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Original

Impact of Sodium Chloride and Heat on Survival Time of *Linguatula Serrata* Nymphs in vitro: An Experimental Study

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Abstract

Introduction: *Linguatula serrata* is a zoonotic parasite, belonging to the class Pentastomida. The major aim of this study was to evaluate the impact of sodium chloride (NaCl) and heat on survival time of *Linguatula serrata* nymphs.

Materials & Methods: Thirty nymphs (10 in triplicate) were separately transferred to plastic tubes, containing different concentrations of NaCl solution (2%, 5% and 10%). Meanwhile, 30 nymphs in tubes containing Phosphate Buffer Saline (PBS) were separately treated by +50°C, +60°C and +72°C. As control group, thirty nymphs were stored in PBS at +4°C. The effects of different conditions on survival time of the nymphs were evaluated by observing their motility in different periods of time.

Results: The survival time of the nymphs stored in 10% NaCl solution was too short and all of them were dead after 3 hours. But the other ones maintained in 2% NaCl solution were significantly more resistant ($p<0.05$) and were survived for 2 days. All the nymphs pertaining to each +60°C and +72°C treatments were found dead after first 5-minute storage interval; the nymphs stored at +50°C died totally after 20 minutes. The nymphs maintained in PBS at +4°C (control group) showed the longest survival time ($p<0.05$); all of them were alive until day 4 and the last ones died on day 34.

Conclusion: It is concluded that salting and heating have significant parasiticidal effects on *L. serrata* nymphs and could be used as disinfecting methods in processing of meat products especially liver. However, refrigeration at +4°C increases the resistance of the nymphs in meat products and therefore might endanger the food safety.

Keywords: Pentastomida, Survival Rate, Sodium-Potassium-Chloride Symporters, Hot Temperature, Food safety

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Introduction

Linguatula serrata is a zoonotic parasite, belonging to the class Pentastomida. The adult male and female measure respectively about 20 and 100-130 mm in length and inhabit in the canine respiratory system as final hosts. Infective eggs are discharged into the environment by nasopharyngeal secretions of the canine. Once the eggs are swallowed by herbivore intermediate hosts such as cattle, goat, sheep, camel etc., they hatch in digestion tract and larvae are released. After six to nine molting steps, the larvae reach to the mesenteric lymph nodes (MLNs), liver, lung and other internal organs, developing to infective nymph with a length of 4-6 mm [1-4].

L. serrata infection is more prevalent in tropical and subtropical regions of the world. Human beings may be infected following eating infected organs of herbivores such as liver, lung and MLNs, a condition called nasopharyngeal linguatulosis or Halzoun syndrome. Human infection often occurs through fauces, throat, larynx, pharynx and nasal passages. The symptoms include coughing, sneezing, nasopharyngitis, headache, dysphagia, dyspnoea and asphyxiation [5-7]. Thus far, human infection has been reported from different countries, including Iran [8-13]. In Lebanon, nasopharyngeal linguatulosis in human population is related to eating undercooked internal organs of the ruminants such as MLNs and liver. In the Sudan it is connected with consumption of a popular dish, called Marrara, prepared form raw visceral organs without any heating process [7,14]. In Iran, some human cases were recorded following consumption of barbecued liver (Kebab). Eating undercooked liver by pregnant woman and children is common in some parts of Iran especially rural regions; since there is an opinion that undercooked liver is more nutrient than well-cooked one because of having more iron and vitamins [4,5]. Recently, *L. serrata* infection of a young boy patient in Tehran, Iran with a history of consumption of undercooked liver was reported [15]. Salting and heating are of the most important procedures for food preservation and processing since ancient times. Sodium chloride (NaCl) that is also named as common salt and table salt is the oldest known seasoning and food preservative. Raw meats were the early foods which preserved only by salting and heating. Now, these are common and cheap methods for preservation of meat and meat products in poor areas with no chilling and freezing equipments [16,17].

In spite of high significance of linguatulosis in public health in endemic areas, there is no comprehensive and standard disinfecting method in meat processing. On the other hand, this parasite is mostly located in the internal parenchyma of the infected organs and therefore is not easily seen by the eye during routine inspection at slaughterhouses. So, finding efficient disinfecting methods (such as slating and heating) to increase meat products safety is a necessity. To do this, it is essential to know how long the nymphs remain alive and infective at various concentrations of NaCl and different temperatures. The major aim of this study was to evaluate the impact of NaCl and heat on survival time of *Linguatula serrata* nymphs as efficient disinfecting methods.

Materials & Methods

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-*Collection of Linguatula serrata nymphs*

Mesenteric lymph nodes (MLNs) samples were taken from slaughtered domestic ruminants in Ehsan-Rey slaughterhouse, Tehran, Iran (5-10 samples from each animal). The samples were transferred to laboratory and after that isolation of *L. serrata* nymphs from the samples was immediately begun. Each MLN was cut longitudinally, put in petri dishes containing sterile PBS (Phosphate Buffer Saline) for about 5-10 minutes. Then, the isolated fresh nymphs were washed in sterile PBS and utilized for storage trials [3].

