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Araştırma Makalesi

Research Article

Some Morphological Data of Various Stages of Linguatula Serrata

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Abstract

Linguatula serrata is a zoonotic parasite causing visceral and nasopharyngeal linguatulosis in humans. Dog and other canines are the main definitive hosts while most of herbivores, including ruminants serve as intermediate hosts for linguatulosis. The current abattoir survey aimed to assess some morphological characterizations of different stages (egg, nymphs, male and female adults) of *L. serrata*. Upper respiratory tract of stray dogs and mesenteric lymph nodes (MLNs) of domestic ruminants (goats, buffaloes, cattle and sheep) were examined for the presence of *L. serrata*. Eggs, adults and nymphal stages of *L. serrata* were analyzed for morphometric traits. No significant differences were observed in the size of various parts of nymphs collected from different in compared to the nymphs. Eggs were ovoid shape with semi-thickness shell. Probably, semi-thickness shell in eggs protected them from environmental condition and help to transmission of infections to intermediate hosts.

Introduction

The phylum Pentastomida contains a highly specialized taxon with more than 120 species which infect the respiratory tracts of reptile and other vertebrates (Mehlborn, 2008; Riley, 1994; Riley and Henderson, 1999). *Linguatula serrata*, a zoonotic and obligated endoparasite, is a member of phylum *Pentastomida* (Gosling, 2005; Nourollahi-Fard et al., 2010a). The adult form of this parasite is found in the nasal airway, frontal sinus and tympanic cavity of dogs and cats (Alcala-Canto et al., 2007; Bowman et al., 2004; Muller, 2002).

The adult females grow up to 13 cm, while males reach only 2 cm. Also, they are flat or annulated and have 4 hooks surrounding a central mouth (Mehlborn, 2008). They keep attached at the wall of the respiratory system by means of their mouth hooks. Females excrete thousands (up to 5,000,000) eggs per day (Mehlborn, 2008; Tajik et al., 2008). These eggs, which contained fully developed larvae, are discharged from the definitive host's nasopharyngeal secretions and then ingested by the plant feeder animals (including human). Nymphal stages of *L. serrata* in intermediate hosts grow

up to 0.6 cm and have 4 hooks, mouth, annular rings and spines (Hami et al., 2009; Mehlborn, 2008). If these nymphs are eaten by the final hosts, the larvae invade the nasal system and reach maturity within 6-7 months and live for about 15 months (Mehlborn, 2008).

Although the exact pathogenesis of linguatulosis is not clear, but it has been shown that proteolytic enzymes may have an important role (Alcala-Canto et al., 2007). Clinical signs associated with infections have not been well described in the intermediate hosts (Mehlborn, 2008). In human, linguatulosis can be caused by either eggs (visceral form) or nymph stage of the parasite (nasopharyngeal form) (Tavassoli et al., 2007). Nasopharyngeal linguatulosis, known as Halzoun or Marrara syndrome, is often occurs after consumption of raw or undercooked viscera (liver, lung and lymph nodes) of infected animals (Beaver et al., 1984; Drabick, 1987; El-Hassan et al., 1991). Rarely, human become infected as definitive host (Mehlborn, 2008; Oryan et al., 2008). Linguatulosis in human has been reported from Africa, Southeast Asia and the Middle East (Acha and Szyfrez, 2003; El-Hassan et al., 1991; Lazo et al., 1999; Yagi et al., 1996). Based on literature review, there are several reports of nasopharyngeal linguatulosis in Iran, following consumption of liver kebab (barbecue) (Fata et al., 1994; Sadjadi et al., 1998). Additionally, the prevalence of *L. serrata* in dogs has been reported by several researchers in different regions of Iran (Meshgi and Asgarian, 2003; Oryan et al., 1997; Oryan et al., 2008), camels (Oryan et al., 1993; Tajik et al., 2007), buffaloes (Sisakumar et al., 2005; Tajik et al., 2008), sheep (Esmail-Nia et al., 2000; Nourollahi-Fard et al., 2011; Ravindran et al., 2008; Shekarforoush et al., 2004; Tavassoli et al., 2010b; Tajik et al., 2006) and goats (Nourollahi et al., 2010a; Razavi et al., 2004; Saiyari et al., 1996).

Most reported documents in linguatulosis are parasitological studies that have shown a high prevalence of *L. serrata* in many of countries and also some aspects of histopathologic changes due to this parasite. Therefore, the present study was designed to determine the some morphological characterizes of various stages of *L. serrata* including; egg, nymph and adult collected from different hosts.

