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# Practical Approach for DNA Extraction of Food Born Linguatula Serrata Nymphs: An Analytical Method

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## Abstract

**Background:** Isolation of high quality genomic DNA is one of the most important steps in molecular biology studies related to food borne parasites. Usually, various protocols are used for different tissues but so far, there is no common, simple and cost benefit procedure for genomic DNA extraction of Linguatula serrata (L. serrata.) larvae as a Pentastomida endangering food safety. One of the procedures used in other studies is using commercial kits that are very expensive especially for developing countries which have this health problem. This study investigated a simple and cost-benefit method to extract genomic DNA form L. serrata. **Materials and Methods:** In this study, after collection of larvae from sheep, washing was done with phosphate buffer saline. The samples were grinded and incubated in lysis buffer at 56ºC overnight. The precipitation was done in absolute ethanol. Extracted DNA was analyzed using Agarose gel Electrophoresis and Spectrophotometery. **Results:** Results indicated that the mean concentration of extracted DNA was 59.3±2.84 ng/µl, and the mean ratio of A(260)/ (280) was 1.6±0.3. It seems that the efficacy of this modified extraction method for L. serrata is appropriate. **Conclusion:** In conclusion, this simple, cost-effective, fast and easy to use method could replace inexpensive commercial kits in molecular laboratories for DNA extraction of Pentastomida and some other similar tissues. So, application of this analytical method could be useful to improve the safety of food especially liver and other meat products.**[GMJ.2014;3(2):115-19]**

**Keywords:** DNA Extraction; Linguatula serrata; Food safety

## Introduction

Detection and identification of any organisms based on molecular techniques require extracted high quality DNA. Usually, various protocols are used for different tissues but so far, there is no common, simple, and cost benefit procedure for genomic DNA extraction of Linguatula serrata (L. serrata) larvae as a Pentastomida. Linguatula serrata is a zoonotic food borne arthropoda which lightly has a convex dorsal part, and is flattened ven-



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trally and is also known as tongue worm [1-3]. Two-host life cycle of L. serrata occurs in canids and herbivores as final host and intermediate host, respectively. The eggs containing larvae are produced by female parasite and then scattered in the environment by nasopharyngeal secretion of the canids, especially domestic dogs as final hosts. When intermediate hosts such as sheep, goat, camel, cattle, buffalo, etc. swallow the eggs from contaminated pastures and water sources, the larvae release in intestinal cavity of the mentioned animals. Then, the larvae migrate to different internal organs particularly mesenteric lymph nodes (MLNs), liver, lung, and kidney, where after several molting stages the larvae develop to infections nymphs with a length of about 5 mm and milky-white in color [4-8]. Human infection named 'Halzoun' may occur after ingestion of raw or semi-cooked infected edible organs of herbivores in which human acts as a final host. Halzoun syndrome is more prevalent in the Middle East countries due to nutritional habit of consumption of raw or undercooked edible offal of domestic animals [9].

To this date, the identification, species delimitation and classification of Pentastomida have almost been based on morphological and biological characters [10-13], and only a few species have so far been subjected to molecular characterization of portions of their either genomic or mitochondrial DNA. Recently, the molecular studies have suggested a close relationship between the Pentastomida and the crustacean subclass Branchiura [14-19], but the systematic position and taxonomic rank of the Pentastomida have not been fixed yet [12, 13]. One of the present problems regarding molecular studies is DNA extraction from this metazoan with a hard tegument using handy, simple, and cost benefit methods. Therefore, the need for setting up an appropriate procedure is urgent especially when hundreds of samples are to be analyzed molecularly.

The purpose of the current study was to develop a practical approach for DNA extraction of L. serrata nymph as a food borne parasite. Also, morphological characteristics of infected MLNs were studied.

## Materials and Methods

### *Samples*

Mesenteric lymph nodes (MLNs) of slaughtered sheeps were taken from an abattoir. In laboratory, at first, the morphological characteristics of each MLN were carefully recorded. After that, the samples were cut longitudinally and were immersed in a glass Petri dish containing saline for about 5-10 minutes [6]. Subsequently, the isolated fresh L. serrata nymphs were washed in saline twice and put inside 70% alcohol and -20 °C for long term storage till the next performance.

### *DNA Extraction*

DNA extraction was performed based on phenol-chloform method described by Sambrook and Russel (2006) [20] with some modifications. Samples were removed from freezer and were dried under condition of 37°C till alcohol was removed thoroughly. Six groups containing five larvae worms were selected and were crushed after having been washed with saline buffer,. Extraction buffer (25mM Tris-HCl, pH=7.6; 100 mM NaCl; 20 mM EDTA, pH=8; 1% SDS) was added to 10 folds. Twenty microliters proteinase K (20mg/ml) was added and incubated at 56ºC overnight. After complete lysis, purification was done using phenol, chloroform, isoamyl alcohol (P:C:I; 25:24:1), briefly, after adding PCI, samples were mixed thoroughly and followed by centrifugation at 12,000 rpm for five minutes at room temperature. The aqua phase was transferred to a new sterile 1.5 ml microtube and equal volume of chloroform was added to the solution. After mixing thoroughly in about three minutes, it was centrifuged at 12,000 rpm for three minutes at room temperature. The upper phase was transferred into a new sterile 1.5ml microtube and DNA was precipitated with cold ethanol and 0.3M sodium acetate (pH=5.2) and incubation at refrigerator for at least 20 minutes. The next step was followed by centrifuge at 4ºC for 5 minutes. The final washing steps were done by 70% ethanol and after drying the pellet, sterile double distilled water was added and incubated at

65ºC for 10 minutes. Quantity and purity of DNA samples from this modified procedure were checked using spectrophotometer at absorbance of 230, 260, 280 and 320 nm. Also, its quantification analyzing was performed using 0.8% Agarose gel Electrophoresis and visualized using gel documentation (E-Gel® Imager, life technologies).
Data are analyzed by using SPSS software version 15.0 and are expressed as mean±standard deviation (SD).

