



## Prevalence of *Linguatula serrata* (Order: Pentastomida) nymphs parasitizing Camels and Goats with experimental infestation of dogs in Egypt

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### Abstract

*Linguatula serrata* is an arthropod of the class pentastomida, found worldwide. It has a zoonotic importance to humans either by ingestion of nymphs (nasopharyngeal linguatulosis) or by ingestion of eggs (visceral linguatulosis). This study aimed to record the prevalence rate of this zoonotic parasite in camels and goats as well as experimental infestation of dogs to collect and identify the adults male and female as well as eggs. Four hundred slaughtered camels and goats (200 Goats, 200 Camels) were inspected from Cairo abattoir, of different sex and age at the period of January to December 2015. One hundred donkeys were also inspected on postmortem examination for infestation with *L. serrata* nymphs from slaughterhouse at Giza zoo, Egypt. Fecal samples, nasal swab were collected from 150 stray dogs for coprological detection of natural infestation with adult *L. serrata* with negative results. The total prevalence rate of infestation in herbivorous animals was 22.8% with *L. serrata* nymph. The highest infestation rate occurred in goats (30%) while, the lowest one was in donkeys (8%). The highest intensity of *L. serrata* nymphs was 50 in goats, while the lowest one was in donkeys (1N/MLN). The highest infestation rate was reported at age ranged from (> 24 months) in goats (50%). However, all inspected animals were found negative for *L. serrata* nymphs in the age less than 12 months. Nineteen adult *L. serrata* male and 2 female were recovered from experimental infestation of puppies, morphological parameters of nymphs, adults male and female as well as eggs were recorded.

**Keywords:** *Linguatula serrata*; experimental infestation; Halzoun, Visceral linguatulosis; Prevalence; Egypt..

### 1. Introduction

*Linguatula serrata* is an arthropod of the class pentastomida (Hendrix and Robinson, 2006). The life cycle of *L. serrata* involves two hosts: Dogs, cats, foxes and other carnivores are the definitive hosts of this cosmopolitan parasite. Transmission to intermediate hosts occurs through contamination of nasal secretions and fecal matter of dogs contains eggs of this parasite. Intermediate hosts include human

beings and herbivores animals such as cattle, goats, sheep and other ruminants that have ingested grass plants contaminated with *L. serrata* eggs. They are worm like parasites of the upper respiratory system, inhabits the nasal cavities, turbinate and frontal sinuses of carnivorous animals as definitive hosts (Oryan et al. 1993; Rahman et al. 2009). The eggs are expelled from the respiratory passage of the final

host therefore contaminate pastures and water resources. When the infected eggs are swallowed by suitable herbivorous animals, the larvae reach the mesenteric lymph nodes, liver, lungs, spleen, rarely the eyes and other organs (Youssef and Hadizadeh-Moalem, 2015).

Humans may be infested with *Linguatula* spp. either by ingestion of nymphs resulting in a condition called nasopharyngeal linguatulosis or Halzoun syndrome or by ingestion of infective eggs which develop in internal organs resulting in visceral linguatulosis. Halzoun syndrome may arise via consumption of raw or undercooked liver and lymph nodes (Yao et al., 2008; Tappe et al. 2009). Human linguatulosis is reported from various parts of the world such as Egypt (Morsy et al. 1999) and Sudan (Yagi et al. 1996).

The occurrence of the parasites in domestic and wild animals used as a food source to humans including cattle, buffaloes, sheep, goats, pigs has been well documented (Khalil, 1976; Saiyari et al. 1996; Tavassoli et al. 2007). Canine acquire the parasites by ingestion of raw or undercooked viscera of infected animals with nymph stage (Rajabloo et al. 2015).

In Egypt, there are few reports of linguatulosis in dogs (Khalil 1970; 1972; 1973). For this purpose, the present study, aimed to investigate the prevalence of *L. serrata* nymphs in mesenteric lymph node (MLNs) of herbivores animals at Cairo abattoir, Egypt and experimental infestation of puppies with isolated nymphs to investigate the life cycle.

