

## Prevalence and Pathology of Nasal Myiasis in Camels Slaughtered in El-Zawia Province-Western Libya: with a Reference to Thyroid Alteration and Renal Lipidosis

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**Abstract:** Camel botfly, *Cephalopina titillator*, causes severe economic losses to the camel industry in many camel-producing areas of the world. This investigation was carried out randomly on camels slaughtered in El-Zawia abattoir, Western Libya, on 589 camels 149 females and 440 males. The investigation has not only focused on the prevalence and pathology of that disease, but also on its other impacts like thyroid alteration and its subsequent expositions. After slaughtering, the head was dissected and grossly inspected for presence of *C. titillator* larvae and other gross abnormalities. It was found that 79% were infested with this larvae (68.4% in males and 44.9% in females) and the rate of infestation was significantly greater in the colder months (68.8%) compared to those of warmer ones (31%) and in males (65.0%) compared to those of the female camels (45.60%). The prevalence rate was lower in camels younger than 2 years old (39.8%) compared to those of 2-6 (61.5%) and over 6 years old (62.8%). Proper tissue sections from nasal cavity, pharynx, turbinates and frontal sinuses of 30 infested camels were examined histopathologically and showed serious tissue alterations of the mucosal epithelium of the nasopharyngeal region varied from inflammatory reaction, degeneration, necrosis and cystic dilatation of the submucosal glands with various degrees of reparative processes. Focal to diffuse meningitis and conspicuous colloid goiter was observed in the thyroid sections of the corresponding cases. All accompanied with renal lipidosis. Serum samples analysis of infested camels revealed significant decrease in T3 and T4, non-significant decrease in TSH, significant decrease in HDL as well as a significant increase in LDL and cholesterol levels. It was concluded that camel nasal botfly infestation is responsible for the pathological changes and functional alterations in the thyroid gland of camels with its subsequent impacts.

**Key words:** Camel botfly • Prevalence • Pathology • Thyroid alterations • Cholesterol • Lipoproteins

### INTRODUCTION

The oestrid fly or the camel nasal botfly *Cephalopina titillator* (*C. titillator*) is an insect that causes health hazards and severe economic losses to camel industry in many camel-producing areas of the world [1]. No doubt, camels are very important livestock particularly in arid and semi-arid lands. Nevertheless, the control of the adult *C. titillator* and its larval instars is a must for the sake of the animal and for the human welfare [2].

Camel nasopharyngeal myiasis is caused by larvae of *C. titillator*. The larvae of this fly are deposited in the nostrils of camels. They moult twice while attached to the nasal cavity and pharynx [3] causing extensive irritation, tissue damage and respiratory disorders [4, 5]. Infestations impair animals' welfare, reduce host

physiological functions, destroy host tissues and cause significant economic losses to livestock through abortion, reduction of milk production and losses in terms of weight gain, infertility and low hide quality [6, 7]. Infested camels lose their appetite, show difficulty in breathing, snort, sneeze and expel the larvae from their nostrils. Occasionally the larvae may reach the cranial cavity causing meningitis. Hussein, *et al.* [4] referred to the appearance of neurological signs resembling cranial coenurosis. Infested camels may also die from meningitis caused by secondary infections [8]. It is also mentioned that the mechanical damages such as penetrating the ethmoid bone by the larvae may assist in the introduction of bacteria and viruses to the cerebrospinal canal [3]. The intensity of clinical signs depends on the amounts of damage by migrating larvae.

The available literature contains few contributions on the localization of these larvae in the head of infested camels in Sudan [5], Saudi Arabia [9] and Egypt [10]. However, no studies on the location of larval moults have been carried out. Information on the migration route inside infested heads was inadequate.

This investigation was premeditated to record the prevalence together with gross, histopathological changes and the possible subsequent alterations in thyroid gland due to *C. titillator* infestation in camels slaughtered in El-Zawia abattoir Western-Libya.

## MATERIALS AND METHODS

This investigation was carried out randomly on camels slaughtered in El-Zawia abattoir, Western Libya, on 589 camels 149 females and 440 males during the period from September 2007 to August 2008. Diagnosis of *C. titillator* infestation was based on gross observation. The slaughter house was visited once weekly. These animals included both imported camels and those have been brought from different arid and semiarid areas in western Libya.

