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Original article

Linguatula serrata, a food-borne zoonotic parasite, in livestock in Bangladesh: Some pathologic and epidemiologic aspects

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ABSTRACT

Food-borne parasitic zoonoses are major threats to human health and only next to the 'big three' such as AIDS, tuberculosis and malaria. *Linguatula serrata* is a food-borne zoonotic pentastomid that affects a wide range of animals including humans. Dogs and other carnivores are the final hosts while most herbivores, including domestic ruminants serve as intermediate hosts. Here, the study was conducted on some epidemiologic and pathologic aspects of *L. serrata* infection in cattle and goats through a slaughterhouse based survey. A total of 302 samples, consisting of 257 mesenteric, 26 hepatic and 19 pulmonary lymph nodes (LNs) of cattle and goats were collected. Out of 302 LNs, 136 (45.0%) were infected with the nymphal stage of *L. serrata* (50.7% of cattle and 31.0% of goats). Significantly, a higher (P = 0.002) rate of infection was detected in cattle. Sex, but not seasons, is a prominent determinant of the infection. The infection was mostly detected in the mesenteric LNs (MLNs) (50.9%) but the parasite was also detected in the hepatic (3.9%) and pulmonary (21.1%) LNs. Grossly, the affected LNs were enlarged, edematous and soft. Cut surface of the LNs showed spongy appearance, and sometimes nymphs were found to crawl out. Histopathological studies revealed severe damage in parenchyma of LNs, characterized by the loss of typical pattern of lymphatic follicles and trabecule. Massive infiltration with eosinophils was detected. Collectively, the data suggest that *L. serrata* infection is endemic in food animals and has significant pathological impacts on livestock, and people of the country are at high risk to linguatulosis.

1. Introduction

Food-borne parasitic diseases are major threats to human health. According to the latest WHO-estimation, disease burden (DALY, Disability Adjusted Life Year) of food-borne parasitic diseases is only next to the so called 'big three' such as AIDS, tuberculosis and malaria (Lustigman et al., 2012). *Linguatula serrata*, a pentastomid, is a crustacean-related but helminth resembling zoonotic, food-borne endoparasite affecting humans and animals (Oluwasina et al., 2014; Sulyok et al., 2014). It is a tongue shaped parasite, and rudimentary legs are present only at its larval stage. Due to its characteristics morphology, it is known as a tongue worm (Soulsby, 1982). The lifecycle stages include eggs, larvae, nymphs and adults, and the parasite has an indirect lifecycle involving several intermediate and definitive hosts. Adults inhabit the nasal airways, frontal sinuses and tympanic cavity of carnivores such as dogs, wolves, foxes and cats. Herbivorous animals, such as sheep, goat, cattle and rodents, act as intermediate hosts. Humans may act as both intermediate and accidental definitive hosts (Oryan et al., 2011; Tappe and Buttner, 2009; Tappe et al., 2006). In intermediate hosts, the parasite lives mainly in the MLNs but also in the liver and lungs, and less frequently in the brain, intestine, eye and prostate glands (Koehsler et al., 2011; Soulsby, 1982).

L. serrata is distributed throughout the globe and the infection has been recorded in many countries in Asia, including India (Sudan et al., 2014) and Bangladesh (Mohanta and Itagaki, 2017). Humans get infection through infected or contaminated food and water and/or by handling infected pets, which causes both nasopharyngeal (Halzoun-Marrara syndrome) and visceral form of the disease. Both nymphs and eggs can cause infection in humans through contaminated food and water. Linguatuliasis, either in visceral or nasopharyngeal form, is fatal

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for humans and in some cases the migrated parasite reaches up to the eyes resulting complete and permanent impairment of vision (Koehsler et al., 2011; Mourra et al., 2010). In Bangladesh, there are a lot of street dogs, cats and wild carnivores, which have unrestricted access into the animal premises and herds; thus, our livestock are very prone to the linguatula infection. In livestock, the nymphal stage of the parasite mainly and badly damages the MLNs (Sudan et al., 2014) and severely hampers the functions of the innate and adaptive immunity.

In Bangladesh, many people, especially young boys and girls, love to eat fast food or junk food, in which improperly cooked meat or meat offals are used. Unfortunately, with few exceptions, slaughterhouse management and meat handling are substandard throughout the country. In addition to a large number of street dogs, companion animal keeping is increasing day by day; thus, people of Bangladesh are at high-risk to the pentastomid infection. Considering these points, the present work was designed to study the prevalence, pathology and risk factors of linguatulosis in cattle and goats, the main food animals of Bangladesh.