## 2. Materials and Methods

This study was assessed and approved by faculty of Veterinary Medicine, Cairo University Ethics Committee and being performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

### 2.1. Collection of samples

#### 2.1.1. *Linguatula serrata* nymph

Mesenteric lymph nodes (MLNs) were collected from 500 slaughtered herbivorous animals (200 Goats, 200 Camels) of different ages and sexes, from Cairo abattoir, Egypt. One hundred donkeys were also inspected on postmortem examination for infestation with *L. serrata* nymphs from slaughterhouse at Giza zoo, Egypt; during the period from January – December 2015. Three to five MLNs were examined for the presence of nymphal stage from each inspected animals.

### 2.2. Examination of mesenteric lymph nodes (MLNs)

Obtained MLNs were examined immediately for the infestation of the nymphal stage of *L. serrata* according to (Sinclair, 1954). All lymph nodes were transferred to Parasitology lab of Faculty of Veterinary Medicine, Cairo University in separate polyethylene bags. For gross examination, lymph nodes were longitudinally dissected, placed in petri dishes and massaged in warm water for 15 minutes. The samples were then examined under the stereomicroscope. The total numbers of nymphs per lymph nodes were recorded, collected in normal saline and counted to be used for experimental infestation puppies.

### 2.3. Examination of fecal samples and nasal swabs

Fecal samples collected from stray dogs collected in Faculty of Veterinary Medicine, Cairo University. In the laboratory, preparation and examination of the fecal samples occurred in the same day of collection using the concentration floatation technique by saturated salt solution (NaCl) as described by (Pritchard and Kruse, 1982). The nasal swabs were put in saline then centrifuged and the sediment was examined under microscope for detection of *L. serrata* eggs.

### 2.4. Examination of stray dogs heads for detection of adult *Linguatula serrata*

The collected stray dogs were fasted for 12 h and euthanized using a lethal dose (130 mg/kg body weight) of sodium pentobarbital (Ethanol®, Virbac Corp., and Fort Worth, TX) according to (Yong Kil et al. 2010) and their heads were cut sagittally. The nasal passages were examined for the adults *L. serrata*.

### 2.5. Experimental infestation of puppies with *L. serrata* nymphs

Twelve puppies of three weeks old were obtained from laboratory kept bitches and assigned to two main groups (Mgp); 1<sup>st</sup>Mgp; 10 puppies assigned to one puppy in each Subgroup (Sgp) were used for the experimental infestation with *L. serrata* nymphs, while, the 2<sup>nd</sup> Mgp (2 puppies) were kept as non - infested control group. Puppies were supplied with clean milk, bread and eventually small amount of cooked minced meat and hygienically kept till they reached 6 months age. The puppies of 1<sup>st</sup> Mgp were fed 10 free viable nymphs (living & contact). The puppies were carefully watched after infestation (vomition of the nymphs) for day. The feces of the

puppies (1<sup>st</sup> Mgp & 2<sup>nd</sup> Mgp) were inspected weekly for the presence of *L. serrata* eggs by using floatation technique according to Soulsby, (1986). The experimentally infested dogs (2-6) months after infestation were fasted for 12 h and euthanized using a lethal dose (130 mg/kg body weight) of sodium pentobarbital (Ethanol®, Virbac Corp., and Fort Worth, TX) according to (Yong Kil et al. 2010) and their heads were cut sagittally. The nasal passages were examined for the adults *L. serrata*.

### 2.6. Identification of nymphs and adult of *L. serrata*

The obtained nymphs and adults were washed in saline, relaxed, flattened, fixed, stained, mounted and identified according to (Pritchard and Kruse, 1982).

### 2.7. Statistical analysis

Prevalence of infestation was compared between paired animal species using  $2 \times 2X^2$  contingency tables and 95% confidence intervals (CI) for prevalence and analyzed using Chi square test. Significance was considered when  $P \leq 0.05$ . All analyses were performed using the SPSS v.11.0.

## 3. Results

### 3.1. Prevalence rate among herbivorous animals and dogs

The total prevalence rate of infestation with nymphal stages of *L. serrata* was 22.8%. The highest infestation

rate was detected in MLNs of goats (30%), while the lowest one was detected in donkeys (8%) **Table 1**. High infestation rate in male than females in goats while in Camels females is more than males but the effect of host sex on the total number of nymph infesting herbivores animals could not be estimated during each month due to the great variation in the number of the examined animals of both sexes. However, the overall prevalence according to sex during the study period (one year) showed no significant difference ( $P > 0.05$ ) between the infestation of male and females with *L. serrata* nymphs ; **Table 2**. The highest infestation rate was reported at age ranged from (>12-24) & > 24 months (23.3%, 50%) in goats. However, all inspected animals were found negative for *L. serrata* nymphs in the age less than 12 months, Table 2. No statistical differences between age groups in goats, camels, donkeys. The highest intensity of *L. serrata* N/MLN (nymph/ mesenteric lymph node) was 50 N/ MLN of goats, while the lowest one was detected in donkeys (1N/MLN), **Table 3**.

