasons, Table (5)., Graph 8..

C. Prevalence of third larval instars

With respect to the monthly prevalence, the highest infestation rate with third larval instars was reported during April (54%) while the lowest one was during December, July (1%) and January (0.1%). The rate of infestation with third larval instars was 0% during January, June and November, Table (5), Graph 5. L/D of L3 in April (7.04±1.73) was significantly increased ($p<0.05$) than that of other months except March (4.22±1.42) and August (5.44±2.11), Table (5)., Graph 6. and Fig (1).

With regard to the seasonal prevalence, the highest infestation rate with third larval instars was recorded during spring (25.87%) while the lowest

one was during winter (1.36%), Table (5), Graph 7. L/D of L3 in spring (4.08 ± 0.83) was significantly increased (p<0.05) than that of other seasons, Table (5)., Graph 8..

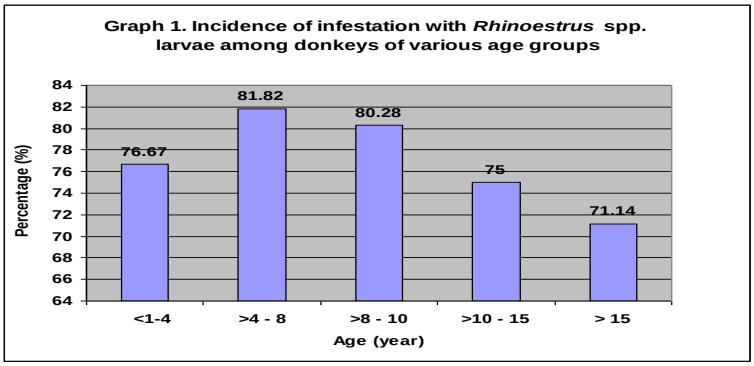
From Table (1 , 2, it was obvious that 227 donkeys' heads were infested with 8388 *Rhinoestrus* spp. larvae [7221 L1 (86.08%), 545 L2 (6.49%), 622 L3 (7.41%)] giving mean number of 27.68 ± 2.43 larvae per head. Also it was observed from the larval burden that L1 were present during each month of the study period while L2 and L3 were absent during June and November.

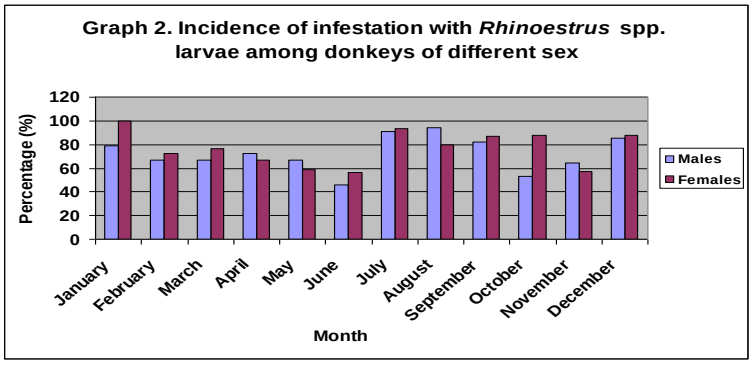
The highest number of *Rhinoestrus* spp. larvae recovered from one donkey's head was 248 (247 L1 and 1 L3) in the 2nd week of July while the lowest one was 1. The highest number of L1 (247) was obtained in the 2nd week of July while the lowest was 1. The highest number of L2 (43) was obtained in the 4th week of August while the lowest was 1. The highest number of L3 (34) was obtained in the 3rd week of April while the lowest was 1. Monthly larval burden and number of larvae per donkey's head (L/D) were the highest (1722) and (46.54±10.04), (43.95±10.34) during July and January, respectively, while the lowest were (328) and (13.08±2.87) during April. Seasonal larval burden and number of larvae per donkey's head (L/D) were the highest (3277) and (35.25 ± 5.15) in summer followed by (2132) and (36.14 ± 5.21) in winter while the lowest were (1125) and (15.83 ± 3.80) in spring. The overall L/D and range of collected larvae were 27.68 ± 2.43 and (1-248); whereas those of L1, L2 and L3 were 23.83±2.34 and (1-247), 1.80 ± 0.33 and (1-43) and 2.06 ± 0.32 and (1-34), correspondingly.

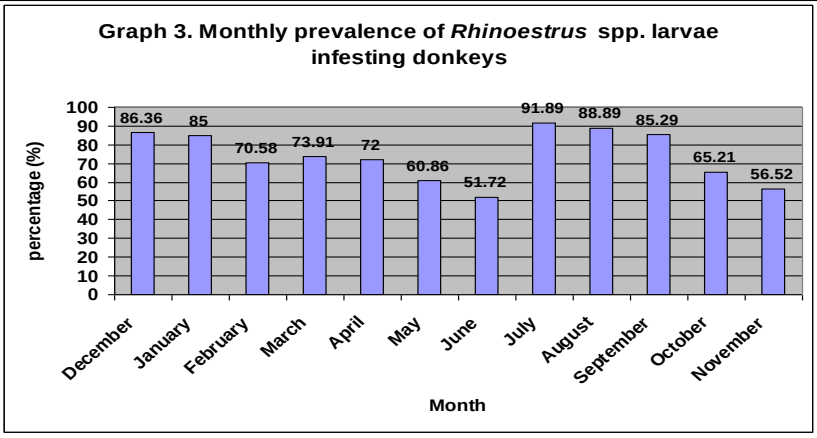
Concerning to the prevalence of *Rhinoestrus* spp. larvae among donkeys from different Governorates, donkeys admitted from Giza presented the highest infestation rate (89.09%), while the lowest rate (66.66%) belonged to donkeys admitted from El-Fayom. The highest monthly prevalence (100%) was in donkeys admitted from Giza and El-Fayom during September, November and December, September, respectively, while the lowest one (0%) was in donkeys admitted from Giza during October, Table (3), Graph 9. The highest seasonal prevalence (94.44%) was in donkeys admitted from Giza during summer, while the lowest one (64%) was in donkeys admitted from El-Fayom during autumn, %), there was no significance (p<0.05) between number of larvae in donkeys from different Governorates except there was significant increase in number of 2nd larval instars in donkeys admitted from Giza (4.18± 1.22) than those from other Governorates, Table (3, 4.), Graph 10.