Material and Methods

For morphometric analysis of nymphal stages of *L. serrata*, the mesenteric lymph nodes of the slaughtered animals (sheep, goats, cattle and buffaloes) were examined grossly for the presence of nymphal stage of this parasite in the municipal abattoir of livestock animals, in Urmia (the capital of Azerbaijan province, northwest of Iran) between March 2009 and October 2010. At least, three infected MLNs were collected from each animal and transferred to the laboratory of Parasitology Department.

Lymph nodes were cut longitudinally and were examined for the presence of *L. serrata* nymphs under a dissecting microscope. Finally, the number of collected nymphs was recorded.

To determine the morphological characteristics of the adult stages of *L. serrata*, twenty stray dogs were examined for the presence of this parasite from August 2009 to September 2010 in the northwest of Iran. The dogs were euthanized using Pentobarbital (20% solution) 150 mg/kg according to the approval of the state committee on animal ethics, Urmia University, Urmia, Iran (89/105/DE). At necropsy, the nasopharynx area, nasal turbinate's, paranasal sinuses, Eustachian tubes, trachea, and brain were examined for the presence of the adult form of *L. serrata*. The parasites were collected in PBS and transferred to Department of Parasitology for further studies.

For the nymphal morphometric analysis, several factors including body length, body width, mouth,

hooks, spines and annuli size and some other morphological characterizations were measured using the classic methods of parasitology. For this purpose, 50 nymphs from each domestic ruminant were evaluated and their morphological data recorded.

For adult parasites, morphological investigations and micromeasurements were done on a number of 20 samples from each sex. In addition, 100 eggs collected from females uterus analyzed for this purpose.

Statistical Analysis

Differentiation of morphometric characterization of nymphal stage collected from different intermediate hosts were statically analyzing using Chi Square and Backward elimination tests (SPSS version 17.0). The data with P values equal or less than 0.05 were considered significant.

Results

Examined nymphs appeared tongue-shaped with an anterior swollen body and posterior narrow end. The mean length of nymphs' body collected from goats, cattle, sheep and buffaloes were 4.82 (3.88-5.54) mm, 4.77 (3.98-5.24) mm, 4.88 (3.88 - 5.42) mm and 4.73 (3.99-5.22) mm, respectively.

The mean size of the body width in the apical part of nymphs was 1.04 mm in nymphs collected from goats, cattle, and sheep and 1.05 in nymphs collected from buffaloes. The body width in the end part of nymphs was 0.33 mm at a mean (from 0.28 to 0.38 mm) for all nymphs groups. The mouth flanked by two pairs of hooks that surrounded by separated suckers (Figure 1). Nymphs had segmented body with transversely striated spines and an average of 42 per segment, at the posterior edge of each abdominal segment beside of the origin of nymphs. The total number of abdominal segments was 84 to 86 for different groups of nymphs. The results of the histomorphometric study of nymph forms of *L. serrata* are shown separately in Table 1 to 4 for each animal.

The male parasites were similar to nymphs in shape but differ in size and some internal organs. The mean length of this form of parasite was 18 (14-22) mm. Maximum and minimum body width was 3.04 (2.55-3.53) mm and 0.42 (0.4-0.5) mm at the mean, respectively. The absence of spines over the male and female bodies was the most different characters among various forms of this parasite. Other morphometric data from male *L. serrata* are shown in Table 5.

Female parasites were different in shape and specially size of nymphs and males. Presence of the uterus containing thousands of eggs resulted in increasing in size of the parasite in length and width. The mean length of this form of parasite was 88 (85-93) mm in evaluated cases. The mean size of the body width in the apical and end parts of females was 9 (6.5-12) mm and 1.72 (1.5-2.23) mm, respectively. Absent of spines and present of hooks and annuli were the other characters in this form of parasite. Other data of morphological characters of evaluated female parasites are summarized in Table 6. The differences between the mouth and hook size of the parasite in different forms are shown in Figures 2 and 3, respectively.

Table 1. M	1orphological	data of L.	serrata nym	phs collected	from MLNs	of cattle	(50 nyr	nphs evaluated)	
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Measured Region	Minimum	Maximum	Average	SEM
Number of annuli	74	93	85	6.9
Body length, μm	3986	5240	4777	498
Maximum body width, μm	924	1096	1040	54
Minimum body width, μm	288	358	337	23
Intestine length, µm	2830	3734	3423	363
Intestine width, μm	82	96	92	6.4
Mouth length, μm	132	168.5	146	12.2
Mouth width, μm	70	84	78	5.5
Hook diameter, µm	105	120	115	4.7
Hook width, μm	76	81	79	2.3
Total hook length, μm	145	164	156	6.6
Inter segments space, μm	42	67	57	9
Distance of mouth from apex, μm	168	195	183	11
Distance of mouth from margin, μm	268	328	298	20
Spine length, µm	28	32	30	1.5
Spine width, μm	7.2	9.1	8.2	0.6
Intestine length/total length	0.70	0.73	0.717	0.01

Table 2. Morphological data of *L. serrata* nymphs collected from MLNs of sheep (50 nymphs evaluated).