All examined specimens from stray dogs (150 fecal samples and 50 skulls, 50 nasal swabs) were found negative for *L. serrata* for both eggs and adults.

**Table 1: Prevalence of *L. serrata* nymphs in examined MLNs of herbivorous animals**

Animals	No. Ex.	No. Inf.	%
Goats	200	60	30
Camels	200	50	25
Donkeys	100	8	8
<b>Total</b>	500	114	22.8

No. ex.: Number of examined animals.

No. inf.: Number of infested animals.

%; percentage of infestation.

**Table 2: Prevalence of *L. serrata* nymphs in relation to sex and age of inspected herbivorous animals**

		Goats		Camels		Donkeys		Total	
Sex		No. Ex.	No. Inf (%)	No. Ex.	No. Inf (%)	No. Ex	No. Inf (%)	No. Ex	No. Inf (%)
	Male	150	47(31.3)	160	38(23.7)	74	8 (10.8)	384	89(23.1)
	Female	50	13(26)	40	12(30)	26	0(0)	116	25(21.5)
Age	6-12	0	0	0	0	8	0	8	0
	> 12- 24	150	35(23.3)	80	15(18.8)	32	0	262	50 (19)
	> 24 M	50	25(50)	120	35(29.2)	60	8(13.3)	230	64 (27.8)

**No.Ex.:** Number of Examined animals.

**No. Inf.:** Number of Infested animals.

**%:** Percentage of infested animals

**Table 3: Intensity of infestation rate with *L. serrata* N/MLN.**

Animal	No. of <i>L. serrata</i> N/ MLN		
	Min.	Max.	Aver.
<b>Goats</b>	5	50	<b>39</b>
<b>Camels</b>	2	5	<b>3</b>
<b>Donkeys</b>	<b>1</b>	<b>1</b>	<b>1</b>

N/MLN: (Nymphs per mesenteric lymph nodes)

Min.: Minimum

Max.: Maximum

Aver. : Average.

### 3.2. Morphological parameters of nymphs, adults and eggs

#### Nymphs

Macroscopically, the freshly recovered nymphs (**Fig.1**) were whitish in color, convex dorsally and slightly concave ventrally, tongue shape with broader anterior end and attenuated posterior end. Living nymphs are actively motile through successive body wave begin posterior and end anteriorly, with four hooks appeared retractile in a small pits or fossetts on the ventral surface anteriorly. The body is divided into several rings.

Microscopically showed that *L. serrata* nymphs (**Fig.1 and 2; Table 4**) in all animals examined differed in length from 2.3- 5.5 mm. (4 mm.). The maximum breadth ranged from 0.7 - 1.6 mm. (1.1 mm.). Cuticle of the tongue shape body of the nymph was annulated

or segmented into 81 - 99 cuticle segments (average 90 cuticle segments). Each cuticle segment was provided posteriorly with a single transverse row of closely arranged spines; each spine measured 16 - 40µm. (Average 28 µm.) in length. The oral opening is subterminal, quadrangular with rounded angles, located on the ventral surface anteriorly; it measured 105 - 175 µm. in length (average 140 µm.). Around the oral opening there are two pairs of complex hooks arranged in a trapezoidal form. Each one has a sharp, curved, biarticulated main hook with a small accessory one. Length of the main hook varied from 425 - 544 µm. (Average 494.5 µm.). A pair of frontal papillae, one on either sides of the most anterior part of the nymph is present ventrally. The alimentary or intestinal canal in form of a simple tube runs along the whole body length and opens to exterior by a terminal anal pore. A single transverse row of minute pores present on each segment.



