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The Occurrence of Nymphal Stage of *Linguatula serrata* in Water Buffaloes (*Bubalus bubalis*): Nymphal Morphometry and Lymph Node Pathology

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With 5 figures and 1 table

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Summary

The mesenteric lymph nodes (MLN) of buffaloes ($n = 100$) were examined for the presence of parasitic infection. The nymphal stage of *Linguatula serrata* was observed in two buffaloes. A single white-coloured nymph with transversely striated spines on a segmented body, two pairs of oral suckers and hooks was observed in the MLN. The morphometrics of the nymphs were studied. The affected lymph nodes were grossly enlarged with cyst and showed pathological lesions of fibroblastic reaction with a mild underlying inflammatory zone.

Introduction

Water buffaloes are major dairy animals of traditional village farming systems significantly contributing to agricultural economy in Asian and African countries. Water buffaloes in India, which account for 50% of the total buffalo population in the world, produce 150 million tons milk and 10 million tons beef annually and provide draught power for agriculture and transport (FAO, 2001). The buffalo has been preferred to dairy cattle among the Asian countries, because of its adaptability to the hot tropics and its ability to perform optimally under relatively adverse environmental conditions. Despite the importance of buffaloes, there have been relatively few studies on them in relation to parasitic diseases having zoonotic importance.

Linguatula serrata Frohlich, 1779, well known as 'tongue worm', is an aberrant cosmopolitan arthropod parasite, which infests the nasal sinuses and nasopharynx of carnivorous mammals belonging to the families of Canidae, Hyaenidae and Felidae (Khalil, 1970, 1973; Riley, 1986). Eggs of *L. serrata* are infective to a range of mammal intermediate hosts, particularly large grazing herbivores (Sachs et al., 1973). *Linguatula serrata* infection has been reported in most domestic and wild animals including cattle, sheep, goats, pigs, camels, lion, leopard, giraffe and kudu, and rodents (Sachs et al., 1973; Singh et al., 1973; Krishna et al., 1975; Muraleedharan and Zaki, 1975; Young, 1975). Human pentastomosis has also been reported in certain parts of the world, particularly in countries of the Middle East, America, Africa and in South-East Asia including the Indian subcontinent (Roy and Ganguly, 1940; Lazo et al., 1999; Acha and Szyfres, 2003). Barring two reports, each one from Pakistan and Egypt (Choudhary and Dewan, 1967; Khalil, 1976), there was limited information on the occurrence of *L. serrata* in water buffaloes. This short communication reports the occurrence of the nymphal stage of *L. serrata* in water buffaloes, morphometrics of nymphs and pathology of affected lymph nodes.

Materials and Methods

Mesenteric lymph nodes (MLN), collected from water buffaloes ($n = 100$) slaughtered at the Bareilly slaughterhouse (North India), were examined grossly for pathological alterations and for the presence of parasites. The gross lesions and orientation of nymph in the cystic cavity, and nymphal morphometrics such as body size, number of abdominal segments, arrangement pattern of spines, and number of hooks and suckers were recorded. Based on these morphometric characteristics, the parasite was identified as the nymphal stage of *L. serrata* (Sachs et al., 1973; Riley, 1986). Pieces (0.5 cm thickness) of MLN preserved in 10% neutral-buffered formalin were embedded in paraffin blocks; sections of 4–5 μm thickness were cut and stained with haematoxylin and eosin.

Results

A single encysted nymph was observed in the MLN of two buffaloes (Fig. 1). These lymph nodes were grossly enlarged and oedematous. On cut section, a small cyst with haemorrhagic surface filled with viscid fluid was observed in the medullar region. The size of the cysts was 0.6 and 0.4 cm in diameter respectively. The morphometric analyses of the nymphs are given in Table 1. The nymphs were greyish-white in colour measuring 4 and 3.4 mm in length respectively. Nymphs appeared tongue-shaped with an anterior swollen

