



## COMPARATIVE EVALUATION OF SOMATIC PROTEINS OF THE NYMPHAL STAGE OF *LINGUATULA SERRATA* ISOLATED FROM GOATS AND CATTLE IN NORTHWEST IRAN

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### Summary

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Because the nymphal stages of *L. serrata* isolated from different animals have different strains, it was assumed that their somatic proteins would be different. The present study was conducted to evaluate and characterise the somatic proteins of *L. serrata* nymphs, isolated from goats and cattle mesenteric lymph nodes. Nymphs were collected from the infected mesenteric lymph nodes of slaughtered cattle and goats. Somatic proteins were prepared by homogenising nymphs and then were electrophoresed using SDS-PAGE. Following SDS-PAGE, somatic proteins of the nymphal stage of *L. serrata* in our samples were characterised by the presence of 6 protein bands with molecular weights of 14.4, 32, 36, 48, 75 and 100 kDa in goats and 7 protein bands with molecular weights of 14.4, 32, 36, 48, 75, 100 and 120 kDa in cattle. The evaluation of somatic proteins showed slight differences in the mesenteric lymph nodes of goats and cattle. The findings of this study can be used both for further evaluation of their antigenicity for diagnostic purposes and for development of protective vaccines.

**Key words:** cattle, goat, *Linguatula serrata*, SDS-PAGE, somatic proteins

### INTRODUCTION

As one of the parasitic zoonoses, *Linguatula serrata* inhabits the canine respiratory system as its final habitat. Discharged eggs from the definitive host's nasopharyngeal secretion are ingested by the grazing herbivores where they hatch into larvae which migrate into the mesenteric

lymph nodes, liver and lungs, where eventually they develop into infective nymphs (Rezaei *et al.*, 2011). The pathogenesis of linguatulosis is not clear, but it has been shown that proteolytic enzymes may have a role in it (Alcala-Canto *et al.*, 2007). According to Yagi *et al.* (1996) and

Siavoshi *et al.* (2002), linguatulosi in men can be caused by either the egg (visceral form) or nymph stage of the parasite (nasopharyngeal form). Nasopharyngeal linguatulosi, known as Halzoun or Marrara syndrome, often occurs after consumption of raw or undercooked viscera (liver, lung and lymph nodes) of infected animals. The low mortality/high morbidity typical of helminth infections caused by gastrointestinal worms, schistosomes, nematodes or cestodes, presumably reduces the interest and thus focused investigation into their control and treatment. At the same time, however, these infections typically provoke severe disablement through pathological lesions and malnutrition. Thus a better understanding of the host-parasite inter phase in helminth infections is urgently required and, given the recent technical innovations in molecular biology and cellular immunology, quite feasible. The new era of recombinant antigens, T-cell clones and anti-idiotypic reagents is ideally suited to the immediate objectives of reliable diagnosis, vaccine production and alleviation of immune pathological consequences. The mother technology in the field of molecular biology has caused new trend in the research which enables us to diagnose the infectious stages of parasites (Parkhouse & Harrison, 1989). One of these methods is identifying the somatic proteins of the parasites by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Somatic proteins whose identification is essential for an early diagnosis of the parasitic infections, are considered as surface and secreted proteins are the most likely targets for protective immune responses (Pritchard, 1986). A thorough study of parasitic helminths somatic proteins is a pre-requisite for control programmes, which are intended to diminish pathologi-

cal sequel and are based on accurate immunochemical diagnosis, protection by vaccination, and perhaps immune modulation (Parkhouse & Harrison, 1989).

To date, the existing research has focused on the somatic proteins in isolated *L. serrata* nymph from mesenteric lymph nodes of goats (Youssefi *et al.*, 2014). Therefore, the objective of this study was to compare the somatic proteins of the nymphal stage of *L. serrata* isolated from goats and cattle mesenteric lymph nodes.

## MATERIALS AND METHODS

### *Study area*

This study was conducted on slaughtered goats and cattle in Slaughterhouse of Tabriz, East Azerbaijan, in 2015. The city of Tabriz is located in the Northwest of Iran (geographical coordinates 38° 4' N and 46° 18' E) with a human population of almost 1.5 million. The studied region has a moderate climate in winters and mild in summers, with an annual rainfall of about 388 mm.

### *Preparation of the samples*

The mesenteric lymph nodes from 130 goats (95 females and 35 males) and 80 cattle (60 male and 20 female) were examined for *L. serrata* nymphs between May and January 2015 after collection of mesenteric lymph nodes of goats and cattle slaughtered in Tabriz slaughterhouse. They were transferred to the Laboratory of veterinary parasitology, Faculty of Veterinary Medicine, Urmia University. Each lymph node was cut into small pieces and immersed in the Petri dish containing tepid water and left for 15 min to allow nymphs to come out from the tissue. Then, apparently normal lymph nodes were digested in 200 mL of digestion solution

