Ecological and Immunological Studies on *Rhinoestrus purpureus* infecting Donkeys in Egypt and Its Control with Doramectin and Ivermectin

Mohamed Y. Ramadan [1], Abla D. Abd El mageid [2] and Mohamed G. Abdelwahab [3]

[1] Department of Parasitology, Faculty of Veterinary Medicine, Benha University.
[2] Department of Entomology, Faculty of Science, Benha University
[3] Department of Animal Medicine, Faculty of Veterinary Medicine, Benha University
mgahassan@yahoo.com

Abstract: The objectives of this study was to determine the prevalence, habitat and control of parasitic larvae of *Rhinoestrous purpureus* in donkeys. Forty donkeys were necropsied to search larvae in their nasal cavities. The results indicated that 26 out of 40 examined donkeys were infected by *R. purpureus* larvae and the percentage of infection was 65%. Female donkeys showed higher infection rate (77.78%) compared to (54.55%) in males. 263 R. purpureus larvae were collected from all investigated donkeys; 143 from females and 120 from males. The mean burden was 10 in male and 10.1 in females. L3 were more common than L2 (149 L3 compared to 114 L2). Most of L2 larvae (86.84%) attack labrynith of ethmoidal bones., moderate number infect sphenopalatine communications and only 2.63% were collected from pharyngeal cavity. On the other hand 53.59 % of L3 was collected from labrynith of ethmoid bones and other half was collected from sphenopalatine communication (23.49%), pharyngeal cavity (8.75%) and common nasal meatus (5.32%). An increase in serum globulin level (6.30 mg/dl) in R. purpures infected animals compared to (4.8 mg/dl) in control was recorded. Gamma globulins in infected animals was (2.97 mg/dl) while it was in (2.53mg/dl) in control. IgM was higher in infected animals (59.20 ng/dl) compared to (23.90 ng/dl) in control. Also IgA showed an increase in its value in infected animals compared to control (191.75, 87.11ng/dl). Concerning control of *R. purpureus* larvae, the results indicated that both doramectin and ivermectin had a larvicidal effect against *R. purpureus* larvae with superiority of doramectin as it caused 100% larval mortalities in vitro within 8 hrs. It was concluded that, *R. purpureus* larvae affect high number of donkeys specially females and the doramectin was selected to be the drug of choice for control in donkeys.
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1.Introduction

Equines still receive more interest and care in many countries as draft animals, source of leather and other related products. Donkeys have a prominent position in the agricultural systems in many developing countries **(Pearson, 1999).** The myiasis caused by larvae of *Rhinoestrus purpureus* has important factor in the equine medicine since it causes severe respiratory diseases, impair animals welfare and cause severe economic losses. The larvae localize in nasal cavities, sinuses and pharynx of equids **(Zumpt, 1965),** inducing inflammation, dyspnea, sneezing and cough. Moreover, lesions of the upper respiratory tract and lungs, damage the olfactory nerves and encephalomyelitis were reported **(Kaboret *et al.*, 1997, Angulo- Valadeza *et al.*, 2010)**. Adult flies may also eject larvae into human eyes inducing opthalmomyiasis or into nasal cavities or mouth **(Rhichard Wall, 2007)**. Knowledge about ecology, biology, immunology and control of R.purpureus is limited in Egypt **(Zayed, 1992; Zayed and Helali 1993; Zayed *et al.*, 1993).** Therefore, the objectives of this study was to determine these parameters in donkeys.

2. Material and Method

1- Collection of *Rhinoestrus purpureus* larvae:

The animals examined for the larvae of *Rhinoestrus purpureus* were from north Cairo (Kalubyia Governorate) and southern Cairo (Giza Governorate). The study was carried out on a total of 40 donkeys. The animals were necropsied at Zoo slaughter house to search larvae in their nasal cavities. The entire nasal cavities of killed animals were opened and examined for the larvae. The larvae obtained from donkeys were placed in a jar containing 70 % alcohol and brought to the laboratory for identification according to **(Dorchiesa *et al.*, 2003)**. The site of parasitism, larvae burden, number and type of larvae per animal, animal sex were investigated.

2- Blood samples:

Blood samples were taken from the investigated animals before killing from jugular vein, kept without anticoagulant to separate serum which

was used for determination protein fractionation, immunoglobulin M (Ig M) and immunoglobulin A (IgA)..

