CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF FIGURES AND PLATES

LIST OF ABBREVIATIONS

Abbreviation	Description
%	Percent
°C	Celsius Degree
µg	Microgram
µL	Microliter
µm	Micrometer

µmol/l	Micromole per liter
4CN	4-Chloro 1-Naphthol
B.WT.	Body weight
BSA	Bovine serum albumin
cm	centimeter
D.W	Distilled water
ELISA	Enzyme linked immunosorbent assay
ESP	Excretory Secretory Product
g	Gram
gm	Gram
hr	Hour
IDT	Intradermal test
IgG	Immunoglobulin G
KDa	Kilodalton
Kg	Kilogram
L1	First larval instar
L1CE	L1crude extract
L2	Second larval instar
L3	Third larval instar
M	Molar
mA	Milliampir
MG	mid gut
mg	Milligram
mg/dl	Milligram to each 100 milliliter
min	Minute
MixCE	Mixed crude extract
ml	Milliliter
mM	Millimol
mm	Milimeter
MW	Molecular weight
n	Number
N	Normality
NaCl	Sodium chloride
Neg-HIS	negative hyperimmune serum
nm	Nanometer
OD	Optical density
OPD	OrthophenyleneDiamine
PBS	Phosphate buffer saline
PBST	Phosphate buffer saline tween
pH	Degree of acidity and alkalinity
PMSF	phenyl methyl sulfonyl fluoride

rpm	Revolution per minute
SDS	Sodium dodecyl sulphate
SE	Standard error
SG	salivary gland
TBS	Tris-Buffer Saline
TBS-T	Tris-Buffer Saline Tween
TEMED	NNN'N' tetra methyl ethylene diamine
V	volt
W	Watt
W/V	Weight to volume
β	Beta

Summary

The present study aimed to early diagnosis of nasal bots infestation in camels and donkeys through determination of the prevalence of infestation with of *C. titillator* and *Rhinoestrus* spp. larvae among camels and donkeys, beside studying their biology, evaluation of the immune response of the animals through ELISA test, characterization of antigens by electrophoresis and immunoblotting, in vitro rearing test and Intradermal test. The study revealed the influence of age, season and sex of the animals on their infestation rate. Also the importance of ELISA, immune-electrophoresis, in vitro rearing and intradermal tests as early tools in diagnosis of *Cephalopina titillator* and *Rhinoestrus* spp. larval infestations.

The results showed that the overall prevalence of infestation with *C. titillator* larvae among camels slaughtered at Toukh city slaughter house was 63.32% (449/709).

Regarding to the effect of age on the prevalence of infestation with *C. titillator* among camels, it was found that camels at all ages were susceptible to *C. titillator* infestation especially those more than ten years old (64.71%).

Dealing with the effect of camel sex on the prevalence, our data revealed that female camels showed higher incidence of infestation with *C. titillator* larvae (75%) than males (63.26%).

Regarding to the monthly prevalence of *C. titillator* larvae among camels, the highest infestation was in September (71.79%). In relation to the seasonal prevalence of *C. titillator* larvae among camels, the highest infestation rate was recorded in autumn (69.38%) and winter (67.04%).

Seasonal data in our study indicated that L1 were prevalent during spring while L2 and L3 were prevalent during summer & autumn, respectively.

In the present study, a total of 23790 *C. titillator* larvae were detected in 449 camels' heads (14020 L1 (58.93%), 7171 L2 (30.14%), 2599 L3 (10.92%)) giving an overall mean of 33.53±2.22 larvae per head.

The highest number of *C. titillator* larvae recovered from one camel's head was 462 and the lowest was one larva. The highest number of L1, L2 and L3 obtained from one camel head was 420, 217 and 46 larva/head, respectively.

The seasonal larval burden / number of larvae per infested camel's head (L/C) was the highest (7448/40.48±5.50) in spring while the lowest was (4480/ 28.01±4.03) in autumn.

With regard to number of generations per year, our data indicated that the evolution of *C. titillator* takes place all year round with many generations, at least three generations.

In the present study we detected that there was a period of diapauses during May that was characterized by prime prevalence (95.44%) to L1 over both L2 and L3, which gave an indication on the time of choice for chemotherapeutic application.

It was the first record to an infestation of 89 (12.55%) of camel head in Egypt with 129 L3 of *Oestrus ovis.*

The overall prevalence of infestation with *Rhinoestrus* spp. larvae Egyptian donkeys slaughtered at Giza zoo slaughter house was 74.91% (227/303).

Regarding to the effect of age on the prevalence of infestation with *Rhinoestrus* spp. larvae among donkeys, it was found that donkeys of all

ages were susceptible to *Rhinoestrus* spp. infestation especially those >4-8 years old (81.82%).

Dealing with the effect of donkey sex on the prevalence, our data revealed that female donkeys showed higher incidence of infestation with *Rhinoestrus* spp. larvae (76.59%) than males (73.45%).

Regarding to the monthly prevalence of *Rhinoestrus* spp. larvae among donkeys, it reached its peak in July (91.89%) followed by August (88.89%).