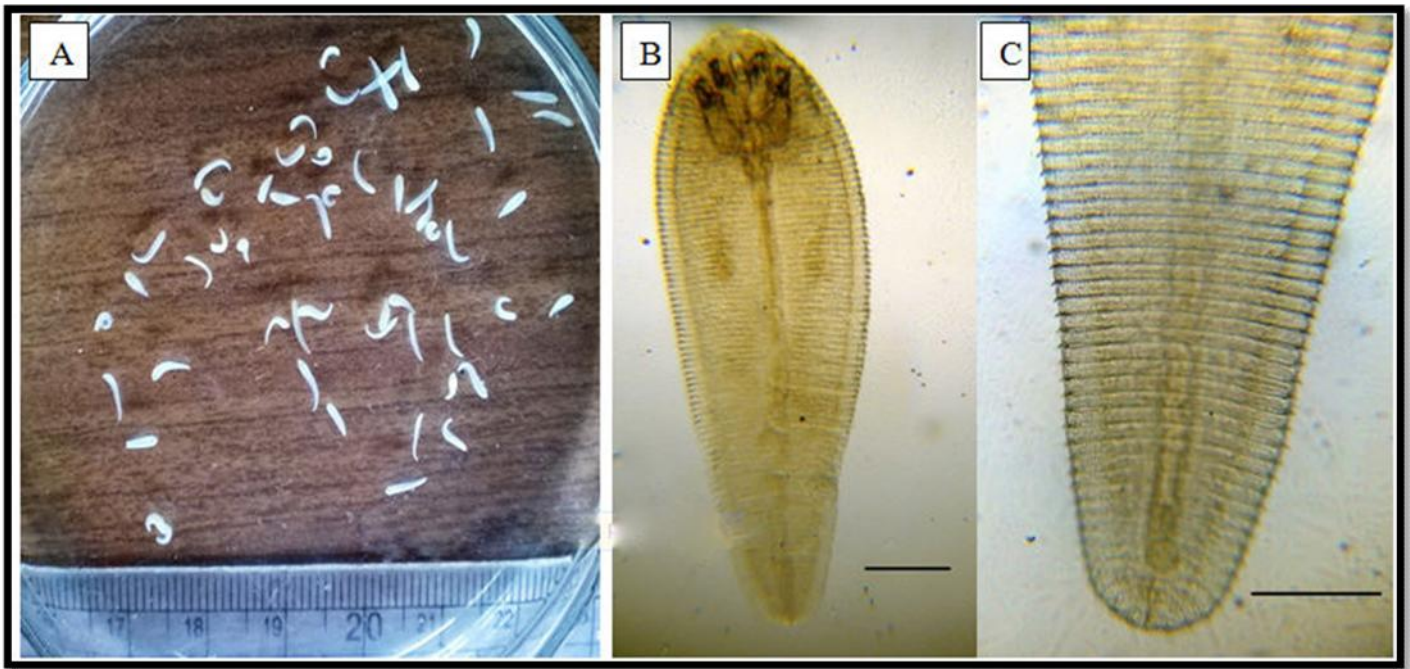


Figure 1: *Linguatula serrata* nymph

A: Fresh specimens of whole nymph, tongue shape body.

B: Permanent specimen of whole nymph, ventral view, tongue shape body, hooks at anterior end.

C: Posterior end, of nymph with distinct cuticular spines and pores of nymph.

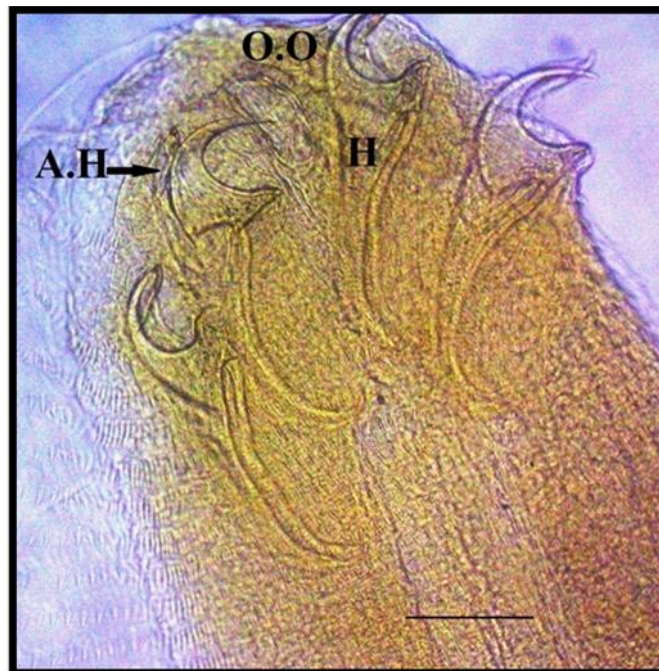


Figure 2: *Linguatula serrata* nymphs (anterior end) showing the shape of hooks (H: main hooks; A.H.: Accessory hooks; O.O.: Oral Opening).