Measured Region	Minimum	Maximum	Average	SEM
Number of annuli	72	92	85	6.5
Body length, μm	3886	5420	4886	498
Maximum body width, μm	936	1116	1041	58
Minimum body width, μm	296	360	335	25
Intestine length, μm	2798	4010	3559	317
Intestine width, μm	86	101	95	6.9
Mouth length, µm	129	171	147	15
Mouth width, μm	71.5	83.5	79	5.5
Hook diameter, μm	108	121	115	4
Hook width, μm	76	83	81	3.3
Total hook length, μm	144	164	154	7.3
Inter segments space, µm	41	69	56.5	10
Distance of mouth from apex, µm	171	202	184	14
Distance of mouth from margin, μm	271	328	301	26.5
Spine length, μm	29	33	30	1.5
Spine width, μm	7.2	9.1	8.1	0.7
Intestine length/total length	0.71	0.74	0.724	0.01

Intestine length/total length

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Measured Region	Minimum	Maximum	Average	SEM				
Number of annuli	73	91	84	6.3				
Body length, μm	3986	5540	4824	573				
Maximum body width, μm	936	1122	1047	58				
Minimum body width, μm	296	360	338	25				
Intestine length, μm	2830	4044	3475	399				
Intestine width, μm	86	100	93	5.9				
Mouth length, μm	129	174	147	14				
Mouth width, μm	72	87	79	5.5				
Hook diameter, µm	108	122	115	4				
Hook width, μm	76	85	80	3.1				
Total hook length, μm	147	168	156	7				
Inter segments space, µm	42	70	58	10				
Distance of mouth from apex, µm	171	200	185	11.5				
Distance of mouth from margin, µm	272	340	302	24				
Spine length, µm	28	32.5	30.5	2				
Spine width, μm	7.2	9.4	8.1	0.7				

0.70

0.74

0.723

0.01

Table 3. Morphological data of L. serrata nymphs collected from MLNs of goat (50 nymphs evaluated).

Table 4. Morphological data of *L. serrata* nymphs collected from MLNs of buffalo (50 nymphs evaluated).

Measured Region	Minimum	Maximum	Average	SEM
Number of annuli	73	91	85	6
Body length, μm	3996	5226	4736	452
Maximum body width, µm	964	1112	1054	51
Minimum body width, μ m	298	364	334	25
Intestine length, μm	2880	4034	3473	399
Intestine width, μ m	80	98	92	6.2
Mouth length, μ m	136	172	146	11.5
Mouth width, μm	72	87	78	5
Hook diameter, μm	108	122	115	5
Hook width, µm	76	82	80	2.4
Total hook length, μ m	148	164	156	7
Inter segments space, μm	41	68	57	9.7
Distance of mouth from apex, μm	177	201	184	9.4
Distance of mouth from margin, µm	274	328	298	22
Spine length, μ m	27	33	30	2
Spine width, µm	7.2	9.3	8.2	0.7
Intestine length/total length	0.71	0.74	0.721	0.01

The eggs were ovoid in shape and had semithickness shell especially in poplars. The mean ratio of length/width in the eggs was 0.6 and most of the internal space of them occupied by unembryonated cells (Figure 4). In fresh samples, there was a swollen transparent membrane around the eggs, but after times, this membrane was flatted over the wall. Morphological data of evaluated *L. serrata* eggs are shown in Table 7.

Table 5. Morphological data of male *L. serrata* collected from dog (20 parasites evaluated).

Measured Region	Minimum	Maximum	Average	SEM
Number of annuli	84	89	87	1.8
Body length, mm	14	22	18	2.9
Maximum body width, μm	2556	3528	3038	322
Minimum body width, μm	404	504	426	50
Intestine length, mm	8	12	10	1.6
Intestine width, µm	972	1044	1001	37
Mouth length, μm	300	360	329	23
Mouth width, μm	199	228	414	12
Hook diameter, μm	314	357	338	20
Hook width, μm	200	228	214	12
Total hook length, μm	386	414	410	18
Inter segments space, µm	129	200	167	24
Distance of mouth from apex, µm	343	371	353	13.5
Distance of mouth from margin, μm	828	900	871	33
Intestine length/total length	0.54	0.57	0.56	0.01

Table 6. Morphological data of female L. serrata collected from dog (20 parasites evaluated).