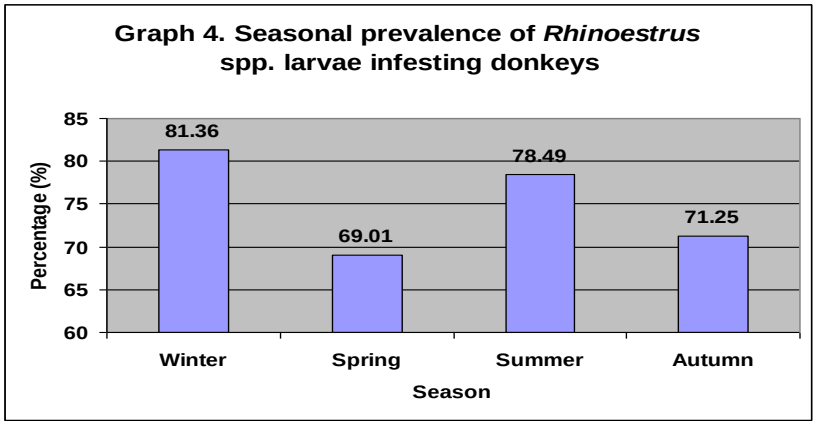
3.2 Localization of *rhinoestrus* spp. larvae

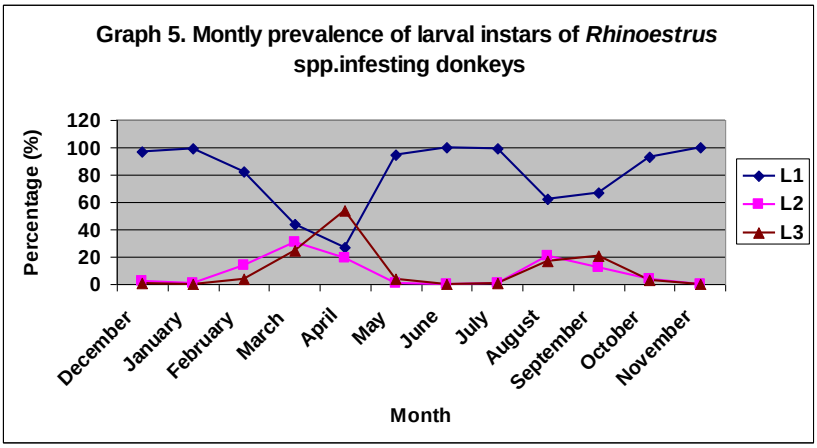
L1were always detected embedded between the turbinate bones and rarely recovered from the adjacent nasal mucosa, L2 were detected mainly in the turbinate and the labyrinth of the ethmoid bone, and frequently in the lamina cribrosa and the pharyngeal cavity while L3 were detected mainly in the labyrinth of the ethmoid bone, the lamina cribrosa and the nasal sinuses, and rarely detected in the pharyngeal and nasal cavities. In one case only, 5 L3 were found in the larynx.