Scale bar : 100µm.

**Table 4: Measurements of *L. serrata* nymphs collected from MLNs of inspected herbivores animals**

Animal species	Aver. Whole length (mm.)	Aver. Max. Breadth (mm.)	Aver. No. of cuticle segments	Oral opening (µm.)	Main hook length (µm.)	Spine length (µm.)
Camels	5.5	1.2	93	140	471	20
Goat	3.9	1.6	99	140	544	40
Donkeys	5.2	1.1	93	175	420	22

Min.: Minimum. Max.: Maximum. Aver. Average

Note: Ten *L. serrata* nymphs from each species of inspected herbivores animals were examined.

### Adults

Macroscopically, the male and female is flattened, white to yellow in colour, The adults male are 1-3 cm. in length broad anteriorly and tapering posteriorly, while female ranged from 6-10 cm. in length, broad and tongue shape anterior and pointed posterior. The male and female was with the same hooks as nymphs (Fig. 3, 4).

### Eggs

Eggs are ovoid in shape, brownish in colour, ranged from 70-80 µm. in diameter, contain fully developed 1<sup>st</sup> instar larvae (Fig.3).

### 3.3. Experimental studies

Experimental infestation of 10 dogs with different numbers of viable *L. serrata* nymphs. Nineteen adult males of *L. serrata* and 2 female (Fig. 3, 4) were obtained. The adults collected from nasopharynx and nasal sinus. None of the control negative had *L. serrata* adults in their nasal passages.

In addition, some clinical observation on dogs after experimental infestation, the dogs after feedings of *L. serrata* nymphs showed different reactions against nymphs, beginning with difficult deglutition (dysphagia), shaking of its head, cough, pain in ears represented by shaking its head into hard object, vomiting (not vomit the nymphs). These reactions were beginning after feeding by 5 minutes and ending for 15 minutes.

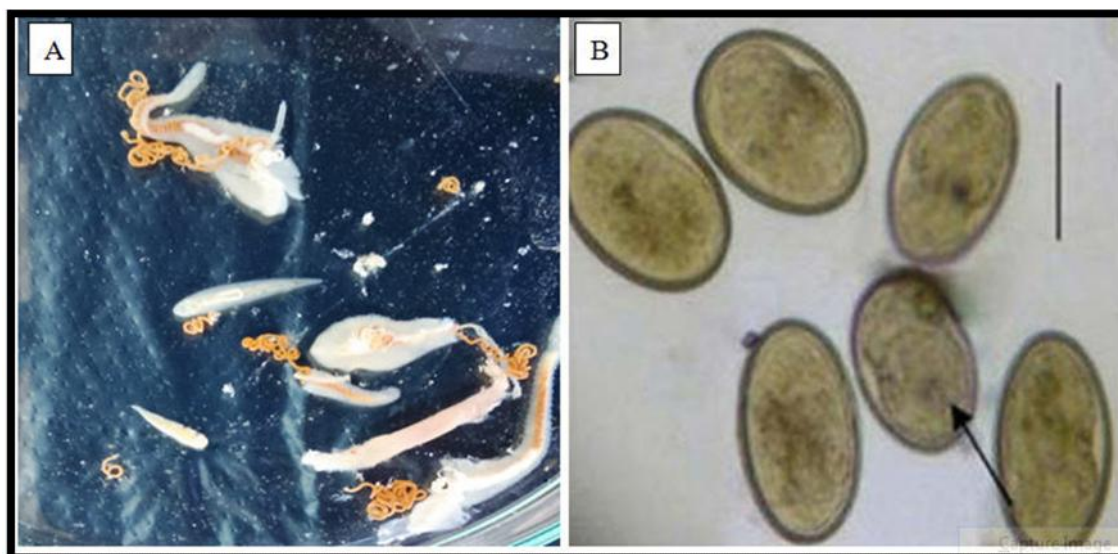


Figure 3: A: Adult male and female *Linguatula serrata* after 6 months of experimental period. B: Eggs of *Linguatula serrata* note its brownish colour and ovoid in shape, contain fully developed 1<sup>st</sup> instar larvae, Scale bar: 100µm.