In relation to the seasonal prevalence of *Rhinoestrus* spp. larvae among donkeys, the highest infestation rate was recorded in winter (81.36%).

Seasonal data in our study revealed that L1 were prevalent in winter while L2 and L3 were prevalent in spring.

In the current study, a total of 8388 *Rhinoestrus* spp. larvae were recovered from 227 donkeys' heads (7221 L1 (86.08%), 545 L2 (6.49%), 622 L3 (7.41%)) giving an overall mean of 27.68 ± 2.43 larva / head.

The highest number of *Rhinoestrus* spp. larvae recovered from one donkey's head was 248. The highest number of L1, L2 and L3 obtained from one donkey's head was 247, 43 and 34larva/head, respectively.

Seasonal larval burden / number of larvae per donkey's head (L/D) was the highest (3277/35.25±5.15) in summer while the lowest was (1125/15.83 ± 3.80) in spring.

This study indicated that donkeys admitted from Giza presented the highest infestation rate (89.09%). Also, there was no significant difference in the number of larvae among donkeys of different head colors.

With regard to number of generations per year, our data indicated that the evolution of *Rhinoestrus* spp. takes place all year round with many generations, at least two generations.

In this present study we detected that there were periods of diapauses during both June and November that were characterized by 100% prevalence for L1 and 0% for both L2 and L3, which could be considered an indication on time of choice for chemotherapeutic application.

As a first record, we detected 1L3 of *Oestrus ovis* dead and calcified in only one donkey's head.

Our study indicated that *C. titillator* full mature 3rd stage larvae had a mean larval prepupal period of 38.93±8.83hr and a pupal period of 27 day. *Rhinoestrus* spp. full mature 3rd stage larvae had a mean larval prepupal period of 24.88±3.90hr, pupal period of 15.75±2.52day, and life span of the adult fly of 8.19±1.89 day. It was noticed that the female emergence followed the male emergence at a rate of approximately 1 male: 3 females. Also, the females had a longer life span (6-15 days) than males.

ELISA assay detected 43.06% and 63.89% infested camels using L1 crude antigen and SG antigen, respectively. The highest antibody level was 168.57% for SG and the lowest was 31.42% for L1CE of *C. titillator*. The highest sensitivity, specificity, positive predictive value, negative predictive value and diagnostic efficacy were 42.24%, 56.28%, 43.87%, 54.63% and 50%, respectively for L1CE.

ELISA assay detected 86.89%, 79.17%, 57.36%, 91.03% infested donkeys using MG extract, SG extract, ESP and Mixed crude antigens of *Rhinoestrus* spp., respectively. The highest antibody ratio was 192.68% for ESP antigen. The highest sensitivity, positive predictive value, negative predictive value and diagnostic efficacy were 97.56%, 60.61%, 84.62% and

62.76%, respectively, for MixCE antigen. While the highest specificity was 36.51% for ESP antigen.

Characterization of *C. titillator* larval antigens using SDS-PAGE revealed 9 protein bands to L1CE of molecular weights 114–6.5KDa and 13 protein bands to SG of molecular weights 143–6.5KDa While characterization of four different *Rhinoestrus* spp. larval antigens using SDS-PAGE revealed 6 protein bands to ESP at 240–32KDa, 15 protein bands to MG extract at 270– 6.5 KDa, 12 protein bands to the SG extract at 240–6.5 KDa and 17 protein bands to the MixCE at 270–6.5 KDa.

The immunoblotting analysis of different hyperimmune sera against their specific *C. titillator* larval antigens on nitrocellulose membrane, revealed 10 immunogenic reactive bands to hyperimmune sera of L1CE at MWs of 171–24KDa and 10 bands to hyperimmune sera of SG extract at 175–21KDa. There were 3 cross reactive bands between L1 and SG antigens at 60, 49 and 24KDa. While analysis of the hyperimmune sera against their specific *Rhinoestrus* spp. larval antigens revealed 5 immunogenic reactive bands to hyper immune sera of ESP at 75–24KDa, 7 bands to hyperimmune sera of MG extract at 175–24KDa, 10 bands to hyperimmune sera of SG extract at 148–20KDa and 14 bands to hyper immune sera of MixCE extract at 175–16KDa. There were cross immunogenic reactive bands: 3 between all antigens at 62, 52 and 24KDa, 2 between MG and Mixed crude antigens at 175 and 45KDa, 2 between SG and Mixed crude antigens at 78 and 37KDa.

Both ELISA and immune-electrophoresis revealed that L1 antigen of *C. titillator* and mixed crude or SG antigens of *Rhinoestrus* spp. larvae are the most efficient antigens in diagnosis of cephalopinosis and rhinoestrosis, respectively.

Early diagnosis of cephalopinosis and rhinoestrosis through in vitro rearing of different larval instars of *C. titillator* and *Rhinoestrus* spp., respectively, on three different media (Agar based, Serum based and Immunoglobulin based media) showed that the only successful inhibition to the larval growth and weight could occur by using the Immunoglobulin based media.

This study recorded for the first time that the intradermal test was superior in early diagnosis of cephalopinosis and rhinoestrosis because it gave fast response after 30min-1hr.