

Clinical, pathological and epidemiological aspects of *Cephalopina titillator* larval infestation in

Camelus dromedaris of Rafsanjan region, Iran

Comment [UdW1]: We propose shortening the title to this form: Clinical, pathological and epidemiological aspects of *Cephalopina* larvae. exciter in *Camelus dromedaris* from the Rafsanjan region, Iran

Abstract

The camel nasal bot fly is an obligate parasite which its larva causes nasopharyngeal myiasis and the 3rd generation of larvae may cause serious nasal mucosal damage. The present study was conducted on *Camelus dromedaris* to determine the clinical assessment, seasonal prevalence and also pathological adverse effect of *Cephalopina titillator* in Rafsanjan region (South east of Iran). Due to history and dental formula, camels were divided into four age groups: less than two years (62 cases), two to five years (86 cases), five to nine (42 cases) and more than nine years (10 cases), which positive cases confirmed in 12 (19.4%), 31 (36%), 40 (95.2%) and 10 (100%) cases, respectively. The major clinical signs of naturally infected camel was categorized in three main parts; Respiratory signs, nervous behavior and general symptoms which the main ones were related to stridor and nasal discharge. Suspected larvae with average 14.83 ± 4.35 was recovered from 93 infested camels and evaluated microscopically for determining the larval stage. Furthermore, the gross and histopathological findings were recorded by using tissue samples. These non sex related findings showed that with increasing the age, susceptibility to this specific parasite infestation increased as well ($p < 0.001$). No significant seasonal changes were recorded, but the incidence rate was higher in March, April and September. Different larval stages were mostly found in nasal cavity, nasopharyngeal and laryngeal areas which was accompanied by local inflammation, regional edema and serosanguinous exudate. Microscopical findings were showed the epithelial damage along with lymphoid hyperplasia, accumulation of inflammatory cells and remnants of necrotic tissue. However, fibroblasts proliferation, connective tissue formation along with some degrees of atrophy and focal calcification were the significant findings of chronic infestation.

These findings indicate the presence of heavy larval infestation in camel of Rafsanjan region which imposes severe economic losses and affect their health, growth and related productions. Therefore, it is recommended to carry out the necessary measures for the biological control of the camel nasal bot fly.

Key words: Iran, Camel, Rafsanjan, Fly, *Cephalopina titillator*larva.

Introduction

Myiasis is the infestation of the host organs or tissues by the larval stages of flies. The camel nasal bot fly is an obligate parasite which its larvae (*Cephalopina titillator*) causes nasopharyngeal myiasis (Wall and Shearer, 2008) in Camelidae. Clark (1797) described an obligate camel parasite for the first time which currently belongs to the *Oestridae* family (Hussein et al., 1982; Yao, et al., 2022). Adult female flies lay eggs on head and around the nostrils of camel from June to September. Larvae hatch spontaneously from eggs in less than a week and migrate to the nasal cavity and sinuses and then be a second stage larvae after 8 to 9 months depending on suitable condition, the temperature and competition with other larvae. Presence of *C. titillator* larvae adversely affects camel breathing and cause some degrees of stridor, damaging epithelial tissue and decrease milk or meat production and death (Mohammadpour et al., 2020). Nearly eleven months after camel infestation, the third stage larvae are formed and are thrown out with sneezing. In suitable condition larvae pupate in soil and emerge into flies (Oryan et al., 2008; El-Hawagry et al., 2020; Yao, et al., 2022). It is well known that camel play a critical role in human's and animal's life in Middle East countries. The roles of camel in milk, wool and meat production and transmission of some infections to human, domestic and wild animals are important as well. Badawy and Elmadawy (2015) stated that the ventral portion of the ventral nasal conchae is a main place of larval migration which causes chronic rhinitis. They have also explained that during migration and due to the large size of the 3rd generation of larvae (33-35 mm), the spread of nasal mucosal damage is serious and because of that the mortality rate increases.

Two mainly factors influence the occurrence of myiasis; those controlling the number of flies and those determining the susceptibility of the host (Scott, 1988). The proliferation of flies is

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seasonal and mainly occur in summer, but the reason for host susceptibility is not well known. Therefore, epidemiological, clinical and pathological studies may help us to find these factors.

Material and Methods

Geographical location

The present research was conducted on 384 slaughtered camel during one and a half year sampling in Rafsanjan region (Figure 1).