2. Materials and methods

2.1. Sampling and sample size

A slaughterhouse based survey was carried out from January 2015 to May 2016 in Mymensingh, Bangladesh (Fig. 1). Sample size was calculated using the following formula

$$n = \frac{Z^2 P(1-n)}{d2}$$

where, n = sample size, Z = Z statistics for level of confidence, P = expected prevalence, d = precession (Daniel, 2009). In Bangladesh, prevalence of linguatulosis in food animals has not been studied yet. However, the parasite has thoroughly been investigated in neighboring countries, including India. Sudan et al. (2014) reported linguatuliasis ~18% animals in India. Therefore, here, we considered the expected prevalence as ~20%, and calculated sample size at 95% level of confidence and 5% precession. Thus, the estimated sample size was 289; however, 302 (215 cattle and 87 goats) samples of both sexes were collected and examined. Mesenteric, hepatic and pulmonary LNs, liver and lungs of cattle and goats were collected randomly from the slaughterhouses. During collection of samples, species and sex of the animals and date of collection were recorded carefully. To study the effects of seasons, samples were collected in the three major seasons (e.g., summer, rainy and winter) of Bangladesh. To avoid the effect of sex, samples were collected by keeping almost equal ratio of male and female in each season. Similarly, to avoid the effect of seasons during calculating the impact of sex on prevalence of linguatuliasis the samples collected throughout the year were included.

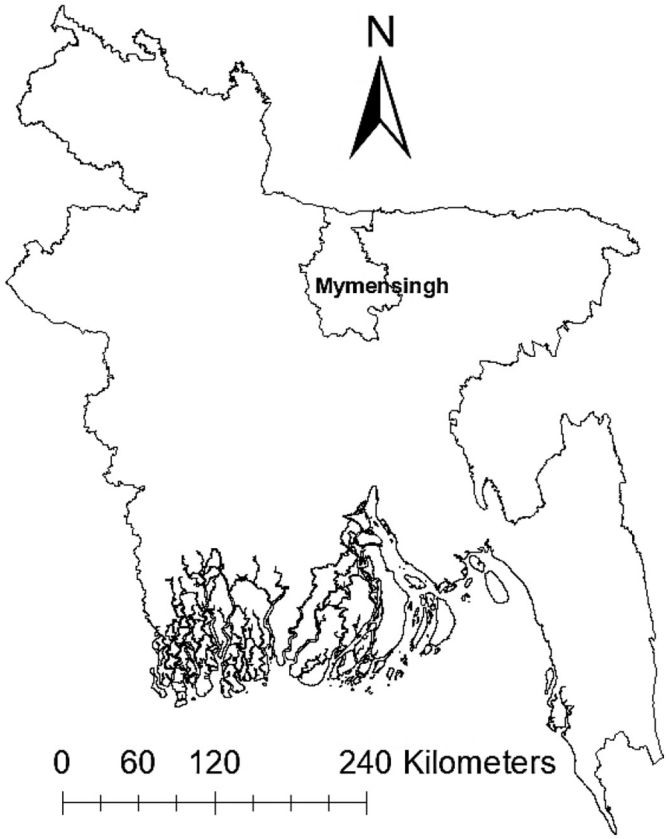

Fig. 1. Study area, Mymensingh, Bangladesh.

2.2. Examination of samples

Immediately after slaughtering, visceral organs (e.g., lungs, liver, and mesentery) of the animals were collected, and examined by close inspection and digital palpation to detect relevant gross pathologies, if any. Suspected LNs were cut into small pieces and placed in a beaker containing phosphate buffer saline (PBS) for two hours to recover the nymphal stage of the parasite. The nymphs were collected with the help of a dropper and preserved in 70% glycerine alcohol. Parasites were identified through morphologic and morphometric analyses preparing permanent slides following the keys and description as given previously (Mohanta and Itagaki, 2017; Soulsby, 1982). Affected tissues were preserved in 4% paraformaldehyde and subjected to histopathological study.

2.3. Histopathological examination

The well fixed tissues were washed in running tap water, trimmed off and embedded in paraffin. Thin sections (5 μm) were cut using a rotary microtome and stained with H&E following the standard protocol as described previously (Luna, 1968). To detect histopathological changes, sections were examined by an investigator blinded to if the LNs were infected or not using 10× and 100× objectives. A total of 5 mesenteric, 5 hepatic and 5 pulmonary LNs were subjected to histopathological examinations and compared with the sections prepared from normal (uninfected) LNs. From each sample, three sections were examined and from each slide four focus areas were evaluated.

2.4. Statistical analysis

Statistical analyses were carried out by Statistical Package for Social Sciences (SPSS) using χ^2 tests or *t*-tests, where appropriate.

3. Results

3.1. L. serrata is fairly common, and cattle are more susceptible

To isolate *L. serrata*, a slaughterhouse based survey was conducted, and a total of 302 visceral samples such as liver, lungs and mesenteric, hepatic and pulmonary LNs from cattle and goats were examined. Of the examined samples, 136 (45.0%) were found infected with *L. serrata*, indicating an overall high prevalence of linguatuliasis (Fig. 2a). Recovered parasites were creamy white, dorso-vetrally flattened, about 5 mm in length with two pairs of rudimentary spines at the anterior part. Cuticle consists of segmentation and each segment contains spines. Anterior part is wide with a gradually tapering posterior part (Fig. 2b&c), conforming exactly to the description given by (Mohanta and Itagaki, 2017; Soulsby, 1982). On an average, 11.1 ± 22.7 (range: 1–90 per LN) parasites per LN were collected (Fig. 2d).