-*Storage solutions*

Different concentrations of NaCl solution (2%, 5% and 10%) were prepared by dissolving NaCl (Merck/ Germany) in distilled water. Sterile PBS was made by dissolving one PBS tablet (Sigma/P4417) in 200 ml distilled water producing 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4, at 25 °C.

- *Survival time of Linguatula serrata nymphs*

Thirty nymphs (10 in triplicate) were separately transferred to 50-ml roundbottom plastic tubes, containing different concentrations of NaCl solution (2%, 5% and 10%) and each one was stored at +4°C. Meanwhile, 30 nymphs (10 in triplicate) in tubes containing PBS were separately treated by +50°C, +60°C and +72°C through water bath. As control group, 30 nymphs (10 in triplicate) were stored in PBS at +4°C.

The effects of NaCl and heat on survival time of the nymphs were evaluated in different periods of time. For this purpose, survivability of the nymphs related to each treatment method was immediately determined after about 1-2 minutes by observing their motility and wriggle under a stereomicroscope (Figure 1). The nymphs that had not any motility and movement were considered as dead.

-*Statistical analysis*

The statistical analysis was performed using ANOVA. The $P<0.05$ level was considered as significant.

Results

As is shown in table 1, the survival time of the nymphs stored in 10% NaCl solution was too short and all the nymphs were dead after 3 hours. But the other ones maintained in 2% NaCl solution were significantly more resistant ($p<0.05$) and they were survived for 2 days.

All the nymphs pertaining to each +60°C and +72°C treatments were found dead after first 5-minute storage interval; the nymph stored at +50°C died totally after 20 minutes (Table 2).

The nymphs maintained in PBS at +4°C (control group) showed the longest survival time ($p<0.05$); all of them were alive until day 4 and the last ones died on day 34 (Figure 2).

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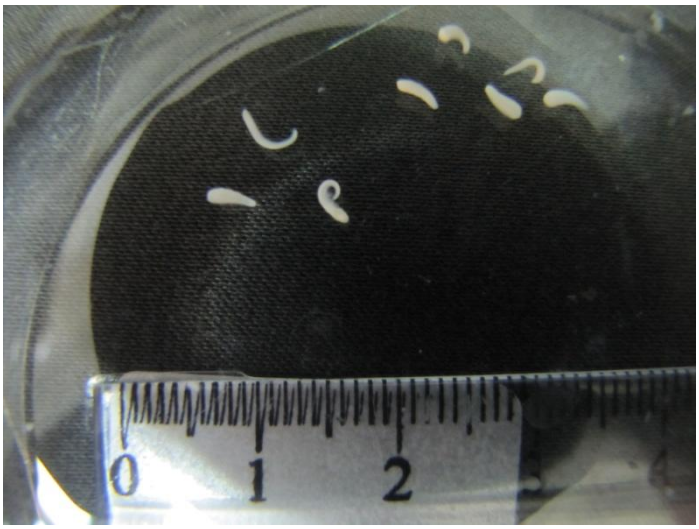


Fig. 1: *Linguatula serrata* nymphs isolated from a domestic ruminant slaughtered in Ehsan-Rey slaughterhouse, Tehran, Iran

Table 1: The survival time of *Linguatula serrata* nymphs in different NaCl solutions

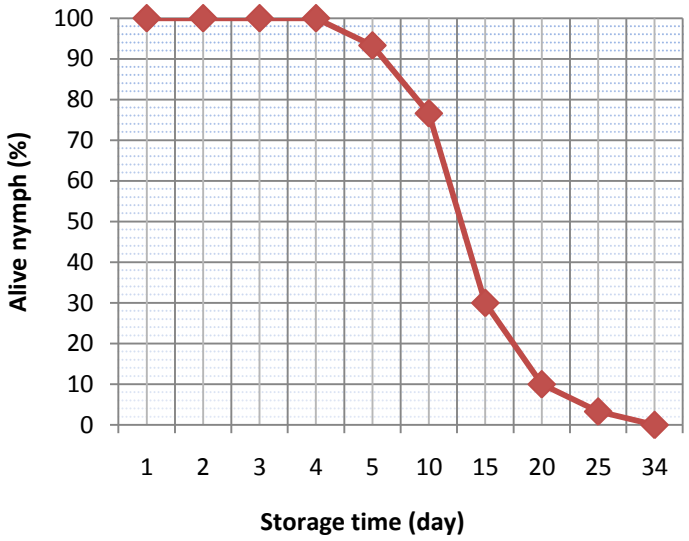
		Alive nymphs number in different periods of time					
NaCl concentration	Triplicate groups	0 h	3 h	6 h	12 h	24 h	48 h
2%	A	10	10	8	1	0	-
	B	10	9	3	3	1	0
	C	10	9	5	1	1	0
	Mean	10	9.33	5.33	1.66	0.66	0
5%	A	10	0	-	-	-	-
	B	10	0	-	-	-	-
	C	10	2	0	-	-	-
	Mean	10	0.66	0	-	-	-
10%	A	10	0	-	-	-	-
	B	10	0	-	-	-	-
	C	10	0	-	-	-	-
	Mean	10	0	-	-	-	-