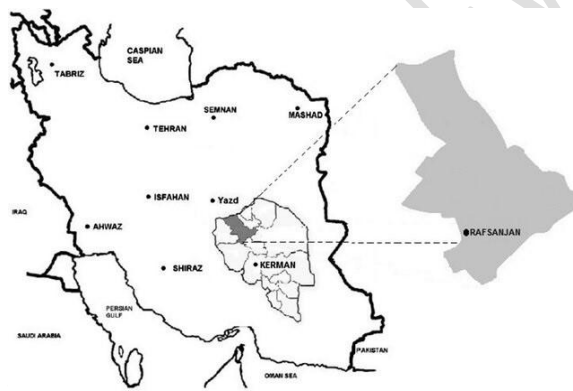


Figure 1: The geographical location of Rafsanjan region in Kerman province, Iran.

Rafsanjan is located in south part of the Lut desert, in north-west of Kerman province. The longitude of this city is 56 degrees east and the latitude is 30 degrees south. Rafsanjan is a semiarid region in central Iran, which has a long, hot summers and sufficient chill in winters. Rafsanjan is located at an elevation of 1760.0 meters above sea level. The district's yearly temperature is 16.55°C and it is -1.88% lower than Iran's averages. Rafsanjan typically receives about 24.01 millimeters (0.95 inches) of precipitation and has 47.29 rainy days (12.96% of the time) annually.

Comment [UdW3]: It is better to say: The city is located at 56 degrees east longitude and 30 degrees south latitude.

Animals

During antemortem examination the presence of stridor, nasal discharge, headache, sneezing, coughing and other signs of upper respiratory tract discomforts were recorded. Totally, two hundred camel were examined and given identification number for further inspection. Based on owner's information and according to dental formula (Al-Ani, 2004), the age was determined and divided into four age groups: less than two years (62 cases), two to four years (86 cases), five to nine (42 cases) and more than nine years (10 cases).

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Comment [UdW5]: It is necessary to maintain the information given in the summary that was for two to five years.

Sampling

Separated heads split longitudinally by band saw and nasal cavity, sinuses, turbinate bone, pharynx, larynx and upper part of trachea was inspected carefully. In affected camels, the number of *Cephalopina titillator* larvae, the site and the extension of gross lesions were recorded.

Recovered larvae was washed with 0.9 percent saline solution and embedded in 70 percent alcohol solution containing 5% glycerol to determine the larval stages (1st, 2nd and 3rd generation) using the method described by Zumpt (1965). For light microscopic examination, labelled tissue samples were fixed in buffered neutral formalin 10% solution and paraffin sections were cut at 5 µm then stained with haematoxylin and eosin (H&E).

Analytical method

The relation between gender, age and other factors with larval infestation were statistically analyzed via using Chi-square test at the level of $p < 0.05$.

Results

Clinical signs

As shown in table 1 the major clinical signs of camel bot fly larval infestation was categorized in three main parts; Respiratory signs, nervous behavior and general symptoms. Clinical signs of respiratory system involvement included mucoid to mucopurulent and sometimes blood-tinged

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nasal discharge (epistaxis), sneezing, coughing and inspiratory stridor, which the relative frequency of severe sneezing and mucoid discharge were higher than that other signs. Nervous behavior was consisted of nasal rubbing (19%) and forced head shaking (12%) which may a part of restlessness due to the presence of an occupying lesion in nasopharynx or sinuses. The other signs were not specific and relate to secondary bacterial infection (fever, lymph node enlargement and increase heart rate) and chronic illness (weight loss and inappetence).

Table 1: Major clinical signs of nasal camel bot fly larval (*Cephalopinatitillator*) infestation in Rafsanjan region

	Clinical signs	Description	Frequency (Relative frequency)
<i>Respiratory signs</i>	Stridor	Sneezing	63 (64.94%)
		Coughing	22 (22.68%)
		Grooming	42 (43.29%)
		Snoring	33 (34.02%)
	Nasal discharge	Epistaxis	15 (15.46%)
		Sticky mucus discharge	60 (61.85%)
	Dyspnea	Difficulty in inspiration	14 (14.43%)
<i>Nervous behavior</i>	Restlessness	Head shaking	12 (12.37%)
		Rubbing nose	19 (19.58%)
<i>General symptoms</i>		Inappetence	29 (29.89%)
		Weight loss	25 (25.77%)
		Fever	10 (10.3%)
		Lymph node enlargement	5 (5.15%)
		Increase heart rate	25 (25.77%)

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Larval collection and Identification

Out of the 200 inspected camels, larval infestation were positively recorded in 93cases, which shows a significant prevalence, equivalent to 48.5% in Rafsanjan region. The average number of isolated larvae was equal to 14.83 ± 4.35 , and the nonsignificant highest number belonged to female camels (Figure 2).