History about previous antiparasitic treatment could not be obtained unfortunately, but considering the general health condition of the animals and their other parasitic fauna; it was unexpected that they had given any drug against *C. titillator*.

**Work Protocol:** The examined animals were categorized into three age groups; less than two years old, 2-6 years old and over 6 years old. Diagnosis of *C. titillator* infestation based on both gross and histopathological examination of collected specimens. Through P.M. examination was carried out on the head of the slaughtered camels after its separation from the rest of the body. The skull was opened sagittally to expose the different regions of nasal and pharyngeal cavities, the labyrinth of the ethmoid bone, the turbinates, the lower nasal meatus and the pharynx. These were all inspected carefully for the presence of *C. titillator* larvae and the possible gross abnormalities accompanied the presence of the larval infestation. Also, the larvae collected from each camel were counted and their size was measured.

**Preparation of Tissue Specimens:** For histopathological examination, representative specimens from the nasal and pharyngeal cavities, frontal sinuses, turbinate bone and meninges were taken from 40 infested camels and 10 non infested ones. Thyroid gland and specimens from kidneys of the corresponding cases were also obtained. Then all

specimens were fixed in 10% neutral buffered formalin and routinely dehydrated in graded alcohols, cleared in xylol, embedded in paraffin, sectioned at 4-5 $\mu$ m and then stained by haematoxylin and eosin for light microscopic examination.

**Preparation of Blood Samples:** Blood samples were collected from the corresponding cases in clean sterile tubes, centrifuged at 3000rpm for 15min. for serum separation. Serum samples were kept frozen at -20°C till used for analysis of thyroid hormones (triiodothyronine, T3 and thyroxine, T4) and thyroid stimulating hormone (TSH) using radioimmunoassay (RIA) technique according to Rodbard and Hutt [11]. Also serum samples were analyzed for total cholesterol (mmol/l), HDL-cholesterol (mmol/l) and LDL- cholesterol (mmol/l) by quantitative enzymatic colorimetric method [12].

**Statistical Analysis:** The obtained data were statistically analyzed using student t-test and analysis variance wherever appropriate [13].

## RESULTS

**Prevalence of Infestation:** In the present study, examination of the nasal cavity, pharynx, turbinates and sinuses of 589 camels; 149 females and 440 males during the period of Sept. 2007 to Aug. 2008 revealed a high percent of infestation among camels in the examined locality.

Table 1 revealing that 465 (79 %) camels were infested with instars of *C. titillator*. Also, as shown in the same table, the prevalence of infestation was significantly high in male (68.4%) than in females (44.9%). Also the prevalence was higher in cold (68.8%) than that in worm (31%) seasons ( $P < 0.05$ ). In addition, the prevalence of infestation was significantly ( $P < 0.05$ ) high in animals of 2-6 years old (63%) and those older than 6 years-old (68%) as compared to those less than 2 years-old (43%). The number of larvae harvested from each camel ranged from 5 to 18 per camel with a maximum infestation number in cold season. It was obvious that different stages of the larvae could be observed all over the surveyed period.

**Results of Biochemical Analysis:** The mean values of serum TSH and thyroid hormones (T3 and T4) of the infested and non-infested camels are presented in Table 2. Both of T3 and T4 showed significant decrease ( $P < 0.001$  and  $P < 0.01$  respectively) in their levels in infested animals. While, serum level of TSH revealed non-significant decrease.

Table 1: The prevalence of *C. titillator* infestation among camels in the examined locality

	Inspected.	Infected.	Prevalence(%)
Total	589	465	79
Males.	440	301	68.4
Females.	149	76	44.9
Cold season.	379	301	68.8
Worm season.	210	76	31.0

Table 2: The mean values  $\pm$  standard deviation of T3,T4,TSH and lipoproteins in serum of control and *C. titillator* infested camels

	T3 (ng/ml).	T4 (mg/dl).	TSH (IU/ml).	HDL (mmol/L).	LDL (mmol/L).	COL. (mmol/L).
Control animals.	126.47 $\pm$ 17.80	15.23 $\pm$ 6.23	0.018 $\pm$ 0.003	0.58 $\pm$ 0.31	1.98 $\pm$ 0.24	2.45 $\pm$ 0.24
Infested animals	34.52 $\pm$ 2.45**	7.88 $\pm$ 1.78*	0.012 $\pm$ 0.002*	0.34 $\pm$ 0.27**	2.16 $\pm$ 0.09**	4.04 $\pm$ 0.16