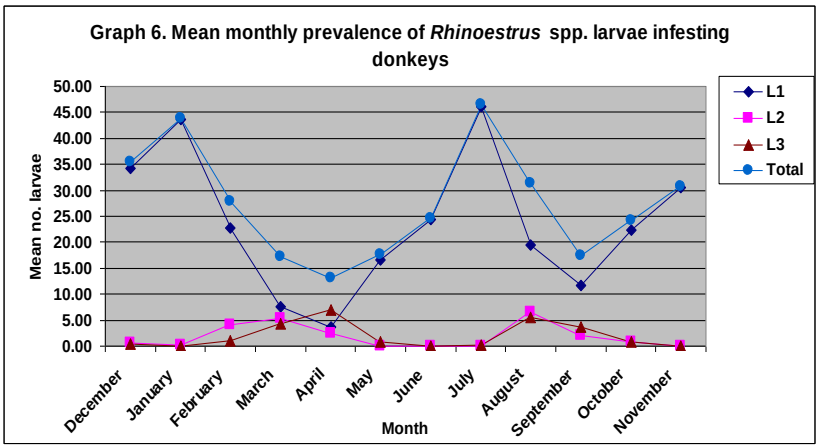


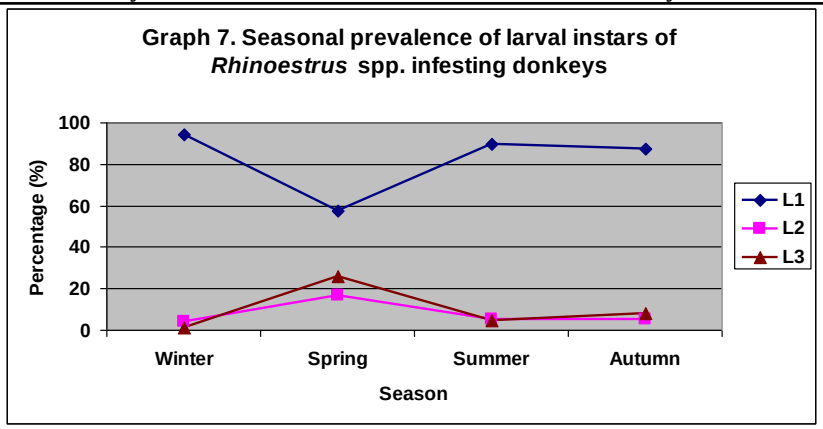


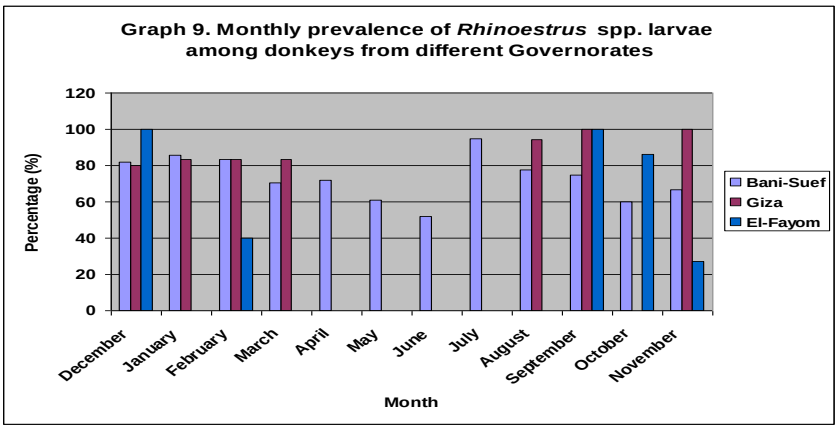

Graph 3. Monthly prevalence of Rhinoestrus spp. larvae infesting donkeys
percentage (%)
100
90
80
70
60
50
40
30
20
10
0
86.36
85
70.58
73.91
72
60.86
51.72
91.89
88.89
85.29
65.21
56.52
December
January
February
March
April
May
June
July
August
September
October
November
Month

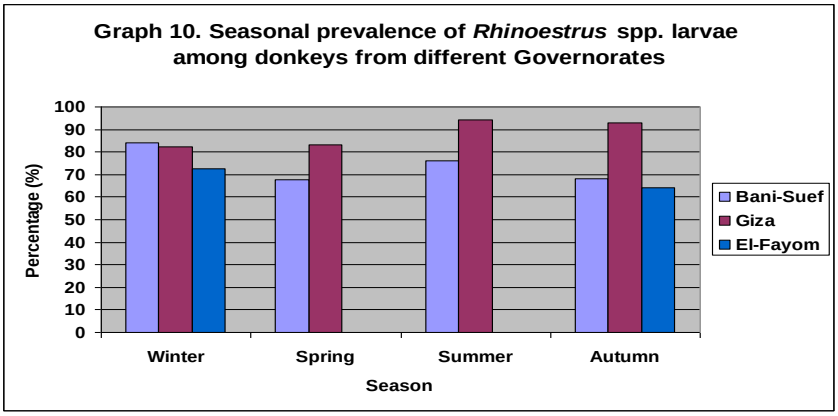

Graph 4. Seasonal prevalence of Rhinoestrus spp. larvae infesting donkeys
Percentage (%)
85
80
75
70
65
60
81.36
69.01
78.49
71.25
Winter
Spring
Summer
Autumn
Season


Graph 5. Montly prevalence of larval instars of Rhinoestrus spp.infesting donkeys
Percentage (%)
120
100
80
60
40
20
0
L1
L2
L3
December
January
February
March
April
May
June
July
August
September
October
November
Month


Graph 6. Mean monthly prevalence of Rhinoestrus spp. larvae infesting donkeys
Mean no. larvae
50.00
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
L1
L2
L3
Total
December
January
February
March
April
May
June
July
August
September
October
November
Month







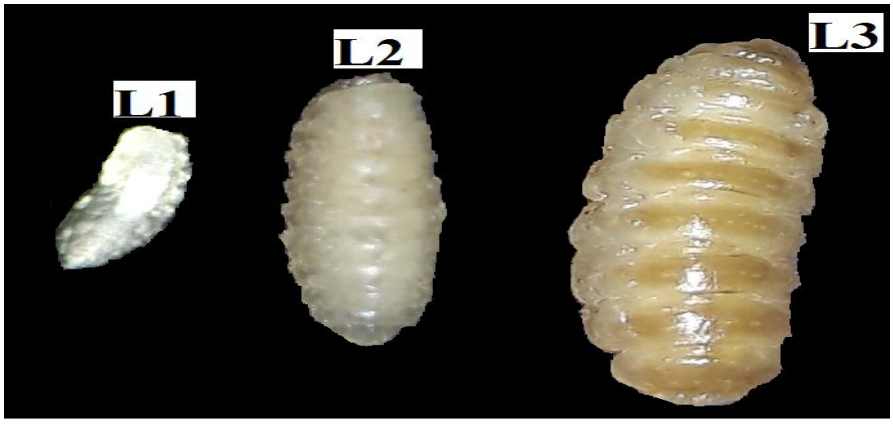


Fig (1) Different stages of rhinoestrus spp. larvae (L1: first stage; L2: second stage; L3: third stage)