II-Experimental study to detect the Larvicidal efficacy of ivermectin and doramectin against *Rhinoestrous purpureus* larvae:

Chemicals:

a- Ivermectin
b- Doramectin
c- Physiological saline

2- *R. purpureus* larvae: 45 active L2 *R. purpureus* larvae were chosen to carry out the insecticidal efficacy of ivermectin.

3- Steps of the experiment

Studying the larvicidal efficacy was done according to **(Pamo *et al.*, 2005)**. One dose of ivremectin and another from doramectine with three replications for each were used *in vitro*. Five larvae per replicate were placed in a clean dry plastic cups with a filter paper, Disc of Whatman No.1 filter paper measuring 62.63 cm2 surface areas, impregnated uniformly with the used concentration of ivermctin and doramectin on the bottom. The drug was dissolved in physiological saline. Bioassays were done at 27±2ºC and 75±5% RH. Larvae were considered alive if they exhibited normal behavior when breathed upon or physically stimulated with a wooden dowels. If larvae were incapable of movement, maintaining normal posture, leg coordination, ability to right themselves, they were considered moribund or dead (Panella *et al.,* 2005). The mortality was initially assessed 30 min. after being subjected to the examined drugs followed by mortality assessment at one hour, 2, 4, 8, 10 and 12 hrs. The used concentrations were 0.1% for each medicaments (Drummond, 1984).

3. Results

Results in Tables (1, 2) indicated that 26 out of 40 examined donkeys were infected by *Rhinoestrus purpureus* larvae. The over all percentage of infection was 65%. Female donkeys showed higher infection rate (77.78%) compared to (54.55%) in males. The table showed also that 263 *Rhinoestrus purpureus* larvae were collected from all investigated donkeys; 143 from she donkeys and 120 from males. The mean burden was 10 in male and 10.1 in females. L3 were more common than L2 (149 L3 compared to114 L2). Most of L2 larvae 86.84% attack labrynith of ethmoidlal bones, moderate number infect sphenopalatine communications and only 2.63% were collected from pharyngeal cavity. The common nasal meatus was free from L2. About one half number of L3 53.59 % was collected from labrynith of ethmoid bones and other half was collected from sphenopalatine communication (23.49%), pharyngeal cavity (8.75%) and common nasal meatus (5.32%).

Results in Table (3) and plates (1, 2) revealed an increase in serum globulin level (6.30 mg/dl) in R. purpures infected animals compared to control (4.8 mg/dl). Gamma globulins in infected animals was (2.97 mg/dl) which was also higher than in control (2.53mg/dl). IgM was higher in infected animals (59.20 ng/dl) compared to control (23.90 ng/dl). Also IgA showed an increase in its value in infected animals compared to control (191.75, 87.11ng/dl) in infected and control animals respectively.

Results in table (4) indicated that, both ivermectin and doramectin had a larvicidal effect against *R. purpureus* larvae compared to control. Larval mortalities started after one hr of exposure to doramectin and increased gradually with time and reach maximum (100%) after 8 hrs. On the other hand ivermectin started to kill larvae after 2hrs and the mortality rate reached 100% after 10 hrs of exposure. All tested larvae remain alive and very active during all time of the experiment in control group.

Table (1): Incidence of *Rhinoestrus purpureus* in male and female donkeys

Sex	No. of ex. Animals	No. of positive animals	Mean burden	No of collected larvae	No. of L2	No. of L3
Males	22	12 (54.55%)	10	120 (5.5/animal)	54	66
Females	18	14 (77.78%)	10.2	143 (7.9/animal)	60	83
Total	40	26 (65%)	10.1	263	114	149