\*\*\* = P<0.05, \*\* = P<0.01

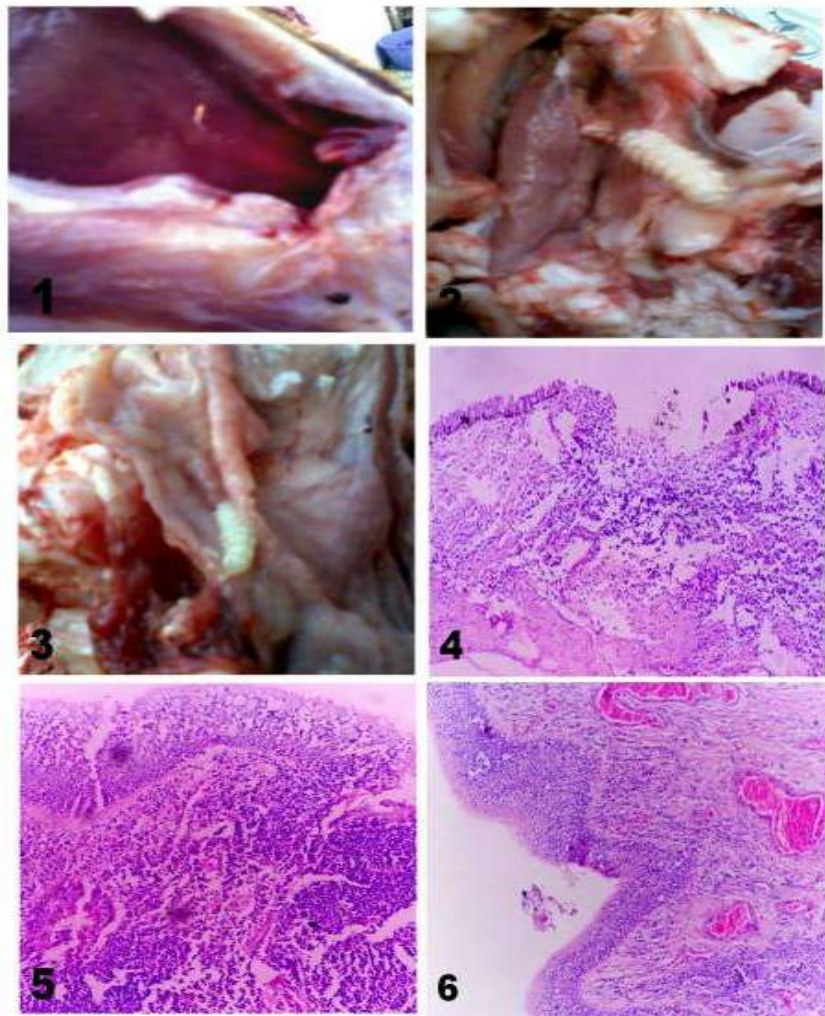


Fig. 1-6: Nasopharyngeal region of *C. titillator* infested camel showing hemorrhagic, swollen and edematous mucosa. Fig. 2 and 3: nasopharyngeal region of *C. titillator* infested camel showing still active and freely crawling about larva with dark brown nodules (2) and others loosely attached to the congested mucosa (3). Fig. 4,5 and 6 the nasopharyngeal region of *C. titillator* infested camel showing desquamation, hydropic degeneration (4) and marked hyperplasia of the mucosal epithelium (5) with Focal or diffused infiltration of the mucosa and submucosal layers by lymphocytes, plasma cells, macrophages, eosinophils and fibroblasts (6). (H&E X200).