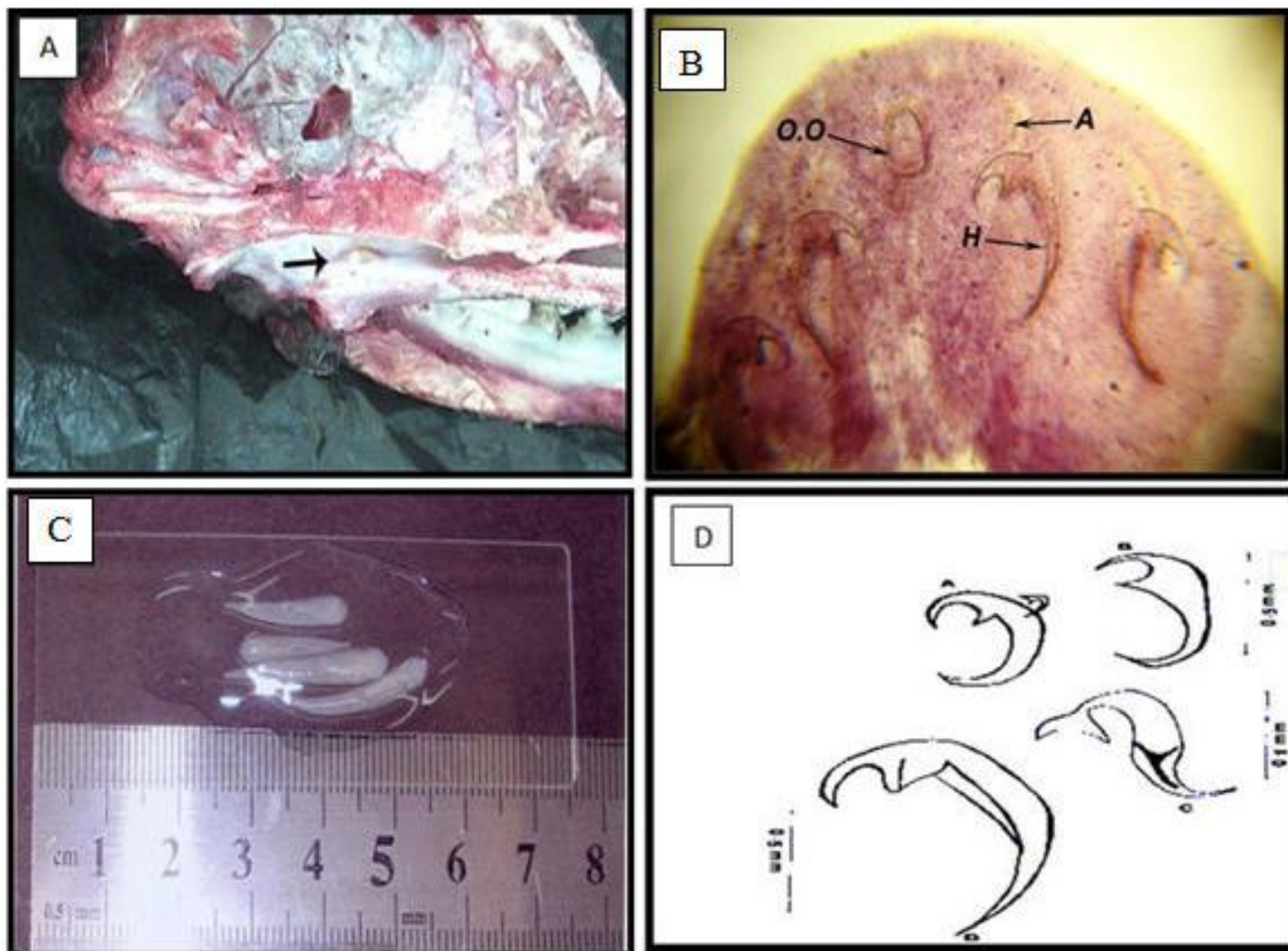


Figure 4: Adult *Linuatgula serrata* isolated from experimental infested dogs of goat origin after two months post infestation