Fig (2) L3 of *Rhinoestrus* spp. in the nasal mucosa of donkey

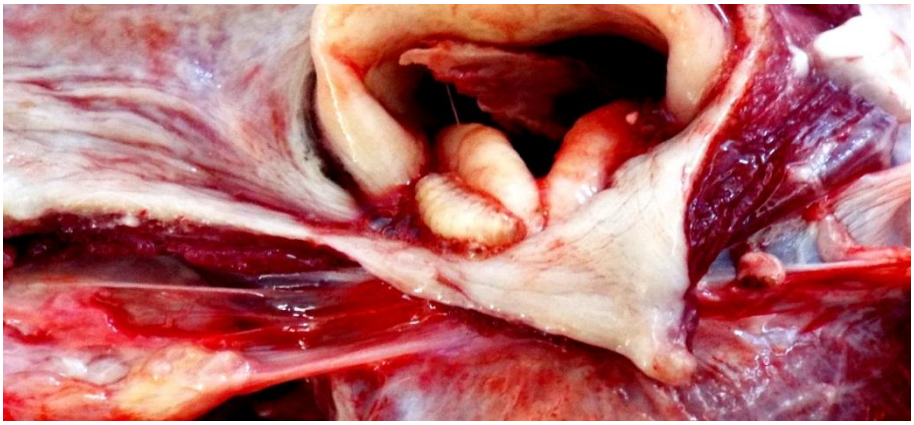


Fig (3) L3 of Rhinoestrus spp. in the larynx of donkey

Table (1) Prevalence of infestation with *rhinoestrus* spp. larvae among donkeys of various age groups

Age /year	No. of examined animals	Infestation		L/D			
		No.	%	L1	L2	L3	Total
<1-4	60	46	76.67	21.82±4.96^{ab}	0.82±0.32^{a}	1.27±0.48^{a}	23.88±4.99^{a}
>4 - 8	11	9	81.82	46.45±20.12^{a}	0.27±0.27^{a}	0.64±0.64^{a}	47.36±19.97^{a}
>8 - 10	71	57	80.28	31.17±4.97^{ab}	1.86±0.77^{a}	1.49±0.53^{a}	34.52±5.42^{a}
>10 - 15	12	9	75	32.33±9.55^{ab}	0.75±0.59^{a}	0.50±0.34^{a}	33.58±9.78^{a}
> 15	149	106	71.14	18.79±3.15^{b}	2.36±0.53^{a}	2.87±0.56^{a}	24.03±3.29^{a}
Total	303	227	74.91	23.83±2.34	1.80±0.33	2.06±0.32	27.68±2.43

-Means with dissimilar alphabetical super scripts in the same columns are significantly different by means of Duncan test at $P<0.05$.
-No.: number of infested donkeys, %: percentage, L1: 1st larval instars, L2: 2nd larval instars, L3: 3rd larval instars, L/D: mean number of larvae /donkey.
- This study had been done during the period from June 2014 to May 2015.

Table (2) Prevalence of infestation with *Rhinoestrus* spp. larvae among donkeys of different sexes

Sex	Number	L1	L2	L3	Total
		Mean±Std. Error	Mean±Std. Error	Mean±Std. Error	Mean±Std. Error
Males	162.00	24.96±3.37	1.76±0.42	2.42±0.52	29.12±3.43
Females	141.00	22.54±3.22	1.84±0.51	1.65±0.36	26.03±3.43

-Means with higher values are significantly different using independent sample T-test at ($p<0.05$)

Table (3) Monthly prevalence of Rhinoestrus spp. larvae among donkeys from different Governorates

Season	Bani-Suef			Giza			El-Fayom		
	No. examined	No. infested	%	No. examined	No. infested	%	No. examined	No. infested	%
Winter	31	26	83.87	17	14	82.35	11	8	72.73
December	11	9	81.82	5	4	80	6	6	100
January	14	12	85.71	6	5	83.33	0	0	0
February	6	5	83.33	6	5	83.33	5	2	40
Spring	65	44	67.69	6	5	83.33	0	0	0
March	17	12	70.59	6	5	83.33	0	0	0
April	25	18	72	0	0	0	0	0	0
May	23	14	60.87	0	0	0	0	0	0
Summer	75	57	76	18	17	94.44	0	0	0
June	29	15	51.72	0	0	0	0	0	0
July	37	35	94.59	0	0	0	0	0	0
August	9	7	77.78	18	17	94.44	0	0	0
Autumn	41	28	68.29	14	13	92.86	25	16	64
September	20	15	75	7	7	100	7	7	100
October	15	9	60	1	0	0	7	6	86
November	6	4	66.67	6	6	100	11	3	27
Total	212	155	73.11	55	49	89.09	36	24	66.66

Table (4) Prevalence of infestation with Rhinoestrus spp. larvae among donkeys admitted from different governorates

Governorate	Number of donkeys	L1	L2	L3	Total
		Mean± Std. Error	Mean± Std. Error	Mean± Std. Error	Mean± Std. Error
Giza	55.00	23.71 ± 4.84^{a}	4.18 ± 1.22^{a}	2.96 ± 0.99^{a}	30.80 ± 5.16^{a}
El-Fayom	36.00	28.11 ± 7.30^{a}	0.64 ± 0.30^{b}	1.14 ± 0.55^{a}	29.89 ± 7.47^{a}
Bani-suef	212.00	23.14 ± 2.85^{a}	1.38 ± 0.33^{b}	1.99 ± 0.37^{a}	26.50 ± 2.95^{a}
Total	303.00	23.83 ±2.34	1.80±0.33	2.06±0.32	27.68±2.43

-Means with dissimilar alphabetical super scripts in the same columns are significantly different by means of Duncan test at $P<0.05$.

- This study had been done during the period from June 2014 to May 2015.