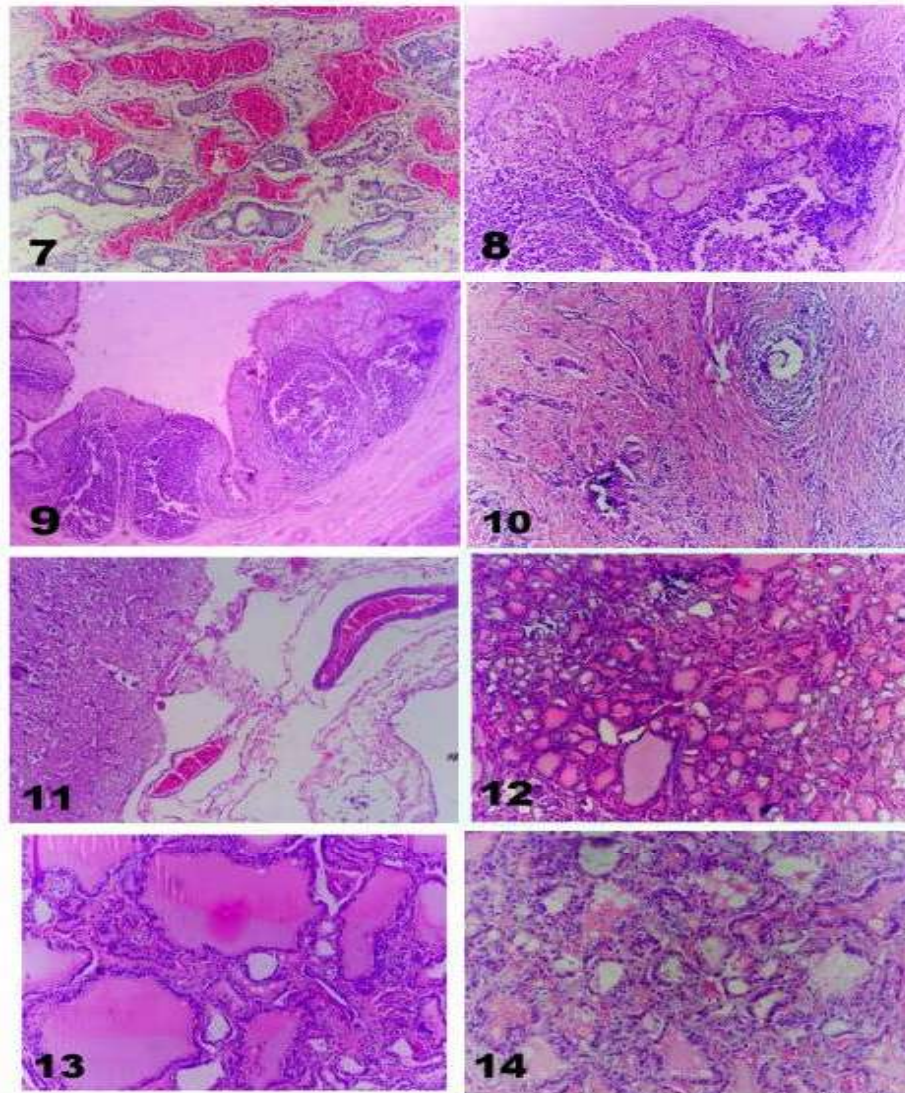


Fig. 7-14: Nasopharyngeal region of *C. titillator* infested camel showing marked congestion of the nasopharyngeal blood vessels (Fig. 7). Degenerative and necrotic changes of the submucosal glands with mononuclear cells infiltration admixed with eosinophilic material (Fig. 8). Many lymphoid nodules with central abscesses at the sites of larval attachment (Fig. 9). Reparative processes including granulation tissue formation, angiogenesis, fibroplasias and focal calcification (Fig. 10). Brain of *C. titillator* infested camel showing diffuse meningitis (Fig. 11). Thyroid glands of *C. titillator* infested camel showing colloid goiter characterized by stretched thyroid follicles with abundant colloid (Fig. 12), or with broken follicular epithelium and escape of the colloid into the space between that epithelium and the basement membrane (Fig.13). Hyperplastic goiter was evident in thyroid gland of some other infested camels (Figure 14). (H&E X200 and 400).

A significant decline ( $P<0.01$ ) was discernible in the HDL as well as a significant increase ( $P<0.01$ ) in the level of LDL and cholesterol.

#### Pathology

**Grossly:** The infestation was obviously restricted to the nasopharyngeal cavity and turbinates. The mucous

membrane of the nasopharynx was congested, swollen, edematous, occasionally haemorrhagic (Figure 1) and infrequently occupied by copious amounts of mucofibrinous thick, dark-coloured exudates in which some larvae were entangled. In some cases, there were ulcer-like lesions and dark brown or black nodules contained pus were seen in the mucous membrane of these structures

representing previous foci of larval attachment. Conspicuously many larvae were still active and crawling about freely (Figure 2), however, some were loosely attached (Figure 3), others were still adhered firmly to the pharyngeal mucosa by their hooks and when removed, firm reddish nodules marked the sites of attachment. Occasionally, degenerated larvae were found embedded between the turbinate bones and few were seen in the turbinate and ethmoid area. The damage in the affected area depended largely on the degree of infestation. Moreover, obvious meningitis was observed in large number of heavily infested animals.

