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Research paper

A behavioral evaluation of the effects of ingestion of *Linguatula serrata* nymphs in rats

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ABSTRACT

Nasopharyngeal linguatuliasis known as Halzoun or Marrara syndrome occurs following ingestion of raw or undercooked viscera, including lymph nodes, liver and lung of infected animals. The present study was aimed to investigate the behavioral changes induced by ingestion of *Linguatula serrata* nymphs in rats. For this purpose, 24 rats were divided into four groups and orally ingested with 0 (control), 15, 30 and 60 *L. serrata* nymphs, respectively. Sneezing, mouth and tongue movements and mouth opening numbers were counted and the duration of mouth and nose grooming was measured at 10-min blocks for 30 min. Ingestion of 0 (normal saline) number of nymph produced negligible behaviors, whereas 15, 30 and 60 numbers of nymphs increased the above-mentioned behaviors when compared to normal saline (0 nymph) group. In this context, 60 number of nymphs produced more behavioral changes than 15 nymphs. We concluded that ingestion of *L. serrata* nymphs can produce behavioral changes in orofacial area in rats.

1. Introduction

Linguatula serrata, a well-known cosmopolitan parasite, is a member of small group of parasites which belongs to phylum pentastomida (Gosling, 2005; Muller, 2002). The adult nymphs usually inhabit in upper respiratory tract of carnivorous mammals, especially Canidae and probably Hyaenidae and Felidae, causing rhinitis. The larvae and nymph can also infect the various visceral organs of herbivores (Lazo et al., 1999). *L. serrata* has four hooks by which can attach to the wall of respiratory tract. The adult male and female parasites measure 1.8–2 cm and 8–13 cm in length, respectively (Soulsby, 1982). Eggs containing larvae can spread into the environment through nasopharyngeal secretions and be ingested by grazing herbivores. The ingested egg hatch in the alimentary canal and the larvae reaches the mesenteric lymph nodes, liver and lung (Berger and Marr, 2006; Khalil and Schacher, 1965; Soulsby, 1982). Humans may also be infected as an intermediate host (visceral linguatuliasis) or on some rare occasions as an accidental final host (nasopharyngeal linguatuliasis) (Prathap, 1981). Human nasopharyngeal linguatuliasis, known as Halzoun or Marrara syndrome, occurs following ingestion of infected raw or undercooked viscera including lymph nodes, liver and lung (Beaver et al., 1984; Drabick, 1987). In these cases, the nymphs anchor to the mucosal epithelium with prominent hooks causing symptoms including throat irritation and pain, dyspnea, dysphagia, vomiting, headaches, photophobia, aural pruritus and yellow nasal discharge (Anaraki et al., 2008; Siavashi et al., 2002; Maleky, 2001; Tabibian et al., 2012). Several studies have investigated the prevalence of *L. serrata* nymph in definitive hosts including humans (Maleky, 2001; Lazo et al.,1999; Rezaei et al., 2011; Yagi et al., 1996) and grazing herbivores such as goat, sheep, camels and etc. (Dincer,1982; Hodjati and Naghili, 1989; Tavassoli et al., 2007). But there is lacking data on behavioral symptoms of infected animals and to best of our knowledge there is no experimental study evaluating the behavioral effects of *L. serrata* in rats. So, in this study, we tried to investigate the behavioral changes caused by ingestion of *L. serrata* nymphs in a rat model of nasopharyngeal linguatuliasis.

2. Material and methods

2.1. Sampling

The mesenteric lymph nodes of goat were collected from Urmia slaughterhouse and transferred to the parasitology laboratory of Faculty of Veterinary Medicine of Urmia University, Iran. After removal of fat

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tissues, the mesenteric lymph nodes were cut longitudinally and placed in a petri dishes containing tap water to allow nymphs to exit from tissue (Tavassoli et al., 2007). Recovered nymphs were reserved in normal saline.

