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Effect of different temperature conditions on survival time of *Linguatula serrata* nymphs

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Abstract. Objective: Linguatulosis is one of the food-borne parasitic zoonoses. Human infection may occurs following consumption of the raw or undercooked infected offal of slaughtered ruminants, especially mesenteric lymph nodes (MLNs), liver and lung. The main objective of this study was to evaluate the effect of different temperature conditions on survival time of *Linguatula serrata* nymphs. Material and Methods: Fifteen infected MLNs (5 in triplicate) were separately put in plastic bags and were treated by +50°C, +60°C, +72°C and -18°C through water bath or freezer. Meanwhile, fifteen infected MLNs (5 in triplicate) were stored in refrigerator (+4°C) as control group. The survival time of the nymphs was evaluated by observing their motility and wriggle under a stereomicroscope in different periods of time. Results: The infected MLNs were mainly edematous, hemorrhagic and dark comparing to the non-infected ones. The survival time of the nymphs stored at the high temperatures (+50°C, +60°C and +72°C) were short and all the isolated nymphs were found dead after maximum 1.5 hours. The other ones stored at freezing temperature (-18°C) were more resistant and died after 3 hours. But, the nymphs stored in +4°C (control group) had the longest survival time and were resistant until day 20. Conclusion: It is concluded that cooking and freezing temperatures have a parasiticidal effect on *Linguatula serrata* nymphs and could be applied as a suitable method in processing of meat products. But refrigeration at +4°C is not a safe method and may increase the resistance of nymphs in meat products.

Key words: *Linguatula serrata* nymph, mesenteric lymph node, survival time, temperature conditions.

Introduction. *Linguatula serrata* is one of the zoonotic and food-borne parasites which belongs to class Pentastomida. The adults live in the respiratory system and sinuses of the carnivores especially dogs as final hosts. Eggs are swallowed by herbivore intermediate hosts such as cattle, buffalo, sheep, goat, etc. Then, larvae migrate mainly to mesenteric lymph nodes (MLNs) and offal (such as liver, lung, spleen, heart, etc.). Following several molting stages, the larvae change to the infectious nymphs (Kaufmann 1996; Oryan et al 2008; Akhondzadeh Basti & Hajimohammadi 2011). Man infection may occur either through eating of infected offal, a condition called nasopharyngeal linguatulosis or via swallowing the eggs in contaminated water and vegetables resulting in visceral linguatulosis. Nasopharyngeal infection happens following the consumption of the raw or undercooked infected offal to *Linguatula serrata* nymphs afflicted by sneezing, coughing, nasopharyngeal inflammation as well as a hypersensitivity reaction of the upper respiratory tract named Halzoun syndrome or Marrara syndrome (in Sudan) (Yagi et al 1996; Slifko et al 2000; Haddadzadeh et al 2009; Oryan et al 2011). So far, many cases of human infection have been reported from different parts of the world, including Iran (Sadjjadi et al 1998; Lazo et al 1999; Maleky 2001; Yeganeh et al 2001; Ma et al 2002; Koehsler et al 2011).

Marrara is a common Sudanese dish prepared mainly from raw offal of small ruminants along with some seasons like chili, salt, lemon and onion. Since, Marrara is usually eaten rawly, there is a great risk of infection to *linguatula serrata* and many cases of human linguatulosis, so-called Marrara syndrome, have been reported. An investigation in Sudan indicated that 20% of the people consuming raw offal of small ruminants had nasopharyngeal inflammation at least one time in their lifetime (Yagi et al 1996).

In Lebanon, consumption of ruminants MLNs is probably a major way of human infection, and nasopharyngeal linguatulosis have been previously reported from this country (Khalil & Schacher 1965; Schacher et al 1969).

In Isfahan, central province of Iran, there is a popular dish preparing from sheep lung named Beryani. In some cases, it may be not properly heated in the process of cooking, resulting in the linguatulosis. Meanwhile, in some parts of Iran, there is an idea that raw liver is more nutrient than cooked ones and have more iron and vitamins; therefore, consumption of raw or undercooked liver is not uncommon particularly by pregnant women and children (Nourollahi Fard et al 2010; Akhondzadeh Basti & Hajimohammadi 2011). Anaraki Mohammadi et al (2008) have reported linguatulosis from a 10-year-old boy in Theran, Iran with a history of consumption of sheep liver.

Although *Linguatula serrata* is a public health problem, there is no standard and comprehensive method for diagnosing the infection in offal during the abattoir inspection. On the other hand, ruminant infection has no considerable clinical symptoms and such animals are major sources of infection for the human beings. Therefore, the definite diagnosis of the infected offal is not easy for slaughterhouse's inspectors. So, it is necessary to find an efficient disinfecting method during the offal processing in order to improve food safety and public health.