Table (5) Monthly prevalence of Rhinoestrus spp. larvae among donkeys

Season	x/n (%)	Sex		TL	L1		L2		L3		Total
		Male (%)	Female (%)		No. (%)	L/D	No. (%)	L/D	No. (%)	L/D	
Winter	48/59 (81.36)	27/34 (79.41)	21/25 (84)	2132	2014 (94.47)	34.14 ± 4.87^{a}	89 (4.17)	1.51 ± 0.78^{a}	29 (1.36)	0.50 ± 0.25^{b}	36.14 ± 5.21^{a}
December	19/22 (86.36)	12/14 (85.71)	7/8 (87.50)	780	754 (97)	34.27 ± 7.48^{abc}	15 (2)	0.68 ± 0.68^{cd}	11 (1)	0.50 ± 0.50^{cd}	35.45 ± 8.15^{abc}
January	17/20 (85)	11/14 (78.57)	6/6 (100)	879	872 (99)	43.60 ± 10.38^{ab}	6 (1)	0.30 ± 0.30^{d}	1 (0.1)	0.05 ± 0.05^{d}	43.95 ± 10.34^{ab}
February	12/17 (70.58)	4/6 (66.66)	8/11 (72.72)	473	388 (82)	22.82 ± 6.16^{abcde}	68 (14)	4.00 ± 2.47^{abc}	17 (4)	1.00 ± 0.58^{cd}	27.82 ± 8.36^{abc}
Spring	49/71 (69.01)	24/34 (70.59)	25/37 (67.57)	1125	648 (57.6)	9.13 ± 3.42^{c}	186 (16.53)	2.62 ± 0.75^{a}	291 (25.87)	4.08 ± 0.83^{a}	15.83 ± 3.80^{b}
March	17/23 (73.91)	4/6 (66.66)	13/17 (76.47)	394	174 (44)	7.57 ± 2.41^{de}	123 (31)	5.35 ± 2.08^{ab}	97 (25)	4.22 ± 1.42^{ab}	17.13 ± 5.28^{bc}
April	18/25 (72)	16/22 (72.72)	2/3 (66.66)	328	90 (27)	3.60 ± 1.96^{e}	61 (19)	2.44 ± 0.65^{bcd}	177 (54)	7.04 ± 1.73^{a}	13.08 ± 2.87^{c}
May	14/23 (60.86)	4/6 (66.66)	10/17 (58.82)	403	384 (95)	16.70 ± 10.03^{cde}	2 (0.4)	0.09 ± 0.09^{d}	17 (4)	0.74 ± 0.56^{cd}	17.52 ± 10.17^{bc}
Summer	73/93 (78.49)	42/52 (80.77)	31/41 (75.61)	3277	2942 (89.78)	31.63 ± 5.11^{ab}	177 (5.40)	1.90 ± 0.66^{a}	158 (4.82)	1.71 ± 0.66^{b}	35.25 ± 5.15^{a}
June	15/29 (51.72)	6/13 (46.15)	9/16 (56.25)	710	710 (100)	24.48 ± 8.47^{abcde}	0 (0)	0.00 ± 0.00^{d}	0 (0)	0.03 ± 0.03^{d}	24.52 ± 8.50^{abc}
July	34/37 (91.89)	20/22 (90.90)	14/15 (93.33)	1722	1709 (99)	46.19 ± 10.01^{a}	2 (0.4)	0.05 ± 0.04^{d}	11 (1)	0.30 ± 0.12^{d}	46.54 ± 10.04^{a}
August	24/27 (88.89)	16/17 (94.11)	8/10 (80)	845	523 (62)	19.37 ± 5.16^{bcde}	175 (21)	6.48 ± 2.04^{a}	147 (17)	5.44 ± 2.11^{ab}	31.30 ± 6.03^{abc}
Autumn	57/80 (71.25)	26/40 (65)	31/38 (81.58)	1854	1617 (87.22)	20.21 ± 4.15^{bc}	93 (5.02)	1.16 ± 0.41^{a}	144 (7.77)	1.80 ± 0.51^{b}	23.18 ± 4.49^{ab}
Septembe	29/34 (85.29)	9/11 (81.81)	20/23 (86.95)	595	398 (67)	11.71 ± 3.83^{cde}	72 (12)	2.12 ± 0.70^{bcd}	125 (21)	3.68 ± 1.01^{bc}	17.50 ± 5.33^{bc}
October	15/23 (65.21)	8/15 (53.33)	7/8 (87.50)	554	515 (93)	22.39 ± 8.30^{abcde}	21 (4)	0.91 ± 0.91^{cd}	18 (3)	0.78 ± 0.78^{cd}	24.09 ± 8.95^{abc}
Novembe	13/23 (56.52)	9/14 (64.28)	4/7 (57.14)	705	704 (100)	30.61 ± 10.20^{abcd}	0 (0)	0.00 ± 0.00^{d}	1 (0)	0.04 ± 0.04^{d}	30.65 ± 10.22^{abc}
Total	227/303 (74.91)	119/162 (73.45)	108/141 (76.59)	8388	7221 (86.08)	23.83±2.34 *(1-247)	545 (6.49)	1.80 ± 0.33 *(1-43)	622 (7.41)	2.06 ± 0.32 *(1-34)	27.68 ± 2.43 *(1-248)

-x/n: number of infested animal/number of examined animals, TL: total number of collected larvae
-%: percentage of infestation, No.: number, L1: 1st larval stage, L2: 2nd larval stage, L3: 3rd larval stage, L/D: mean number of larvae / donkey, *Range of larval burden.
- This study was carried out during the period from June 2014 to May 2015.
-Means with dissimilar alphabetical super scripts in the same columns are significantly different by means of Duncan test at $P<0.05$.