The thyroid gland of infested camels appeared ovoid in shape but few were elongated. The surface of most cases appeared smooth, but few cases showed rough and cystic surface. The cut surface sometimes exuded a sticky jelly like fluid.

The kidneys of the corresponding cases were apparently normal; however in few cases it appeared pale and enlarged.

**Microscopically:** The main histopathologic changes observed in the nasopharyngeal region were desquamation, hydropic degeneration and marked hyperplasia of the mucosal epithelium (Figures 4, 5). Focal or diffused infiltration of the mucosa and submucosal layers by lymphocytes, plasma cells, macrophages, eosinophils and fibroblasts were evident (Figure 6).

Severe congestion of the blood vessels of the nasopharyngeal wall (Figure 7), goblet cell hyperplasia and marked cystic dilatation of submucosal glands were also seen in the heavily infested animals. Many of the later glands showed degenerative and necrotic changes with mononuclear cells infiltration admixed with eosinophilic material (Figure 8).

Formation of lymphoid nodule with central abscesses were evident at the sites of larval attachment (Figure 9). Various degrees of reparative processes were also encountered, including granulation tissue formation, angiogenesis, fibroplasias and scar tissue formation accompanied with atrophic changes and focal calcification (Figure 10).

Focal to diffuse meningitis characterized by congestion and oedematous swelling of the meninges as well as mononuclear inflammatory cells infiltration was observed in heavily infested camels (Figure 11).

As a regards, examination of tissue sections from thyroid gland of infested animals revealed; colloid goiter

in many sections that represented by great enlargement of the thyroid follicles with abundant uniformly dense colloid with complete or nearly complete absence of resorption vacuoles at the periphery of the colloid (Figure 12). The walls of the later follicles were enormously stretched and lined by flattened epithelium, some of which were detached. Occasionally the follicular epithelium was broken with a resultant escape of the colloid into the space between that epithelium and the basement membrane (Figure 13). Obviously many follicles had ruptured and coalesced to form giant follicles with seeping of colloid into the interfollicular spaces. On the other hand some other sections showed evidence of hyperplastic goiter (Figure 14), in which the follicles were of different sizes, irregular shapes and their lumens were devoid of colloid. The lining epithelium of these follicles was hyperplastic and hypertrophic, their cytoplasm was eosinophilic with hyperchromatic nucleus.

Concerning, the examined renal tissue sections of the infested cases revealed mild congestion, glomerular lipidosis and tubular degeneration. The later represented by variable degrees of fatty change in the tubular epithelial cells.

## DISCUSSION

The current study showed that *C. titillator* is a common parasite in camels slaughtered at the official abattoir in the surveyed locality. The overall rate of infestation among camels was 79%. The prevalence of infestation was higher in cold months (68.8%) of the year than in warm ones (31%). Different stages of the larvae could be observed all over the surveyed period which indicated that the flies may be found all year around, but in a varying level of abundance.

Previous reports indicated variable high incidence of *C. titillator* in camels in neighboring as well as other countries. For example the prevalence of infestation was 71.7, 25, 58.1, 100 and 52% in Ethiopia [14], North Sinai, Egypt [2], Iran [1], Western Sudan [5] and Saudi Arabia [9]. It was pronounced that winter and autumn constitute the highest prevalence seasons of infestation.

Morsy, *et al.* [2] reported that in Egypt the highest prevalence month was October and the highest prevalence season was autumn. While, Fatani and Hilali [9] and Oryan, *et al.* [1] reported the highest prevalence during the colder seasons of the year. They attributed that to; in warm seasons the small size of the first stage larvae that may be overlooked and absence of the larger sized, second and third stages larvae.

It was stated that the larval period in the nasal cavity, turbinates and frontal sinuses of the camel is 11 months [15, 16].

It is not clearly known why male camels were more infected than females.