To achieve useful control programs, it is necessary to know that how long the nymphs remain alive at the different temperature conditions during the food processing. The main objective of this study was to evaluate the effect of different temperature conditions on the survival time of *Linguatula serrata* nymphs. In addition, the gross changes of the infected MLNs were studied.

Materials and Methods. Mesenteric lymph nodes (MLNs) of slaughtered ruminants were taken from Ehsan-Rey slaughterhouse, Tehran, Iran (5-10 MLNs from each animal). The samples of each animal were transferred separately to laboratory in a plastic bag within 3 hours. Then, one of the MLNs from each bag (related to each animal) was cut longitudinally in a glass petri dish containing sterile Phosphate Buffer Saline (PBS) and examined by a stereomicroscope after about 5-10 minutes (Haddadzadeh et al 2010). If any *Linguatula serrata* nymph was isolated from the MLN, the other ones from the same animal were considered as infected and were used in the experiment. Sterile PBS was previously prepared by dissolving one PBS tablet (Sigma/P4417) in 200 ml sterile 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.

Fifteen infected MLNs (5 in triplicate) were put in plastic bags and were separately treated by +50°C, +60°C, +72°C and -18°C through water bath or freezer. Meanwhile, fifteen infected MLNs (5 in triplicate) were stored in refrigerator (+4°C) as control group. Evaluation of survival time of the nymphs was done in different periods of time. For this aim, first of all, the nymphs were isolated from each infected MLN as previously explained. Then, survivability of the isolated nymphs were carefully determined by observing their motility and wriggle under a stereomicroscope. The nymphs that had no motility and movement were considered as dead (Mir et al 2009). Finally, the number of alive or dead nymphs isolated from each MLN was recorded.

Results. The survival time of the nymphs stored at high temperatures (+50°C, +60°C and +72°C) were short and all of the isolated nymphs were found dead after maximum 1.5 hours. The other ones stored at freezing temperature (-18°C) were more resistant and died after 3 hours (Table 1). But, the nymphs stored at +4°C (control group) had the longest survival time and were resistant until day 20 (Table 2).

The infected MLNs were mainly edematous, hemorrhagic and dark comparing to the non-infected ones (Figs 1-2). In some infected MLNs, calcification was observed. Principally, normal looking MLNs were not infected. The isolated nymphs had a length of about 5 mm and their color was milky-white; meanwhile, the anterior end of the nymphs was wider than the posterior one (Figure 3).

Table 1

The survival time of *Linguatula serrata* nymphs at high and freezing temperatures

			Isolated nymphs number after different period of time			
Temperature of storage	*Infected MLNs number*		*0.5 hour*	*1 hours*	*1.5 hours*	*3 hours*
+50°C	15 (5 in triplicate)	Alive	39	19	0	-
		Dead	0	0	35	-
+60°C	15 (5 in triplicate)	Alive	6	0	-	-
		Dead	17	63	-	-
+72°C	15 (5 in triplicate)	Alive	0	-	-	-
		Dead	30	-	-	-
-18°C	15 (5 in triplicate)	Alive	51	26	20	0
		Dead	0	0	0	47

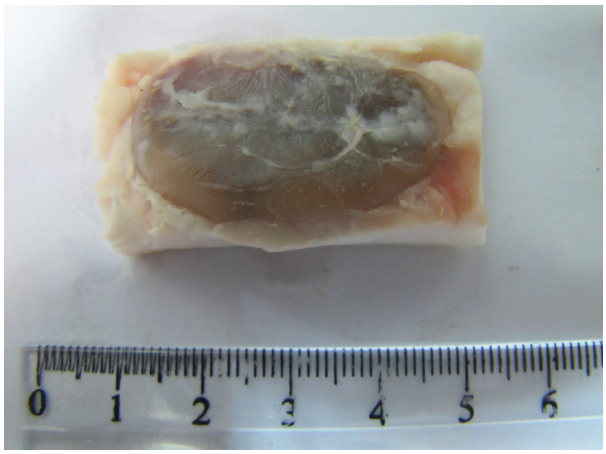

Figure 1. Edema and darkness in an infected MLN to *Linguatula serrata* nymph.