- A: Longitudinal section of dog skull showed adult in nasopharyngeal passage (arrow).
- B: *Linguatula serrata* - anterior end of male, X40, Carmine stain (O.O: Oral Opening, H: Hook, A: Accessory hook).
- C: Males were tongue-shaped, lightly convex dorsally flattened ventrally.
- D: Different shapes of hooks. Using Camera Lucida)
  - A) Nymphal hook with accessory one, X100
  - B) Nymphal hook without accessory one, X100
  - C) Accessory hook, X400
  - D) Adult main hook, X100

#### 4. Discussion

The present study, the highest infestation rate with nymphal stage of *L. serrata* was detected in goats (30%), this result agreed with the result of infestation rate in goats recorded by (Razavi et al. 2004; Khalil and Schacher, 1965), they recorded 40% and 29.9%. In contrast, the highest infestation rate in goats was attributed to the fact that they are scavenger feeders on the ground which brings them more liable to be

infested with the eggs of *L. serrata* from contaminated pasture excreted with nasal discharges or feces of such *L. serrata* harboring dogs. A high rate of infestation in goats suggests possibility of similar high rate of infestation in other animals, and people in investigated area. This in turn emphasizes the need for more preventive measures to reduce the risk of zoonotic outbreaks. In addition, the prevalence rate of infested camels was 25 %.

Moreover, the higher infestation rate in female than males was detected in camels, with in contrast in female goats. This finding was disagreed to results of survey in Iran (Nourollahi et al. 2010) they find the infestation rate in females goats was significantly ( $P < 0.05$ ) higher than males (Nourollahi et al. 2010; Razavi et al. 2004). This difference in infestation rate may due to low number of slaughtered female. Concerning, all inspected animals were found negative for nymphs of *L. serrata* in the age less than 12 months. However, the highest infestation rate was reported at age ranged from (>24 months) in goats (50%). This finding was similar to result of (Nourollahi et al. 2012) in Iran they, reported the infestation rate increased with increased age (< 1, 1-2, 2-3 & < 3 years) 47.4%, 47.9%, 51.9% and 66.6%, respectively with significantly ( $P < 0.05$ ) in goats.

The reason for the high infestation rate in goats wasn't clear but may due to different forage habitat of goats or geographical climate or high exposure to dogs as definitive hosts; close contact between dogs and intermediate hosts such as goat plays an important role in the transmission of *L. serrata*. The high prevalence of infestation observed in herbivores was of concern owing to the zoonotic nature of the parasite and the risk of infestation to humans and other animals. Further investigations about the epidemiology of linguatulosis in other domesticated animals in this area are necessary.

However, this is the first record in donkeys (8%) was in Cairo and Giza Province, Egypt.

The present study revealed; no adults or eggs of *L. serrata* were found in the naturally investigated 150 fecal sample and 50 nasal swabs from stray dogs. This result was similar to (Sinclair, 1954) found no adult or eggs of *L. serrata* in dogs in England. While in Iran; higher infestation rate 143(62.2%) was recorded by (Meshgi and Asgarian, 2003).

In addition, the major number of the obtained nymphs of *L. serrata* in single MLN from goats reaches to 50 nymphs. However (Razavi et al. 2004) recorded 98 N/MLN & 150 /MLN among goats in Lebanon.

Adults of *L. serrata* were obtained after 2 months post infestation of experimental infestation of dogs. This result agreed with the result mentioned by (Meshgi and Asgarian, 2003) who detected most adult reach sexual maturity after 45 day post infestation.

The collected nymphs and adults of *L. serrata* were morphologically similar, with those described by

previous authors (Panaiotova et al. 1999; Sivakumar et al. 2005; Yong Kil et al. 2010).

## 5. Conclusion

A high rate of infestation of *Linguatula serrata* nymphs in goats suggests possibility of similar high rate of infestation in other animals and people in investigated area. This is in turn emphasizes the need for more preventive measures to reduce the risk of zoonotic outbreaks. However, the first record in donkeys (8%) was in Cairo and Giza Province, Egypt. Successful experimental infestation of puppies with collected *L. serrata* nymphs from naturally infested mesenteric lymph node. *Linguatula serrata* should be considered as a risk factor for human beings and other hosts. Dogs shouldn't be around or in contact with herbivores animals. To prevent the Halzoun syndrome it is not advised to consume raw or undercooked liver and lymph node. Since, appropriate cooking of lymph nodes and liver for destroys nymphs.

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