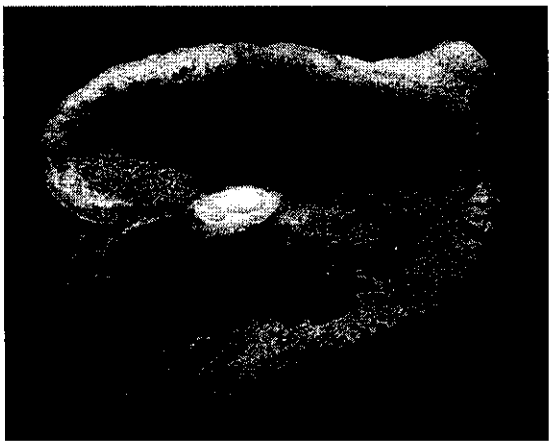

Fig. 1. Mesenteric lymph node: note nymph (arrow head) in the cystic cavity.

Table 1. Results of morphometrics of nymphal stage of *Linguatula serrata*

Morphometric parameter	B15	B21
Total length of nymph (mm)	4	3.4
Total no. of segments	87	85
Average no. of spines in each segment	42	42
Oral suckers	4	4
Oral hooks	4	4

Fig. 2. Anterior part of the nymph: note oral suckers and hooks.

body and posterior narrow end. The anterior part of the nymphs had two pairs of membranous opening-like oral suckers. Two pairs of sickle-shaped hooks were arranged in an arc (Fig. 2). The inner pair of hooks was in closer proximity, whereas the outer pair was distanced. There were no differences in the size and shape of the hooks. Nymphs had segmented body (annulus) with transversely striated spines, an average of 42 per segment, at the posterior edge of each abdominal segment (Fig. 3). The total number of abdominal segments observed in both nymphs was 87 and 85 respectively. The posterior end was not exactly pointed, but rounded with bifurcated spines (Fig. 4). All these features indicated that they

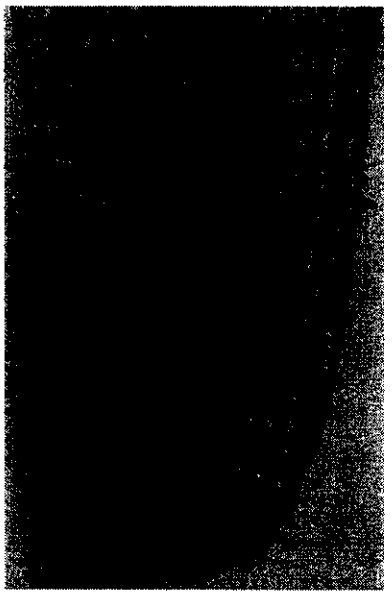

Fig. 3. Posterior part of nymph: note segmented body with downward oriented spines.

Fig. 4. Posterior part of nymph: note bifurcated spines at the posterior end.

were *L. serrata* nymphs (Sachs et al., 1973). Histological sections of the MLN encysted with a nymph revealed a single layer of fibroblastic reaction with a mild underlying inflammatory zone containing mononuclear cells (Fig. 5). There was disruption of cavity lining, a focal area of haemorrhages and congestion of blood vessels in the surroundings of the cystic area. In one case, chronic granulomatous changes were also observed.

Discussion

The present study reports the occurrence of the nymphal stage of *L. serrata* in buffaloes and describes the morphometrics of the nymphal stage and pathology of affected lymph nodes. Occurrence of *L. serrata* was endemic in certain parts of the world with a prevalence of 43% being recorded in Beirut (Khalil and Schacher, 1965), 8% in Cairo (Khalil, 1970) and 25% in Egypt (Khalil, 1973). The prevalence of nymphs in cattle was as high as 72% in certain areas of Britain (Sinclair,

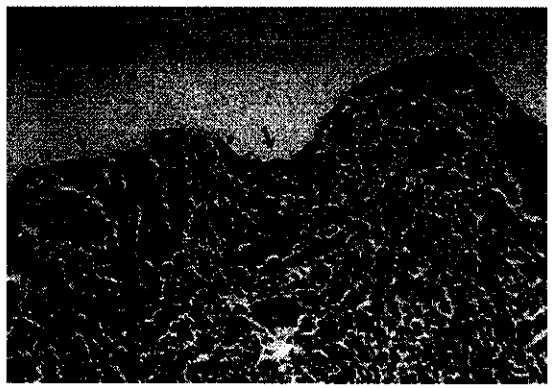

Fig. 5. Mesenteric lymph node: fibroblastic reaction along the cystic cavity with underlying inflammatory zone (H&E 150×).