Concerning to the head color there was no significant difference ($p<0.05$) in the number of larvae among donkeys of different head colors (Table 6).

Table (6) Prevalence of infestation with *Rhinoestrus* spp. larvae among donkeys of different head colors

Head color	Nubmer of donkeys	L1	L2	L3	Total
		Mean ± Std. Error	Mean ± Std. Error	Mean ± Std. Error	Mean ± Std. Error
Light	197.00	25.54 ± 3.18	1.84 ± 0.43	2.27 ± 0.44	29.64 ± 3.30
Dark	106.00	20.65 ± 3.12	1.73 ± 0.46	1.67 ± 0.43	24.05 ± 3.26
Total	303.00	23.83 ± 2.34	1.80 ± 0.33	2.06 ± 0.32	27.68 ± 2.43

-Means with higher values are significantly different using independent sample T-test at ($p<0.05$).

4. Discussion

This study intended for establishing the prevalence of infestation with *Rhinoestrus* spp. larvae among 303 Egyptian donkeys (*Equus asinus*) of different ages and sex slaughtered at Giza Zoo slaughter house from June 2014 to May 2015. The study signified the consequence of age, sex, season and locality of admitted animals on their infestation.

Concerning to the prevalence of infestation, our data demonstrated that rhinoestrosis was established among Egyptian donkeys *Equus asinus*) with an overall prevalence of 74.91% (227/303) in donkeys.

Lower rhinoestrosis prevalence's were reported in Egypt, 61.11% [29] and 65% [15], these differences could be due to changes in the environmental conditions. Similarly, lower incidences were revealed in European countries, 13.6% in Turkey [20]; 6.13% and 4.16% in Apulia region and Sicily, respectively, Italy [12, 14]; 49% in Sardinia, Italy [13]. Likewise, lower incidences were revealed in African countries, 8.1% in Niger [22]; 48% in Dakar, Senegal [2] and 0.002% in Ethiopia [6]. These differences may be related to alterations in climatic and environmental conditions together with the variations in the animals' sample

size between our study and other studies in other countries.

On the other hand, higher rhinoestrosis prevalence's were reported in other studies, 100% in Egypt [8] and 100% in Istanbul, Turkey [23]. All these variations in the prevalence rates between Egypt and the other countries could result from dissimilar climate, sample size, management practices, study periods and immunosuppression that may be elicited by presence of other microorganisms or parasites; the organization of *O. ovis* larvae in the upper respiratory tract of sheep induces high inflammatory cellular activity in the gastrointestinal tract that influencing the progress and productiveness of *Haemonchus contortus* infection in the abomasums [4, 21]. Likewise, [24, 25] designated the presence of an antagonistic interaction between *O. ovis* in the nasal cavitiy and *Trichostrongylus colubriformis* burdens in the small intestine. Moreover, immuno-suppression arises following recurrent *O. ovis* infestation [9].

Regarding to the effect of donkeys age on the *Rhinoestrus* spp. larval infestation, it was revealed that *Rhinoestrus* spp. infest donkeys at all ages especially those more than four to eight years old (81.82%). Similar results were detected for the same age group [7], while our results disagreed with those results obtained by [12, 14] in Italy, where they found that horses under one year in Puglia and Sicily were more infested with a prevalence of (71.22%) and (26.67%), respectively, and [13] in Italy who recorded higher infestation rate (58.3%) in horses up to 2.5 years old.

In our study the mean number of larvae per donkey (L/D) was insignificantly different (p<0.05) except that of 1st larval instars infesting donkeys more than four to eight years old (46.45±20.12) that was significantly increased than that of donkeys more than 15 years old. Nearly same results were obtained by [28] in Egypt; [12-14] in Italy and [7] in Egypt, where they stated that there was no statistical difference in the number of *Rhinoestrus* spp. larvae among horses of various age groups.

The variation in prevalence between various age groups of donkeys may be related to different numbers of animals examined in each age group, immunological resistance that develop with repeated exposure to the infestation and dissimilar local climatic conditions prevailing in each Governorate. Also, [17] observed that *Oestrus ovis* larvae grown-up quickly and in larger numbers in lambs than in older animals exposed to the infestation before, so lambs are liable to this myasis, and [18] stated that adult sheep appear to be less vulnerable to *O. ovis* infestation than young lambs signifying that constantly exposed sheep develop immune competency, [9] found that repeated infestation with *O. ovis* stimulate immunosuppression so there is positive correlation between the age and the incidence of infestation.

Concerning to the effect of donkey's sex on the prevalence of infestation we noticed that the females were more infested with *Rhinoestrus* spp. larvae (76.59%) than males (73.45%). Closer results were revealed by [15] who recorded that she-donkeys were more infested (77.78%) than males (54.55%), and [7] who detected higher number of larvae (842) in females than (502) in males. This variation in prevalence between females and males may be attributed to difference in behavioral habits where the males are more active than females so prevent the adult fly from larvipositing their larvae in their nasal cavity, also she-donkey expose to different physiological situations like pregnancy and lactation that may influence the immunological resistance so harbor more larvae. [15] stated that there may be hormonal differences between both sexes that developed in such difference in infestation.

Our result reflected that there was no significant difference (p<0.05) in number of larvae in both sex except there was significant increase in number of 3rd larval instars in males (2.42±0.52) than that of females (1.65±0.36). Same results were obtained by [28] in Egypt; [12-14] in Italy and [7] in Egypt.