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Table 2: The survival time of *Linguatula serrata* nymphs in different temperatures

Temperature	Triplicate groups	Alive nymphs number in different periods of time: 0 min	5 min	10 min	20 min
+50°C	A	10	8	0	-
	B	10	10	8	0
	C	10	6	4	0
	Mean	10	8	4	0
+60°C	A	10	0	-	-
	B	10	0	-	-
	C	10	0	-	-
	Mean	10	0	-	-
+72°C	A	10	0	-	-
	B	10	0	-	-
	C	10	0	-	-
	Mean	10	0	-	-

Fig. 2: The survival time of *Linguatula serrata* nymphs in PBS at +4°C (control group)



Discussion

Previous researches about linguatulosis in slaughtered herbivores show that this infection is endemic in Iran. The prevalence rate of infection in cattle, sheep, goat and camel have been reported to be 14.8%, 52.5%, 49.1% and 13.5%, in different parts of Iran [3,4, 18,19]. Close contact between dogs and domestic ruminants is a major reason for high prevalence rate of the linguatulosis. It appears that the

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endemicity of *L. serrata* infection in domestic herbivores causes a high risk of infection in the human population of Iran. [5,6]. On the other hand, the epidemiology of nasopharyngeal linguatulosis depends significantly on cultural food pattern of the people, in which nymphs are ingested via raw or undercooked internal organs particularly liver and MLNs [7].

According to the present study, the survival time of the nymphs stored in NaCl solutions comparing to PBS (as control group) was shorter indicating high susceptibility of the nymphs to NaCl. In a research on survival time of *Eimeria tenella* in NaCl solutions, it was revealed that sporozoites survived in PBS at +4°C for 14 days. But, the sporozoites survived only for 3 days in 16% NaCl solution [20].

In this experiment, the survival time of the nymphs maintained at +50°C, +60°C and +72°C was too shorter than control group (+4°C). It shows that the nymphs have no considerable resistance to the heat. Mir et al (2009) observed that *L. serrata* nymphs survived in PBS for 4 days at room temperature [21]. In an examination done by Negrea et al (2009), it was revealed that the maximum survival time of the nymphs stored at +4°C was only 3 days [22], having a significant difference with our results (34 days). This disagreement may be due to differences in the storage methods and/or survivability evaluation process. Previously, Alcala-Canto et al (2007) proved the serine protease activity in the *L. serrata* nymphs [23]. It is known that some protease enzymes can help to expand the survival time of some endo-parasites (e.g. *Clonorchis sinensis* metacercariae) during long-time refrigerated storage [24]. Despite, it appears that the protease enzyme has probably a considerable role in long survival time of *L. serrata* nymphs stored at refrigeration temperature (+4°C), but the detailed mechanism is still unclear.

The killing mechanism of NaCl on the parasites is due to the fact that when they are suspended in high concentration of NaCl, water is removed from the body of the parasites to the external environment. So, plasmolysis and subsequent death will occur. The death mechanism of the parasites after heating is most related to denaturation and coagulation of cellular proteins [17].

It has been approved that *L. serrata* nymphs have inoculative effect and result in transmission of some pathogenic bacteria throughout the migration from alimentary canal to the internal organs [25]. On the other hand, concurrent occurrence of linguatulosis and paratuberculosis bacterial disease (Johne's disease) in goats has been recently reported [21]. So, proper heating and application of common salt as a food preservative in meat products, not only reduces the survival time of *L. serrata* nymphs, but also decreases the risk of food-born microorganisms.

L. serrata is known as an important food safety hazard for the people who consume undercooked internal organs of herbivores. It is concluded that heating and salting have significant parasiticidal effects on *L. serrata* nymphs and could be used as disinfecting methods in processing of meat products. However, refrigeration at +4°C increases the resistance of the nymphs in meat products and therefore might endanger the food safety. In those regions where raw or undercooked organs may be eaten, the people should be warned to the risk of linguatulosis. Whether our results hold accurate under

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field conditions is not clear as yet, but we found that there is a serious risk that shows cold treatment methods currently applied to fresh meat products could not be used to prevent linguatulosis. Anyway, more experiments in food models are needed to acquire detailed data.

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