1954). However, the present study reports only a 2% prevalence on the basis of limited sampling.

Morphometric analyses revealed the similarity between nymphs with respect to body size and shape, segmentation pattern and distribution of spines (Sachs et al., 1973; Lazo et al., 1999). The number of abdominal segments was reported to be in the range of 72–92 (mean 82) in *L. serrata*, 186–232 (mean 210) in *Linguatula multiannulata* and 100–128 (mean 114) in *Neolinguatula muttali*. Body length of larva varied between 3.4 and 4.7 mm (Sachs et al., 1973). The number of annuli (85 and 87) and body size (3.4 and 4 mm) observed in the present study were consistent with the report of Sachs et al. (1973) on *L. serrata* species. The observation of similar number of abdominal segments suggests that a definite number of segments were acquired by the infective nymphal stage, although the number of segments is variable and is probably not consistent until after the last moult (Self, 1969; Riley et al., 1985). Besides, the observation of more or less equal number of segments in both the nymphs in the present study suggests that they were in similar stage of development.

The histological changes observed in the MLN were in accordance with the findings of previous workers (Self, 1972; Singh et al., 1973; Krishna et al., 1975). The presence of a histological lesion surrounding the cystic cavity suggested that infestation with nymphs do not affect MLN extensively; rather, they encysted within a cavity. The observation of only a mild inflammatory reaction in tissues – a rather typical inflammation – suggested that there was an extraordinary degree of compatibility between long established nymph and host tissue as reported by Self and Kuntz (1967). However, the observation of granulomatous lesion in MLN infected with *L. serrata* suggested the concurrent occurrence of pentastomosis and other chronic infections (Lapage, 1965; Choudhary and Dewan, 1967; Muraleedharan and Zaki, 1975). Aberrant parasites preferably invade the tissues already infected by other pathogens because of the failure of protective immune responses, although these tissues are rich in immune cell population. Such an interaction between the parasite and other pathogens, however, needs to be studied in detail.

Larval or nymphal infection in herbivores is thought to be clinically asymptomatic but they are considered to be the main source of infection to definite hosts and humans. Observation of nymphal stages of tongue worm in the MLN tissues of water buffaloes is of more concern in a country like India, where people consume more buffalo meat because of religious concerns and ban on cow slaughter in the northern states of the country. By-products (offal) such as kidney, brain, liver, intestine, heart and tongue are more commonly consumed by people of the economically weaker sections especially in rural and semi-urban areas of the developing countries (FAO, 1978). Ingested nymphs of *L. serrata* lead to a disease called 'Halzoun or Marrara syndrome' in humans and carnivores, which is characterized by inflammation of the upper respiratory tract, swelling of submaxillary and cervical lymph node and abscesses in the eye and ear canal (Khalil, 1976; Self, 1982). Moreover, consumption of nymphal or larval cysts may also lead to pentastomosis infection in humans.

In the present study, determination of the precise place of origin of the buffaloes and the principal source of infection to them was difficult as all these animals were brought from remote villages. However, the role of dogs in spreading the infection could be suspected because the infection rate in dogs was found to be 38% in India and 43% in Lebanon and Mexico (Acha and Szyfres, 2003). In addition, the close proximity of dogs and domestic animals in most household livestock units may favour the contamination of feed and water intended for livestock with eggs of *L. serrata* through excreta of dogs. Ingestion of raw viscera of sheep, goats and other herbivores resulted in the high incidence of pentastomosis in dogs (Acha and Szyfres, 2003). Thus, carnivores and herbivores share the infection mutually (Tavasouli et al., 2001). However, the role of buffaloes in the transmission of the infection needs to be evaluated for better epidemiological data on pentastomosis.

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