Table 2

The survival time of *Linguatula serrata* nymphs at refrigeration temperature (control group, +4°C)

Time period	*No. of isolated nymphs from 15 infected MLNs (5 in triplicate)*	
	Alive	Dead
12 hours	19	0
1 day	21	0
3 days	41	0
6 days	39	0
10 days	28	0
14 days	11	21
16 days	17	7
20 days	0	56

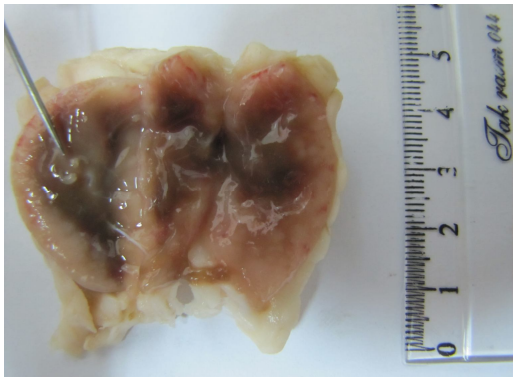

Figure 2. Infected MLN to *Linguatula serrata* nymph (needle).

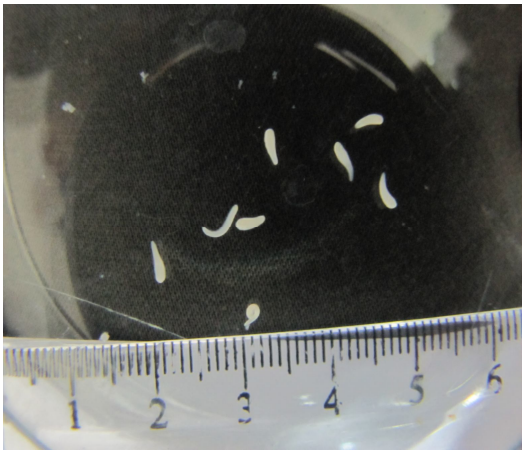

Figure 3. *Linguatula serrata* nymphs isolated from a MLN.

Discussion. Recent studies about linguatulosis in slaughtered ruminants indicate that the prevalence rate of this infection is very high in Iran. The prevalence rate of 14.8%, 52.5%, 49.1% and 13.5% were previously reported in cattle, sheep, goat and camel respectively (Tavassoli et al 2007; Haddadzadeh et al 2010; Nourollahi Fard et al 2010; Youssefi & Hadizadeh Moalem 2010). It seems that the endemicity of linguatulosis in domestic ruminants of Iran results in many infected cases in the human population. The main causes for high prevalence of the infection in Iran are vicinity of dogs and domestic ruminants as well as spreading the stray dogs especially around slaughterhouses. So, proper and standard condemnation of discarded offal particularly MLNs in order to avoid dogs to get the infected offal is a suitable way to reduce the infection rate (Oryan et al 2008; Nourollahi Fard et al 2010).

According to Oryan et al (2011), the MLNs of camels infected to *Linguatula serrata* are grossly enlarged and their cross-sectional areas are coarse, firm and edematous. In another study on infected sheep, MLNs were hemorrhagic and necrotic (Miclaus et al 2008) which is similar to our findings.

Considering the results obtained from the present study, the survival time of the nymphs stored at +50°C, +60°C and +72°C comparing to +4°C (control group) was too short, indicating susceptibility of the nymphs to cooking temperatures. In a research done by Mir et al (2009), it is observed that *Linguatula serrata* nymphs survived in PBS for 4 days at room temperature. Negrea et al (2009) revealed that the nymphs kept at -18°C were totally destroyed in the first 24 hours of observation. But they did not indicate further details. In our study, we found that storing the nymphs at -18°C results in dying all of them in the first 3 hours of the study. On the other hand, in the mentioned experiment, the maximum survival time of the nymphs stored at +4°C were reported 3 days, having a considerable difference with our findings (20 days). Serine protease activity in the *Linguatula serrata* nymphs was previously demonstrated by Alcala-Canto et al (2007). It has been approved that some protease enzymes can help to increase the survival time of some parasites like *Clonorchis sinensis* metacercariae during long-time refrigerated storage (Li et al 2006). Although, it seems that protease enzyme has probably a significant role in long survival time of *Linguatula serrata* nymphs stored at refrigeration temperature, but the detail mechanism is not clear.

It is known that *Linguatula serrata* nymphs have inoculative effect and therefore result in transmission of some pathogen microorganisms during the migration from intestines to offal. In a histological investigation on infected sheep MLNs, bacteria (bacillary and cocci) were detected (Miclaus et al 2008). In another research, concurrent occurrence of linguatulosis and paratuberculosis in goats has been reported (Mir et al 2009). So, application of suitable temperatures during the food processing and cooking destroys the *Linguatula serrata* nymphs and accordingly decreases the risk of other food-borne microorganisms.

Conclusions. It is concluded that cooking and freezing temperatures have a parasiticidal effect on *Linguatula serrata* nymphs and could be applied as a suitable method in processing of meat products. But refrigeration at +4°C is not a safe method and may increase the resistance of nymphs in meat products.

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