## Results

*Morphological characteristics of infected MLNs*
The color of infected MLNs was mostly darker than non-infected MLNs. Also, in some cases, we found that hemorrhagic surface could be a differentiation key to the diagnosis of infected samples (Figure-1).

*DNA Extraction*
After sampling, genomic DNA was extracted and their quality and quantity were measured by Agarose gel electrophoresis (0.8% Agarose; Figure-2) and spectrophotometer, respectively.
The efficiency of the DNA extraction is illustrated in Table-1 and Figure-2. Briefly, the mean concentration of extracted DNA was 59.3±2.84 ng/µl, and the mean ratio of A (260)/ (280) was 1.6±0.3. Also, the mean absorbance of the extracted DNA at 230 and 320 nm were 0.015±0.001 and 0.013±0.002, respectively, that showed appropriate purification of the genome from phenol and external particles. Figure-2 shows the result of quality analysis of extracted DNA on %0.8 Agarose gel electrophoresis with high quality due to the single and pure band. This method was considered as a rapid, simple, and cheap method for DNA extraction from one of the Metazoan parasites with a hardness tegument.

**Table 1.** Efficiency of the present DNA extraction method as determined by spectrophotometry at 230, 260, 280 and 320 nm. values are mean± standard deviation of six measurments.

| DNA concentration (ng/µl) | $A_{230}$ | $A_{320}$ | A260/280 |
|---|---|---|---|
| 59.3±2.84 | 0.015±0.001 | 0.013±0.002 | 1.6±0.3 |

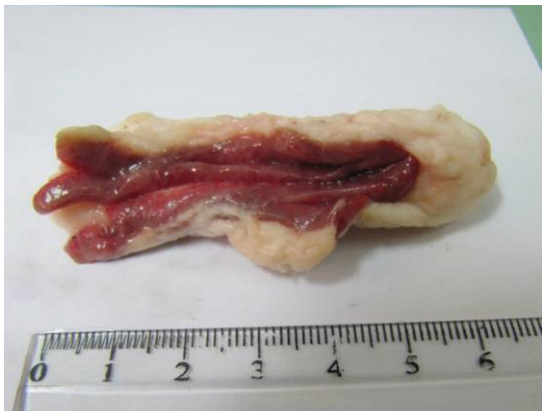


**Figure 1.** Hemorrhagic Surface in MLN of an Infected Sheep

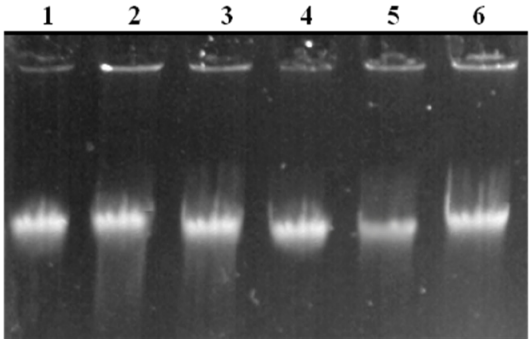


**Figure 2.** Agarose gel electrophoresis (0.8%) of the genomic DNA. Lane 1-6: extracted genomic DNA from L. serrata by used method in this study

## Discussion

One of the best ways to diagnose the infectious diseases is molecular techniques based on Polymerase Chain Reaction (PCR). The success of the PCR depends in particular on the accurate extraction of high quality DNA. There are a few studies about molecular identification of L. serrata that may be due to not having a handy, simple, and cost-benefit DNA extraction method especially in developing countries because of expensive relative kits. Based on our knowledge, there has not been considerable progress in DNA extraction method from L. serrata. Therefore, the need for setting up an appropriate procedure is urgent especially when hundreds of samples are to be analyzed molecularly. This study demonstrates the successful recovery of Linguatula DNA. Our protocol could allow the rapid analysis of molecular epidemiology of this organism.

Lysing the tegument using enzymes is time-consuming, expensive, and complex. These include overnight incubations with enzymes [21] and perhaps the use of liquid nitrogen with many intermediate steps [22]. These methods are not practical in the context of a routine diagnostic laboratory where high-throughput, reproducible results are required. The high level of technical expertise is required and the time-consuming nature greatly increases the labor costs associated with extraction. The use of enzymes and consumables such as liquid nitrogen also significantly increases the cost of the method. The increased number of steps associated with these methods increases the chance of contamination occurring.

As mentioned before, our protocol showed the satisfactory competency in quick genomic DNA extraction from L. serata, inexpensive, and vigorous. An appropriate DNA extraction method will be good for analyzing key genetic markers. The cost, time, final template volume and the purpose for DNA extraction should be considered when choosing extraction methods. Although simple elution is the cheapest and fastest alternative, it is a crude method of DNA extraction and its use may be limited by the choice of PCR [23]. Conversely, DNA extraction using commercial kits is a high throughput method providing high quality DNA, but at a substantial cost and requiring specialized equipment.

In conclusion, according to the results obtained from this study, we represent a rapid, effective and cost-benefit method for DNA extraction of L. serrata nymph. So, application of this analytical method could be useful to improve safety of food especially liver and other meat products; However, in future, more investigations should be done regarding the application of this method for DNA implication during PCR analysis of this parasite.

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**Conflicts of Interest**

None

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