2.2. Animals

Healthy adult male Wistar rats weighing between 220 and 250 g were used in this study. Rats were maintained in polyethylene cages with food and water available ad libitum, in a laboratory with a controlled ambient temperature (22 ± 0.5 °C) and a 12 h light-dark cycle (lights on at 07:00 h). Before testing, the rats were allowed to be accustomed to the new environment and human handling. The experimental protocol was approved by the laboratory animal care and use center of the Faculty of Veterinary Medicine of Urmia University, Iran.

2.3. Infection procedure

Twenty-four rats were divided into four groups. Control group, *L. serrata* nymph 0 (LN0) received orally 1 ml of normal saline. Other three groups, *L. serrata* nymph 15 (LN15), *L. serrata* nymph 30 (LN30) and *L. serrata* nymph 60 (LN60) were orally infected with 15, 30 and 60 numbers of *L. serrata* nymphs, respectively. The rats were kept in food- and water-deprived condition for 4h. Then the nymphs were orally ingested using 1 ml sterile syringe attached to a small plastic tube as described previously (Atcha et al., 2010).

2.4. Behavioral assessment of infected animals

Each rat was placed in Plexiglass observation chamber (30 × 30 × 30 cm) with a mirror mounted at 45° beneath the floor for 30 min adaptation period. Thereafter, each rat was restrained using a towel and normal saline and nymphs were orally ingested. Then, the rats were placed in observation chamber and the behaviors of the animals were recorded using video monitoring system for 30 min (Montazeri et al., 1997). The number of sneezing, mouth and tongue movements and mouth opening was counted and the duration of mouth and nose grooming was measured at 10-min blocks. The total 30 min behaviors were also measured. These behaviors were observed immediately after oral administration of nymphs.

2.5. Statistical analysis

Data obtained from 10-min blocks and total 30 min data were analyzed using two way analysis of variance (ANOVA) followed by Tukey's test. Data were expressed as mean ± Standard error of mean (SEM). P value < 0.05 was considered as statistically significant.

3. Result

LN0 group showed no sneezing at the first, second and third 10-min blocks. At first 10-min block, LN15, LN30 and LN60 groups significantly ($p < 0.05$) sneezed more than LN0 group. In addition, LN30 and LN60 groups sneezed more than LN15 group ($p < 0.05$). At this time block, the highest sneezing frequency was observed in LN60 group. At the second 10-min block, only rats in LN60 group showed significant increase in number of sneezing ($p < 0.05$). No different was found in sneezing frequency at the third 10-min block (Fig. 1).

Tongue movement numbers in LN0 group were 1.2 ± 0.68, 0.17 ± 0.17 and 0.00 ± 0.00 at the first, second and third 10-min blocks, respectively. Significant difference was observed in LN15, LN30 and LN 60 groups at the first 10-min blocks ($p < 0.05$). At second 10-min block, LN30 and LN60 groups showed significant increase in tongue movements ($P < 0.05$). No difference was found in tongue movements at third 10-min block (Fig. 2).

The number of mouth openings in NL0 group was 0.5 ± 0.03, 0.00 ± 0.00 and 0.00 ± 0.00 at first, second and third 10-min blocks, respectively. Mouth openings at the first 10-min block were significantly ($p < 0.05$) increased in LN15, LN30 and LN60 groups. At this 10-min block, LN60 showed more mouth openings than LN30 and LN30 groups ($p < 0.05$). No significant difference was observed among all groups at the second and third 10-min blocks (Fig. 3). The number of mouth openings at the total 30 min was 0.5 ± 0.3 in LN0 group. LN60 group showed the most mouth openings at the total 30 min.

At the first, second and third 10-min blocks, mouth and nose grooming durations were 3.5 ± 1.6, 0.00 ± 0.00 and 0.00 ± 0.00, respectively, in LN0 group. Mouth and nose grooming durations in LN15, LN30 and LN60 groups were significantly higher than LN0 group at the first 10-min block ($p < 0.05$). The highest mouth and nose grooming duration belonged to LN60 group. At the second 10-min block, all LN15, LN30 and LN60 groups showed significantly higher mouth and nose grooming durations ($p < 0.05$). No significant differences were observed among groups at the third 10-min blocks (Fig. 4). The duration of mouth and nose grooming was 3.5 ± 1.6 min at the total 30 min in LN0 group. Significant difference was observed between LN15, LN30 and LN60 groups with LN0 group ($p < 0.05$). LN60 group showed higher mouth and nose grooming durations than LN15 and LN30 groups ($p < 0.05$).