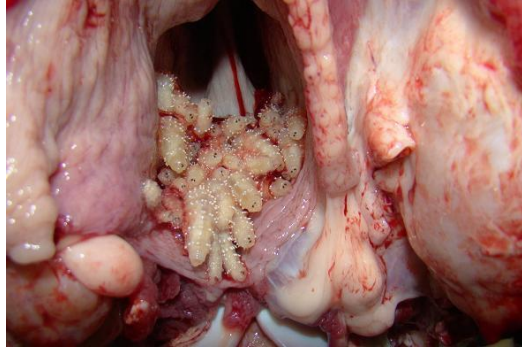


Figure 2: Post mortem view of heavy infestation with *Cephalopina titillator* larvae in pharyngeal region

Different larval stage were mostly found in nasal cavity, nasopharyngeal and laryngeal areas which was accompanied by local inflammation, regional edema and serosanguinous exudate (Figure 3).



Figure 3: Ventral view of a third-stage larvae with sharp black mouth hooks recovered from a camel

The relation between clinical symptoms and the larval generation stage were also considered and our results showed that with increasing in number of 2nd and 3rd larval infestation, occurrence of forced inspiration as well. As shown in figure 2, a significant part of the nasopharyngeal space is occupied by *Cephalopina titillator* larvae, which leading to dyspnea and coughing.

As shown in table 2 and due to history and dental formula, positive cases confirmed in 12 (19.4%), 31 (36%), 40 (95.2%) and 10 (100%) cases. These non sex related findings showed that with increasing the age, susceptibility to this specific parasite infestation increased as well ($p<0.001$). No significant seasonal changes were recorded, but the incidence rate was higher in March, May and September.

Table 2: Age related nasal camel bot fly larval (*Cephalopinatitillator*) infestation in Rafsanjan region

Age (Years)	Frequency	Larval infestation	
		Positive	Negative
<2	42 (21%)	12 ^a (19.4%)	50 ^a (80.6%)
2 - 5	96 (48%)	31 ^a (36%)	55 ^a (64%)
5 - 9	52 (26%)	40 ^b (95.2%)	2 ^b (4.8%)
9<	10 (5%)	10 ^a (100%)	0
Total	200	93 (46.5%)	107 (53.5%)
P value	NS	<0.001	

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Histopathological findings

Larval infestation was mostly observed in nasal cavity and nasopharyngeal region with limited extension to the sinuses. The alive *C. titillator* larva were firmly attached to mucosal membrane which was accompanied by irritation, serosanguinous secretion and local hemorrhage. The underlying mucosa was thicker and darker than normal condition and also inflamed. In microscopical examination some degrees of mucosal damage, hyperemia, necrotic debris, ulceration, lymphoid hyperplasia, accumulation of inflammatory cells and fibrotic tissue formation were recorded. Focal calcification, cell atrophy and fibroblastic cell proliferation were also observed (Figure 4).

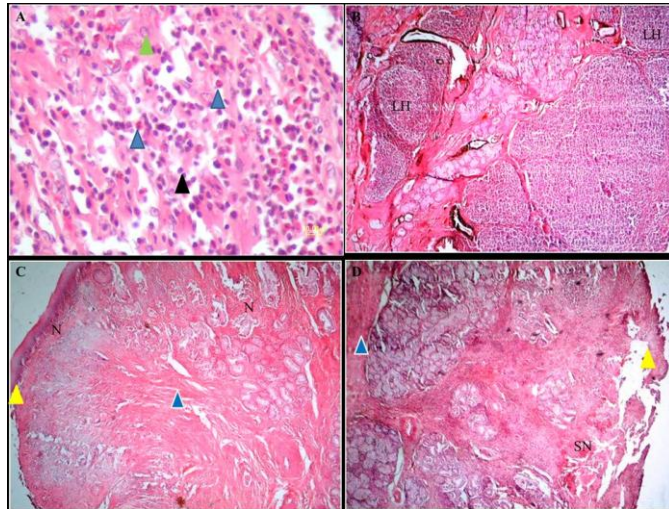


Figure 4: Histopathological findings; **A:** Submucosal aggregation of mononuclear cells dominantly lymphocytes (black arrow head), plasma cells (green arrow head) eosinophils (blue arrow head), **B:** Lymphoid hyperplasia (LH), **C:** Ulcerative damage (yellow arrow head) and scratching of the mucous surface associated with some degrees of necrosis (N) and scar formation (blue arrow head), **D:** Ulcerative damage (yellow arrow head), the destroyed mucosal gland and replaced by fibrotic tissue formation (blue arrow head) and sever necrosis (SN)