Measured Region	Minimum	Maximum	Average	SEM
Number of annuli	85	93	88	2.8
Body length, mm	51	92	68	13
Maximum body width, mm	6.5	12	9	1.8
Minimum body width, μm	1512	2232	1722	259
Intestine + uterus length, mm	38	65	49	8.4
Intestine +uterus width, μm	2592	3024	2754	130
Mouth length, μ m	504	576	540	27
Mouth width, µm	396	432	417	18.5
Hook diameter, μm	612	648	633	18.5
Hook width, μm	396	468	423	27
Total hook length, μm	684	720	705	24
Inter segments space, μm	540	900	705	129
Distance of mouth from apex, μm	432	504	465	28.5
Distance of mouth from margin, μm	864	936	885	28.5
Intestine length/total length	0.70	0.74	0.72	0.01

 Table 7. Morphological data of L. serrata eggs collected from female parasites (50 eggs evaluated).

Measured Region	Minimum (μm)	Maximum (µm)	Average (µm)	SEM
Length, μm	96	106	101	3.6
Width , μm	54	67	61	3.7
Shell thickness in polar region, μm	11.5	14.3	12.5	1.12
Shell thickness in lateral region, μm	5.5	7.2	6.5	0.77
Length/Width	0.6	0.64	0.61	0.01
Polar shell thickness/lateral shell thickness	0.5	0.55	0.52	0.025



Figure 1. Mouth and 4 hooks of *L. serrata* nymphs in anterior part of body (schematic figure).



Figure 2. Comparison of mouth size of *L. serrata* in different forms of parasite. a: Nymph, b: Male and c: Female.



Figure 3. Comparision of hook size of *L. serrata* in different forms of parasite. a: Nymph (present of spines) b: Male (absent of spines) and c: Female (absent of spines).



Figure 4. Schematic figure of *L. serrata* eggs collected from female uterus.

Discussion

Based on morphometric analyses, the isolated nymphs from different animals were almost similar to each other.

On the basis of our observations, the mean length of nymphs' body collected from goats, cattle, sheep and buffaloes were varied from 4.73 to 4.88 mm. The mean size of the body width in the apical part and the end part of nymphs was 1.04 and 0.33 mm for all nymphs groups. The total number of abdominal segments was 84 to 86 for different groups of nymphs.

Some morphometric characterizes of *L. serrata* (adult and nymph) and other species of *Linguatula* were investigated previously.

For example, some ultrastructural characteristics of adult stage of *L. arctica* collected from the reindeer and nymphal stage of *L. serrata* collected from MLNs of goats were reported by Banaja (1983) and Nikande and Saari (2006).

In other studies, some morphological data of nymphal stage of *Linguatula* spp. were measured and recorded. For example the number of abdominal segments was reported to be in the range of 72-97 (mean 82) in *L. serrata* and 186-232 (mean 210) in *L. multiannulata*. Also, body length of *L. serrata* nymph has been reported between 3.4 to 4.7 mm (Sachs and Rack, 1973).

In another study, the number of annuli was 85, body length was between 3.4 to 4 mm and average number of spines on each segment was 42 in collected nymphs from MLNs of slaughtered buffaloes (Sisakumar et al., 2005). Pourjafar et al. (2007) reported similar data in the nymphal stage of *L. serrata* collected from MLNs of slaughtered camels.

In the current study, the number of annuli (84-86) and body length (4.7-4.8 mm at mean) were consistent with previous reports; additionally, the other morphological characterizes such as size of mouth, spines, hooks and intestine, the body width in the apex and base part of nymphs, were analyzed in our survey.

Significant differences were not observed in the size of various parts of nymphs collected from different hosts. It seems that species and size of the hosts don't affect the size and morphological characters of *L. serrata* nymphs.

For adult parasite, similar nymphs, the length of body more variable than that other measured part of parasites. Size of the body and absent of covered spines in adult forms of parasite (male and female) were the major differentiation between them and nymphs. Also, development of reproductive organs in adult forms affected the some morphological factors. Probably, age and the degree of parasite growth in each form are the most effective factors on body size. For eggs, present of semi-thickness shell can protect them from unfavorable environmental conditions and help to transmit the infection to intermediate hosts.

Conclusion

The results of this study showed that there are some differences in the morphology of *L. serrata* nymphs among different species and also between various geographic areas in comparison with other studies. Although, this survey provides baseline data for the future comparison, more studies are suggested to be carried out to collect more data about the morphology

of this parasite and factors that can effect on morphological characteristics.

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