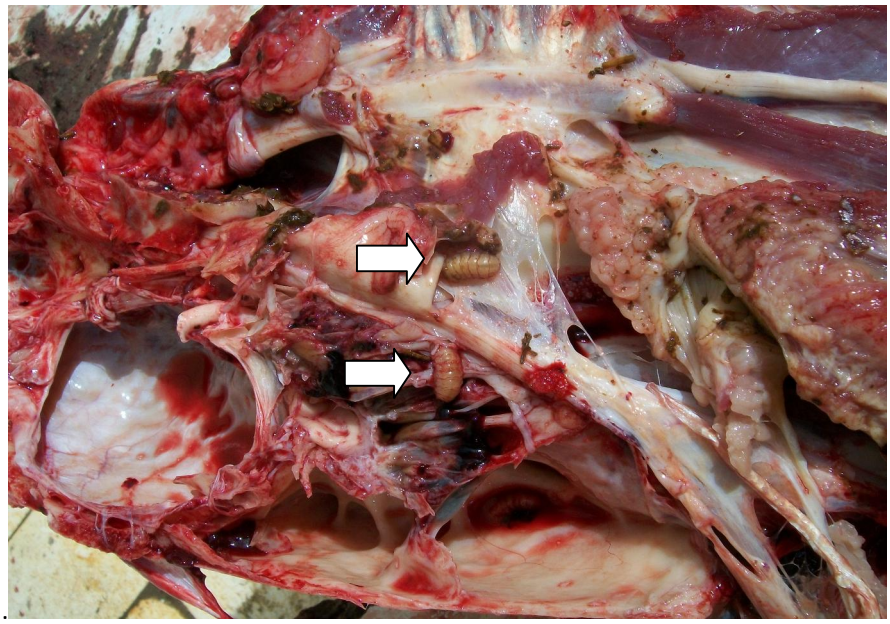

Figure (1): Showing 3rd larval stage of *R. purpureus* inhibit the area of the ethmoid bones.

Table (2): Distribution of Rhinoestrus purpureus in air passages of donkeys

Larvae	Total	Labrynith of ethmoid	Sphenopalatine communication,	Pharyngeal cavity	common nasal meatus
L2	114	99 (86.84%)	12 (10.53%)	3 (2.63%)	---
L3	149	80 (53.59%	35 (23.49%)	20 (13.42%)	14 (12.28%)
Total	263	179 (68.06)	47 (17.87%)	23 (8.75%)	14 (5.32%)

Table (3): Serum protein electrophoresis, IgM and IgA in Rhinoestrus infected donkeys

	Infected	Control
Albumin (gm/dl)	2.3	3.1
Globulin (gm/dl)	6.30	4.8
Alpha1 globulin (gm/dl)	0.24	0.29
Alpha 2 globulin (gm/dl)	0.99	0.96
Beta Globulin (gm/dl)	2.1	1.02
Gamma globulin (gm/dl)	2.97	2.53
IgM (µg/dl)	59.20	23.90
IgA (µg/dl)	191.75	87.11

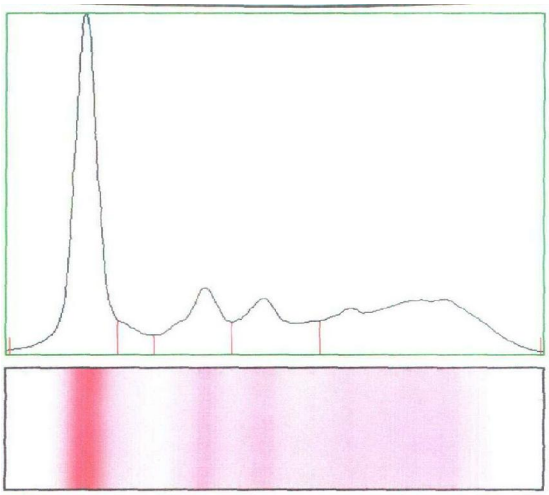

Plate (1): Protein electrophorsis in non infected donkeys

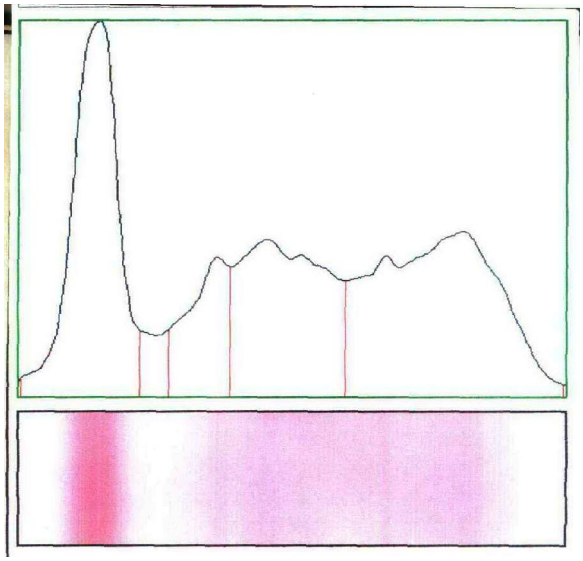

Plate (2): Protein electrophoresis in infected donkeys

Table (3): Mortalities of Rhinoestrous purpureus after treatment with ivermectin and nitroxynil