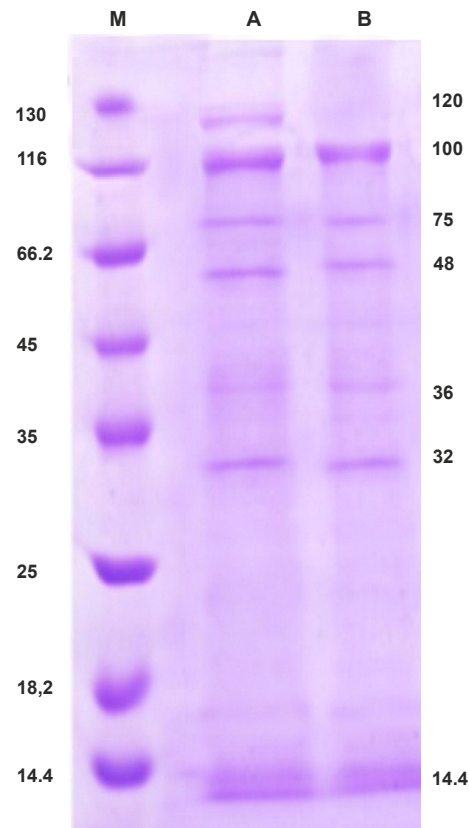
containing 5 g of pepsin and 25 mL hydrochloric acid in 1000 mL distilled water, and were incubated at 37 °C for 24 h (Razavi *et al.*, 2004). Recovered nymphs were washed three times in phosphate-buffered saline (PBS, pH=7.2; Sigma), sonicated in five cycles of 70 kHz (30 s each) at 4 °C and centrifuged at 4,000 g for 15 min. The supernatant was collected and filtered through 0.4 µm sieves and stored at -80 °C until analysis. The protein concentration was measured according to Bradford (1976).

#### *Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)*

Somatic proteins were separated by SDS-PAGE as described by Laemmli (Brant *et al.*, 2010), using a mini protein II cell (Bio-Rad). The proteins were heated in a water bath at 100 °C for 10 min. Then, they were added to each well of a 7% stacking gel and 12% separating gel. SDS-PAGE was carried out at 120 V, for 4 h. Gels were stained with 0.05% Coomassie brilliant blue staining (G-250, Cat. No: B0770, Sigma Chemicals). The molecular weights of proteins were determined by comparing their migration distance against that of a known molecular marker. For size estimation in SDS-PAGE, a pre-stained protein marker at a range of 14.4-130 kDa molecular weight (PS10 plus) was used.

#### RESULTS

Findings showed that 120 out of 130 (92.3%) goats and 40 out of 80 (50%) cattle were infected with nymph stages of *L. serrata*. The range, indicating the number of nymphs collected from each infected lymph node, varied from 50 to 400 for goats and 5-15 for cattle. The evaluation of the lanes containing somatic pro-



**Fig. 1.** SDS-PAGE pattern of somatic proteins in nymphal stage of *Linguatula serrata*. Lane A – cattle, Lane B – goat; Lane M –molecular weight marker (kDa).

tein of *L. serrata* nymphs isolated from mesenteric lymph nodes of goats and cattle revealed six (14.4, 32, 36, 48, 75 and 100 kDa) and seven (14.4, 32, 36, 48, 75, 100 and 120 kDa) protein bands, respectively.

#### DISCUSSION

Linguatulososis is of a veterinary and public health importance in the world. As intermediate hosts, goats and cattle, like other ruminants, are essential in the life cycle of *L. serrata*. A close contact between dogs

and the intermediate hosts plays an important role in transmission of *L. serrata* in our research context. Several studies have been conducted in Iran to determine the prevalence of *L. serrata* in ruminants. For instance, prevalence rates of 44% were recorded in cattle in Urmia slaughterhouse, Iran (Tajik *et al.*, 2006). Moreover, a prevalence of 16.22% and 49.1% in mesenteric lymph nodes from cattle and goats respectively was reported in Kerman (Nourollahi Fard *et al.*, 2010; 2012). In the present study, prevalence rates of 92.3% and 50% were observed in goat and cattle mesenteric lymph nodes respectively. High prevalence rate of infection in our research samples may be a manifestation of climatic parameters that enhance survival of parasite eggs in vegetables, fruits and water resources.

An important aspect in diagnosis of linguatulosus is the identification of antigens which are specific and highly immunogenic for the infected host. Since this parasite stimulates the host immune system and negatively influences many immune dominant antigens, parasitic infections occur in animals, resulting in diminished productivity. Therefore, an early diagnostics seems to be vital in preventing parasite growth. To aid the development of early diagnostics, information about somatic proteins of the nymph stage of *L. serrata* that may elicit a host's immune response, is required (Parkhouse & Harrison, 1989). In the present study, somatic proteins of *L. serrata* nymphs isolated from goats and cattle mesenteric lymph nodes turned out to be different in their protein banding patterns on SDS-PAGE. Our study showed 6 protein bands in goats (14.4, 32, 36, 48, 75 and 100 kDa) and 7 protein bands in cattle (14.4, 32, 36, 48, 75, 100 and 120 kDa). Out of these bands, the 36 and 48 kDa ones were

more prominent. Our study results are incompatible with the findings of Youssefi *et al.* (2014) who researched on electrophoretic pattern of somatic proteins of *L. serrata* nymph isolated from goat mesenteric lymph nodes and found out 6 protein bands (19, 20, 36, 48, 75, 100 kDa). However, our research scope and findings were different; we extended our focus from goat to cattle mesenteric lymph nodes and our results indicated 2 different bands of protein. Tavassoli *et al.* (2014) attributed this difference to the fact that the nymphal stage of *L. serrata* has different strains. In their study on *L. serrata* isolated from different farm animals of Iran, the authors showed molecular differences among them by amplifying and sequencing of 18S rRNA of all *L. serrata* isolated from farm animals. Their results revealed that the lowest diversity of nucleotide sequences (98.8%) belonged to sheep, cattle and dogs, while the highest sequence identity (100%) was in camels and goats. Similarly, our findings can provide support for data of Jones & Riley (1991) on frontal glands of a porocephalid pentastomid (*Porocephalus crotali*) in rats as intermediate hosts; in agreement with their investigation, our findings indicated the six protein bands in which the 14.4 kDa band was the same.

On the basis of our findings, we can determine the most important antigenic protein of *L. serrata* in order to design ELISA and relevant vaccines.

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