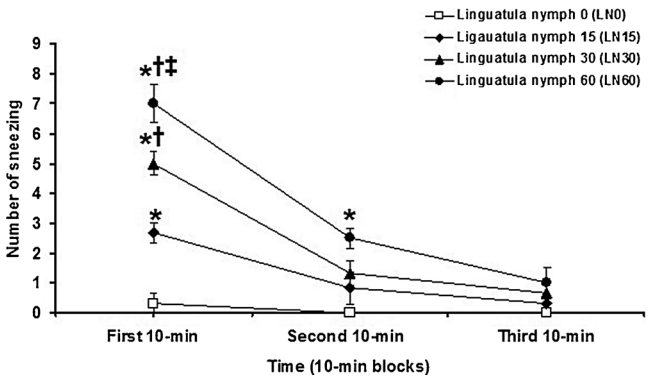

Fig. 1. Sneezing behavior after oral administration of normal saline and *Linguatula serrata* nymphs in rats. Data are expressed as Mean ± SEM from six rats. The number of sneezing was recorded at three 10-min blocks. * $p < 0.05$ in comparison with Linguatula nymph (0 n). † $p < 0.05$ in comparison with normal saline and Linguatula nymph (15 n). ‡ $p < 0.05$ in comparison with normal saline, Linguatula nymph (15 n) and Linguatula nymph (30 n).

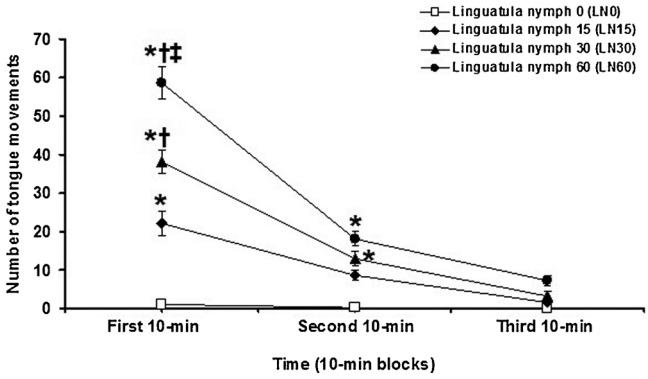

Fig. 2. Tongue movements after oral administration of normal saline and *Linguatula serrata* nymphs in rats. Data are expressed as Mean ± SEM from six rats. The number of Tongue movements was recorded at three 10-min blocks. * $p < 0.05$ in comparison with Linguatula nymph (0 n). † $p < 0.05$ in comparison with normal saline and Linguatula nymph (15 n). ‡ $p < 0.05$ in comparison with normal saline, Linguatula nymph (15 n) and Linguatula nymph (30 n).

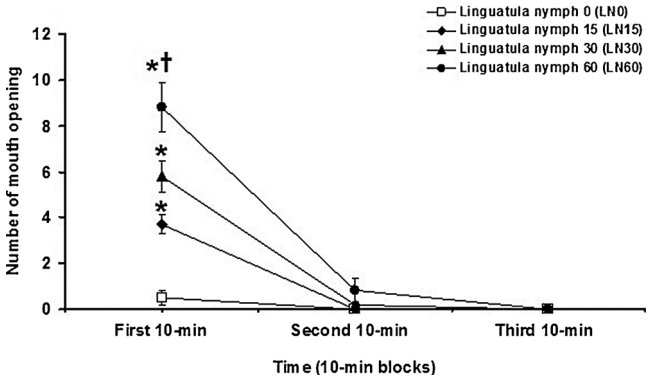

Fig. 3. Mouth openings after oral administration of normal saline and *Linguatula serrata* nymphs in rats. Data are expressed as Mean ± SEM from six rats. The number of mouth openings was recorded at three 10-min blocks. * $p < 0.05$ in comparison with Linguatula nymph (0 n). † $p < 0.05$ in comparison with normal saline (no nymph) and Linguatula nymph (15 n).