Drug	30min	1hr	2hrs	4hrs	8hrs	10hrs	12hrs
Ivermectin							
L	5(100%)	5 (100%)	4 (80%)	2 (40%)	2(40%)	0(0.0%)	0(0.0%)
D	0 (0.0%)	0(0.0%)	2 (20%)	3 (60%)	3 (60%)	5(100%)	5(100%
Doramectin							
L	5 (100%)	4 (80%)	2 (40%)	1(20%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
D	0(0.0)	1(20%)	3 (60%)	4 (80%)	5 (100%)	5 (100%)	5(100%)
Control							
L	5(100%	5(100%	5(100%	5(100%)	5(100%)	5(100%	5(100%
D	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)

L= alive larvae; D= dead larvae

4. Discussion

Rhinoestrus purpureus larvae infestation has great importance in the horse medicine since it causes severe respiratory diseases. Therefore, an accurate diagnosis of rhinoestrosis is essential to study its epidemiology and control so, it is crucial to understand the actual incidence and epidemiology of this myiasis in live animals (**Traversa and Otranto, 2006**).

Our results indicated that, the percentage of infection was 65%. This result agreed with the previous study in Egypt which was carried out by (**Zayed *et al.*, 1993)** who examined heads of 144 donkeys at the postmortem during the period from September 1989 to the end of August 1990 and revealed that (61.11%) donkeys were infested with *Rhinoestrus purpureus*. **(Dorchiesa *et al.*, 2003)** recorded 3 species of nasal bots of equids namely; *Rhinoestrus purpureus* and *R. latifrons* in horses and donkeys (paleartic region) and *R. usbekistanikus* in horses, donkeys (palearctic region) and Burchell's zebra (*Equus burchellii*) in Africa south of Sahara (Ethiopian region). Also, **(Otranto *et al.*, 2004)** & **Otranto and Colwell, 2008)** noted myiasis caused by *Rhinoestrus* larvae recently in Europe, specifically in southern Italy, with a prevalence rate up to 6%. The recorded lower incidence in Italy may attributed to changes in environmental conditions

Higher infection rate (77.78%) was recorded in she-donkeys showed compared to (54.55%) in males. Such higher incidence in females may be attributed either to calm habit of she-donkeys that enable *Rhinoestrous* females to larviposit in their nasal cavities or to hormonal differences between male and female donkeys.

In the present study, L3 predominate L2. Such finding confirmed the previous results of (**Zayed *et al.*, 1993)** in Egypt who recorded an increase in larviposition activity of *Rhinoestrous* females from mid-January to mid-April and highest mean larval number during June.

Concerning habitat of *R purpureus* larvae, it was noticed that, most of L2 larvae inhibit labrynith of ethmoidal bones., moderate number infect

sphenopaltine sinus and the lowest number were collected from pharyngeal cavity. These results agreed with (**Zayed and Hilali, 1993)** in Egypt, who studied the localization and migratory route of *Rhinoestrus purpureus* larvae and recorded 93.9%, of 2nd instar larvae, were found in the labrynith of ethmoid bone, 5.8% in the sphenopalatine sinus, and only 0.3% in the pharyngeal cavity. Such observation suggested that, moulting of the 1st instar larvae occurred only in the labrynith of ethmoid bone. On the other hand, (53.59 %) of L3 was collected from labrynith of ethmoid bones and others were collected from sphenopalatine sinus (23.49%), pharyngeal cavity (8.75%) and common nasal meatus. Our results confirmed the suggestion of **(Zayed and Hilali, 1993)** who reported that, the 2nd instar larvae moulted in the labrynith of ethmoid bone, sphenopalatine sinus and pharyngeal cavity.