Concerning to the monthly prevalence, our records reflected that *Rhinoestrus* spp. larvae infestation rate had 2 peaks, the first was from July to September (91.89-85.29%) and the second was in December (86.36%) and in January (85%). Nearly same results were recorded by [7] in Egypt, where she observed that *Rhinoestrus* spp. infestation had 2 peaks, in January and in August. Also, [29] in Egypt, who detected an infestation rate of 100% during March, July and August and 91.67% during September. Our results disagreed with [22] in Niger, where they found that the highest prevalence was during November (37.5%) and December (33.3%); [12, 14] in Italy, where they observed that highest infestation was during May (38.46%) in Puglia and July (100%) in Sicily and [13] in Italy, who recorded that the intense infestation was in November (73.7%) and April (66.7%).

In our study, the lowest prevalence was in May (60.86%) and June (51.72%). Nearly same results were recorded by [7] who found that the lowest prevalence was during May. In contrast to our results, absence of infestation during both November and December in Egypt [29]; 0% infestation from February to September [22]; disappearance of infestation during the periods from January to March, August to November in Puglia and all over the year except April, July and August in Sicily [12, 14]; and July and September had the lowest prevalence (22.2%) and (16.7%) respectively, in Italy [13].

With regard to the seasonal prevalence of *Rhinoestrus* spp. larvae among donkeys, heavier infestations, aroused in winter (81.36%) followed

by summer (78.49%) while the lowest were in spring (69.01%). Closer results were obtained by [7] in Egypt, where she recorded highest infestation during winter and summer.

Such diversity in the prevalence of infestation with *Rhinoestrus* spp. larvae may attribute to changes in the geographical situation and climatic established environment in other countries. Also, in this study we carefully washed all the nasal mucosa of all examined animals for obtaining all the 1st larval stages but in the antecedent studies, washing of the nasal mucosa was neglected that gave in correspondence such difference in prevalence.

Dealing with the monthly prevalence of various stages of *Rhinoestrus* spp. larvae, the highest infestation rate with first larval instars was reported in June and November (100%) while the lowest was in April (27%) and March (44%). Similar results, L1 was at its highest levels during June and November (100%) to reach its lowest levels in April (15.6%) [7]. Partially agreed results were recorded, the highest infestation with L1 was during June (88.9%) while 0% infestation was noticed from October to December and in May [29]; L1 detected only in November (100%) with absolutely disappearance (0%) all over the year [22] and heavier infestation with L1 was reported from September to December (100%) while the infestation dropped off (19.6%) and (16.1%) in April and May, respectively, [13]. Conversely our results disagreed with other reports, the highest infestation rate with L1 was observed in July with absence of infestation all over the year [12, 14] and absence of L1 during the summer months [15]. These variations in the prevalence of L1 may related to different examined Governorates and the fact that when the climatic conditions in winter become adverse so L1 shows hypobiosis where it arrest its development till the condition become favorable, on the other hand, rise in the ambient temperature in summer months kill the pupae or result in deformed emerged adult flies [26].

Regarding to the monthly prevalence of L2, the highest infestation rate was reported during March (31%) while the lowest one was during May and July (0.4%) and the infestation rate with second larval instars was 0% during both June and November. Partially similar observations were recorded; the peak of infestation with L2 was in March and June while the lowest was in September with absence of infestation in May and from October to January [29]; the highest L2 prevalence was during April (57.7%) while the lowest was during January (1.6%) with absence of infestation in September and from October to December [13] and L2 reached its maximum percentage (42.4) in March and (32.7) in August while low values (6%) were during February with absolute disappearance from September to December and in June, May and January [7]. Dissimilar to our results, the highest infestation rate with L2 was during October and November with 0% infestation all over the year [22] and heavier infestation with L2 was detected during July (29.16%) whereas lower one was during April (25%) with absence of infestation in other months [12, 14].

With respect to the monthly prevalence of L3, the highest infestation rate with third larval instars was reported during April (54%) while the lowest one was during December, July (1%) and January (0.1%). The rate of infestation with third larval instars was 0% during January, June and November. Nearly similar results were obtained, the highest rates for L3 infestation (71.3%) was during April while the lowest was during May (12.3%) together with absence of infestation from October to February and in June and July [7]. Somewhat agreed results, the highest prevalence with L3 was March, August and September whereas the lowest was in May by way of absence of L3 from November to January and in June [29] and the number of L3 was increased during May (37.0%) then sharply decreased during July (3.1%) with absence of L3 from September to June [13]. In contrary to our results, the highest incidence with L3 was during November followed by January and December with full nonexistence in other months [22] and L3 exist only from April to August with maximum infestation during May, in Puglia and during April, July and August in Sicily [12, 14].

Seasonal data in our study revealed that the highest prevalence with L1 was in winter (94.47%) while the lowest was in spring (57.6%), on the other hand, the highest prevalence with L2 and L3 was recorded in spring (16.53%) and (25.87%), respectively, while the lowest one was in winter (4.17%) and (1.36%), respectively. These results were agreed with [13, 7]. Comparable data were reported as the larval instars of *Rhinoestrus* spp. were absent in winter [29] or all over the year except in winter [22]; L1 were prominent only in summer while L2 and L3 were prominent only in spring and summer [12, 14] and only L2 and L3 were detected with absence of L1during summer [13].

Such dissimilarity arises from the variation in the climatic conditions and the number of examined animals together with the method of examination of the nasal mucosa and the development of immune reactions.