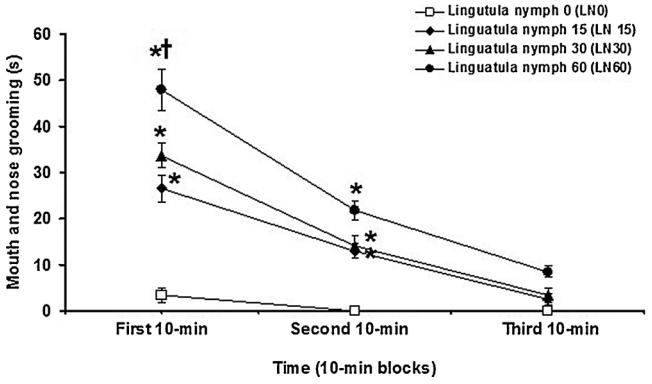

Fig. 4. Mouth and nose grooming after oral administration of normal saline and *Linguatula serrata* nymphs in rats. Data are expressed as Mean ± SEM from six rats. The duration of mouth and nose grooming was recorded at three 10-min blocks. * $p < 0.05$ in comparison with Linguatula nymph (0 n). † $p < 0.05$ in comparison with normal saline (no nymph) and Linguatula nymph (15 n).

4. Discussion

The results of the present study showed negligible sneezing, tongue movements, mouth openings and mouth and nose grooming in rats after oral administration of normal saline. Administration of testing materials directly into the mouth is common in laboratory animals such as mice and rats (Turner et al., 2011a). Normal saline, commonly defined as physiological saline, has been frequently used as a medium for dissolving or suspension of test substances (Turner et al., 2011b).

In the present study, the rats showed sneezing, tongue movements, mouth opening and mouth and nose grooming, after ingestion of *L. serrata* nymph. Sneezing, as an upper airway response, clears upper and lower airways (Tai and Barariuk, 2002). Sneezing has been reported as a symptom of nasopharyngeal linguatuliasis (Yagi et al., 1996). In the present study, tongue movements and mouth opening were observed after ingestion of *L. serrata* nymph. We observed excessive back and forth movements of tongue which was occasionally accompanied by mandible opening. The tongue is a key contributor of swallowing and respiratory function. Brief occlusion of upper airway tracts can increase respiratory derive to tongue muscles and produce tongue protrusion (Lee et al., 2012). Our present results showed excessive mouth and nose grooming behaviors after ingestion of *L. serrata* nymph. Orofacial grooming, in relation to the involvement of orofacial structures, is subdivided into mouth, ear, eye and facial grooming. According to neuronal substrate, grooming behavior is divided into genital grooming, body grooming, maternal grooming and orofacial grooming (Spruijt et al., 1992; Kalueff et al., 2016). Facial grooming is composed of mouth rubbing and facial wiping, and prolonged facial grooming can reflect a pain-related behavior (Hitomi et al., 2015). In the present study, the maximum intensity of these behaviors occurred at the first 10-min block, and continued with very low severity at the second 10-min blocks, and approximately disappeared at the third 10-min block. These indicate that ingestion of *L. serrata* nymph can induce short-term behavioral alterations in rats. In human symptoms appear a few minutes to half an hour after ingestion of infected meal. The variation of incubation period probably depends on the place where nymphs are released from their cysts, as swollen nymphs require more time to migrate to tonsils and nosopharyngeal mucosa (Acha and Szyfres, 2003). Although our time-dependent results are approximately in accordance with findings of Acha and Szyfres (2003), microstructure of orofacial behaviors requires to further evaluation using ingestion of infective food in rats. Symptoms of nasopharyngeal linguatuliasis are nasopharyngitis accompanied by pain, itching of throat and ears, coughing, vomiting, sneezing, bleeding, dyspnea, headache and inflammation (Yagi et al., 1996). Nasopharyngeal linguatuliasis is common throughout the Middle East where it is often known as Halzoun syndrome.

In conclusion, the results of the present study showed that sneezing, tongue movements, mouth opening and excessive orofacial grooming were induced after ingestion of *L. serrata* nymphs.

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