The mean larval burden was 10 in male and 10.1 in females. Indeed **(Biggs *et al.*, 1998)** suggested that any number of larvae above 10 is potentially harmful to the hosts

Absence of first larval instars together with predomination of L3 indicated that there is no larvipostion activity of adult females during the period of the study (summer months). This may be attributed to higher environmental temperature during Summer months which either kill pupae or resulted in deformities in emerged females. In this respect, **(Zayed,1992)** in Egypt studied the pupal duration of *R. purpureus* under variable degrees of temperature and relative humidity (RH) and found that, at 37°C, the pupated larvae failed to pupate and died and their was a deformities in emerged flies. Some necropsy surveys showed that, kinetics of 1st instar larvae is related to adult mating activity **(Tabouret *et al.*, 2001a,b**). An extended pupariation outside has been observed for O. ovis by **(Biggs *et al.*, 1998)** allowing adults flies to wait for the best time for emergence, mating and larviposition. Choosing the right place for burrowing and pupariation is of high importance in this species and extreme temperatures into the soil (for example above 40 ◦C for long periods) being lethal to pupae or leading to low adult weight with high post-emergence mortality rates **(Cepeda Palacios *et al.*, 1998).** For all species of oestrids, mating and seeking activities occur optimal on warm days, sunny and not windy days at temperatures between 20 and 30 ◦C **(Anderson, 2006).**

The present data revealed an increase in serum globulin specially gamma globulin levels in *R. purpures* infected animals compared to control. This is probably caused by a permanent antigenic stimulation during infection as antigenic and some inflammatory products produced by larvae induce inflammatory and hypersensitive reactions in a trail by the host to expel or destruct the larvae. **(Dorchies *et al.*, 2006)** reported that mesoparasites, oestrids display complex host/parasite relationships. Indeed, the hosts develop numerous but often ineffective strategies of expelling them. Frequently there is interstitial pneumonia associated to emphysema and bronchiolitis with many eosinophils. This lesion is firstly related to the effect of parasitic antigens and proinflammatory products liberated by mast cells and eosinophils from nasal cavities inhaled in the lungs. Secondly, it appears that oestrids antigens have an immunosuppressive effect, allowing the actualization of pathogenic effect of virus inducing interstitial pneumonia.

IgM is the first isotype in response to infection, secreted from mucosal surfaces, good agglutinator and fixes complement. IgA is the major isotype at mucosal surfaces, secreted across the epithelial cells, bind to eosinophils, can activate complement by alternative path way and its total % in serum is twice IgM, **(Wakelin, 1996; Scala *et al.*, 2006).** The higher levels of IgM and IgA in infected animals in the present study was in accordance with previous results of **(Suarez *et al.*, 2005; Angulo-Valadez *et al.*, 2009)** who recorded that *Oestrus ovis* infection elicits an IgM and IgG systemic antibody response in both sheep and goats. **Innocenti *et al.*(1995)** demonstrated that salivary glands proteins are the most antigenic *O. ovis* larvae proteins compared to digestive tube contents or cuticular antigens.. Many serological tests have been developed using a crude L2 somatic extract. Sensitivity, specificity, positive and negative predictive values were improved compared to the previous tests but detection of infected animals remains difficult in winter when L1 arrest their development inside the host. Negative correlations among larval establishment and/or larval development as well as intensity of local and systemic IgG responses were found in naturally infected ewes **(Angulo-Valadez *et al.*, 2008).**

With respect to control of *R.purpureus* in vitro, our experiment is the first preliminary trial in this aspect, it showed that both doramectin and ivermecitin had a strong larvicidal effect against its larvae as it cause 100% mortalities. From the middle of the last century control of oestrids myiasis in livestock, relied on the use of the organophosphate products (OPs) which, however, have produced some unsatisfactory results in terms of animal and human safety (**Charbon & Pfister, 1993**). In the past few years OPs have been superseded by macrocyclic lactones such as doramectin and ivermectin **(Sutherland, 1990),** As ivermectin has proved to be highly effective against *Hypoderma* sp. larvae even at dosages as low as 0.2 µg/kg, which is 1/1000th of the

recommended dose **(Drummond, 1984)** so both drugs was suspected to do the same effect against *R. purpureus* larvae in equine.

Conclusion

It was concluded that *R. purpureus* infection in donkeys at 65 % in Egypt with higher incidence in females. The parasitic larvae of *R. purpureus* stimulate immune system. Both of doramectin and Ivermectin had a strong larvicidal effect against *R. purpureus* larvae *in vitro* with superiority of doramectin. Further studies are necessary to determine *R. purpureus* prevalence in the other equine sp. in Egypt as well as studying the effect of ivermectin and doramecin against it in large number of infested equines

It is hoped that this research will help veterinarians, equine owners and others who interested in providing health and welfare to equine to protect equine from such dangerous larvae.

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