Discussion

In the present article authors try to describe the clinical manifestation of nasal bot fly myiasis for the first time. This larval infestation reduces milk production, fertility and other physiological performances and also breathing difficulties which finally accompanied by economic losses. Numerous researchers were called *Cephalopina titillator* larval infestation as a common camel obligate parasite, but none of them never categorized its specific clinical signs. Three major symptoms were recorded in infected camels which mainly related to respiratory distress (nasal discharge, sneezing, coughing and inspiratory stridor), nervous signalments (nasal rubbing and forced head shaking) and general discomforts (fever, enlargement of lymph nodes, increase heart rate, weight loss and inappetence). Serous nasal discharge generally indicates disease conditions

affecting the nasal passages or upper respiratory tract. Furthermore, inflammatory conditions involving the nasopharyngeal region, increased production of glandular secretions which initially are serous but later become mucoid and purulent as secondary bacterial invasion induces an influx of neutrophils (Smith et al., 2020). In these cases the main causes of epistaxis and sanguineous nasal discharge were trauma and erosive or invasive conditions which induced by larval accumulation and irritation. Head shaking along with coughing and sneezing directly relate to the presence of occupying lesion in nasopharyngeal cavity, however anesthesia and blocking the trigeminal or caudal nasal nerve have been resolved the involuntary types of head shaking in horse (Roberts et al., 2009; Aleman et al., 2013).

The overall infestation rate was estimated equal to 46.5% among 200 inspected camel which very close to Al-Ani and Amr (2017) study and lower than others [Yao et al., 2022 (54.2%); Razi Jalali et al., 2016 (52.3%); Oryan et al., 2008 (58.1%); Fatani and Hilali, 1994 (52%)]. These variations between nasal bot fly larval prevalence might be due to the number of samples, season and location of sampling. Rafsanjan is a semiarid region in the center of Iran, which provides suitable conditions for seasonal nasal botfly proliferation, as previously has described by Yao et al (2022). Many investigators revealed that the larval infestation was affected by geographical condition, age, sex and season (Oryan et al., 2008). Our obtained results revealed that the prevalence of larval infestation was not sex related ($P > 0.05$) but increase with the age. Higher prevalence of nasal botfly larva was significantly recorded in camel aged 5-7 years in compare with younger ones ($P < 0.001$). Also, The considerable prevalence rate was observed in camel aged over 9 years which was equal to 90%. These results are closed to Yao et al (2022), Shakerian et al (2011) and Fatani & Hilali (1994) findings. No significant seasonal changes were recorded either, but the incidence rate was higher in March (63.3%), April (51.6%) and September (51.6%) which inconsistent with previous study (Al-Ani & Amr 2016). These authors have stated that the prevalence of larval infestation increased in cold season, because of changes of management and feeding.

The invasion of larvae and their attachment to the mucous membrane leads to hyperemia, edema, erosion, hemorrhage, necrosis and immune system response, which causes lymphoid proliferation and aggregation of inflammatory cells. These observations are somewhat consistent with the findings of Yao et al (2022). Lymphoid hyperplasia is the rapid proliferation of lymphocytic cells

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that resemble lymph tissue which may occur with bacterial, viral and parasitic infections. In response to physical erosion and epithelial tissue traumas, connective and fibrotic tissue formation will be expected which were showed in figure 4. Fibroblasts proliferation, connective tissue formation along with some degrees of atrophy and focal calcification were also the significant findings of chronic infestation.

Ethics approval and consent to participate:

The experimental procedures carried out in this study complied with the guidelines of Shahrekord University (Shahrekord, Iran) for the care and use of animals.

This article does not contain any studies with human participants or animals performed by any of the authors.

Animal welfare/ethical statement:

All procedures used in this experiment were approved by the Shahrekord University ethical committee.

Conclusion

Our findings indicate the presence of heavy larval infestation in camel of Rafsanjan region which imposes severe economic losses and affect their health, growth and related productions. Therefore, it is recommended to carry out the necessary measures for the biological control of the camel nasal bot fly.

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