Owners used males in transportation that makes males move for long distances and visit new places and so are easily exposed to new epidemic areas of *C. titillator*. The present study revealed that infestation with *C. titillator* has resulted in a significant pathology in camels [1]. It ranged from inflammatory and degenerative lesions, healing with fibrosis, scarring, atrophy and calcification in the nasopharyngeal region that extended to meningitis, pituitary-thyroid axis alteration and subsequent renal lipidosis. Similar pathological alterations were more or less previously [1, 4, 14, 17].

Camel nasal botfly has several impacts on respiratory function, feeding, health and productivity of the animals which are all not fully understood, so it is necessary to study the other aspects of this disease and its future economical importance [1].

This investigation has not only focused on the prevalence and pathology of that disease, but also on its other impacts like thyroid alteration and its subsequent expositions. Thyroid gland is one of the endocrine glands that are affected during parasite infestation. The present results indicated that *C. titillator* influenced the thyroid function and caused hypothyroidism that was evidenced by the decrease in T3 and T4 blood levels with a parallel decrease in TSH level. That data may reflect the direct effect of infection on the pituitary-thyroid axis.

There was a decrease in T3, T4 and TSH in camel infested with *Trypanosoma evansi* [18].

The observed impairment of thyroid function possibly explained on the strength of the direct and/or the indirect (through pituitary gland) effects of the parasite on the thyroid gland. The former effect may be a reason of the biologically active factors of parasite origin for example phospholipase A [19], protease [20] and peptidase [21] that causing thyroid dysfunction. On the other hand, the indirect pathological changes that occur during parasite infestation can lead to pituitary dysfunction. Hence, the presence of *C. titillator* within the nasopharyngeal region and its causing bleeding from the nostrils, meningitis and nervous manifestations may perhaps alter the pituitary TSH secretion and in so doing disrupt thyroid function. Additionally, an indirect antithyroid effect could have been mediated through activation of epithelial cells, macrophages and monocytes in the immune system during infection releasing cytokines

which activate and down regulate the endocrine system. The results of [22, 23] suggested that IL-1 might be involved in the regulation of pituitary function.

It has been postulated that the metabolic defect in hypothyroidism may dwell in the mitochondrial membrane [24] since both of the synthesis of long chain fatty acids from glucose and other carbohydrate precursors as well as their oxidation were reduced in hypothyroidism. It is well known that T3 and T4 have a pronounced physiological effect on respiration and energy metabolism as well as the biogenesis of mitochondria as thyroid gland regulates metabolic pathways to modulate oxygen consumption, basal metabolic rate and lipid, CHO and protein metabolism [25].

Lipogenesis and lipolysis were reported to be largely affected by thyroid hormones [25], which explain the observed significant hypercholesterolemia, decrease of HDL and increase of LDL in the infested animals in the present work. These results are similar to those previously recorded [26 - 28]. They described that these changes may be due to a lowering of the basal metabolic rate resulting from hypothyroidism. The changes of HDL, LDL and cholesterol serum levels may be a result of lipolysis. One particular effect of thyroid hormones is the tendency to reduce plasma cholesterol levels. This appears to involve both increased cell uptake of low-density lipoproteins with associated cholesterol molecules and a tendency for increased degradation of both cholesterol and low-density lipoprotein [29].

Histopathological examination revealed glomerular lipidosis and mild to moderate degrees of degenerative changes in tubular epithelium. Renal glomeruli became plugged with lipid in hypothyroid animals, resulting in progressive renal failure [30]. However, lipidosis was not reported in the kidney of hypothyroid sheep [31]. In addition, thyroid glands of the infested animals revealed different degrees of either colloid goiter as the thyroid follicle were distended with uniformly dense colloid or hyperplastic goiter at which the follicles showed hyperplastic epithelium. This observations are agreed with those of Belshaw [32] who mentioned that colloid goiter is observed in hypothyroid state dogs secondary to TSH deficiency with grossly normal or slightly enlarged thyroid glands.

In conclusion, the present investigation revealed that *C. titillator* infestation in the surveyed locality constitutes a great percent and problem among camels. Additionally, it indicated a dysfunction in the servomechanism of the pituitary-thyroid axis during the

state of *C. titillator* infestation in camels through their multiple functions to cause decrease in thyroid hormones. Hence, the result of this study may provide a rational starting point for planning treatment and control measures against the fly and the larvae and may even shed light on some aspects of the life cycle pattern and ecology of that parasite.

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