In the current study, total of 8388 *Rhinoestrus* spp. larvae of which 7221 L1 (86.08%), 545 L2 (6.49%), 622 L3 (7.41%) were recovered from 227 infested donkey's heads. Similarly, [29] recovered 2302 *R. purpureus* larvae of which 988 (42.92%) were L1, 392 (17.03%) L2 and 922 (40.05%) L3 and [7] recorded higher percentage of L1 (66.07) than L3 (21.65) and L2 (12.28). Partially closer results, 2108 larvae were collected of it 66% (64.68) were L1, 22% (20.24) L2 and 12% (11.14)

L3 [13]. Higher L1 in our study was related to the careful washing of the nasal mucosa and to that the 3rd stage larvae adapt themselves to drought by a prolonged pupation period in the soil (external hypobiosis), [22]. On the other hand, higher numbers of L3 as compared to L2 and L1 were obtained by [23] (94L3, 34L2 and 0L1); [22] (10L3, 2.2L2 and 1L1); [12, 14] (93L3 in Puglia; 83L3, 22L2 and 11L1 in Sicily) and Ramadan *et al*. (2014) (149 L3, 114L2 and 0L1). Predomination of L3 was supported by [19] who found that there was a synchronous diapause in that the developed L3 affect the other larval instars development, so, the larval profile contain many L1, fewer L2 and even fewer L3 and vice versa. Also, such difference in the L1 related to the fact that in adverse climatic conditions L1 goes in hypobiosis.

In our study, the overall mean larval burden was (27.68 ± 2.43 larvae per head), quite similar results (23.31±19.81) were recorded by [29] and (7.15 in Puglia and 23.2 in Sicily) by [12, 14]. Alternatively, lower values for overall mean larval burden (7.9) was detected by [13] and (10.1) by [15]. This variation may attribute to lower L1 values or sometimes its absence in these studies, which consequently ends to lower overall burden.

In the present study, the monthly larval burden and the number of larvae per donkey's head (L/D) of L1, L2 and L3, were the highest (1722), (879) and (46.54±10.04), (43.95±10.34) during July and January, respectively, while the lowest were (328) and (13.08±2.87) during April.

Similar results, higher larval burden and mean burden were (61) and (36) during July and lower were (4) during April, were observed by [12, 14] in Sicily. Variable results opposing our findings were reported, the augmented mean larval burden (57.86) during June while lesser one (3) during January [29]; higher burden and mean burden (51) and (10.2), respectively, were in May and lower (10) and (3.3), respectively, were in July [12, 14] in Puglia; highest burden and abundance (158) and (13.2) were in January and August, respectively, whereas lowest ones (65) and (5.4) were in May, [7] and heavier burden and mean burden were (421) and (24.08) during June and March, respectively, [13].

With respect to our seasonal L/D of L1, L2 and L3 data, we established that the highest burden and L/D were (2132) and (36.14 ± 5.21) in winter followed by (3277) and (35.25 ± 5.15) in summer while the lowest (1125) and (15.83 ± 3.80) were in spring. Similarly, the highest number of larvae and mean burden were in summer (61) and (36) and the lowest were in spring (4), [12, 14] in Sicily. Partially agreed records, higher L/D was in summer (117.2) and lower burden in winter (11.50), was detected by [29]. In contrast to our result, increase in larval number and mean burden (86) and (18.7) were during spring while it decreased to (25) and (8.3) during summer [12, 14] in Puglia, and heavier larval numbers and abundance (377) and (31.4) were during summer while lower values (267) and (22.3) were in autumn, [7].

The documentations of this study assigned that the overall L/D of L1, L2 and L3 were 23.83±2.34, 1.80 ± 0.33 and 2.06 ± 0.32, respectively. Very similar results, 14.95±17.63, 7.42±5.37 and 12.37±7.74 for L1, L2 and L3, respectively, were recorded by [7].

In relation to the size of infestation our reports demonstrated that the highest number of L1, L2, L3 and total number of all larvae recovered from one head was 247, 43, 34 and 248 larvae, respectively, while the lowest was 1larva. Lower values concerning the size of infestation were counted by other researchers, the highest number of larvae collected from one donkey was 150 and the lowest was 1 larva [29] and the maximum number of larvae collected from one head was 50 while the minimum was 1 larva , [7].

Dealing with the number of generations per year, regularity of *Rhinoestrus* spp. flies reproduction and development mainly depends on the climatic conditions. Our study postulated that there was more than two generation per year as L1 were recovered all over the year with peak of infestation with during June and November (100%) suggesting existence of high numbers of adult flies during late May and October. Similar result was recorded in previous studies, two generations per year were recorded in March and June in Egypt [29]; two or more generations were observed in Sicily, Italy [12, 14]; there were evolution of L1 all over the year in Sardinia, Italy [13] and in Egypt there were two generations through the year (in January and June) [7]. In contrast, there was one generation in the year in Niger [22].

All differences in prevalence, burden, generations per year between our study and the other studies were attributed to variations in: the number of examined animals, localities examined, immunity level that affected by other parasitic infections, local climatic conditions, examination procedure and lack of understanding of rhinoestrosis signs that hinder the chemotherapy.,

L1 and L2 were always detected embedded between the turbinate bones and in the ethmoid while L3 were detected mainly in the labyrinth of the ethmoid bone, the lamina cribrosa and the nasal sinuses, and rarely detected in the pharyngeal and nasal cavities. Same results were revealed by [16]; [28]; [13]; [15] and [7].

In this study we detected that there was a period of diapauses during both June and November that was characterized by 100% prevalence to L1 and 0% for both L2 and L3,

which gave an indication on the time of choice for chemotherapy.

5. Conclusion

In this study we demonstrated an overall prevalence of 74.91% for rhinoestrosis amongst Egyptian donkey's, which gave an indication on how such myasis should be taken into major consideration. Our results emphasize that there were more than two generations per year and during the period of diapauses in June and November, it is recommended to treat the animals against rhinoestrosis to avoid the moulting of L1 to L2 and then L3.

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