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A case of pulmonary linguatulosis in a rabbit

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Abstract

Linguatula serrata can infect most ruminants and cause accidental infections in humans. It is a causative parasite of linguatulosis, a disease that not only produces economic losses in cattle but also represents a public health risk due to its zoonotic nature. This study aimed to explore the clinical and pathological findings of pulmonary linguatulosis in a rabbit. The most striking clinical findings in the deceased rabbits were wheezing and labored breathing. Grossly, the most prominent morphological changes in the lungs were well-circumscribed, flat or slightly raised, solitary grayish-white nodular lesions, and consolidated areas. The characteristically tongue-shaped developmental forms of parasites were observed on the cut surface of the lung. Histopathologically, the most noticeable morphological changes in the lung parenchyma were diffuse thickening of the inter-alveolar septum, fibrinoid necrotic vasculitis, medial smooth muscle cells hypertrophy of the arteries, alveolar emphysema, longitudinal and transverse sections of *L. serrata* nymphs and extra-medullary hematopoietic foci (megakaryocytes). The morphological appearance of the nymphs showed multiple transverse grooves, saw-like cuticles, peri-buccal hooks and acidophilic glands. In conclusion, these findings reveal the etiopathological diagnosis of linguatulosis and suggest that the lungs might be a target organ in addition to the liver and lymph nodes.

Introduction

Linguatula serrata is a pentastomid, cosmopolitan and zoonotic parasite belonging to the *Pentastomida phylum*.[1] In end hosts (dogs, wolves, foxes and cats) adult forms inhabit the nasal airways, frontal sinuses and tympanic cavities, causing irritation, bleeding and inflammation in these areas. Thus, cough, sneezing and purulent rhinitis occur clinically (nasopharyngeal linguatulosis or Halzoun syndrome).[2] Eggs are excreted from the final hosts in coughs and nasal discharges, and the eggs being swallowed are also excreted in the feces. Eggs ingested orally by herbivorous intermediate hosts (herbivores) such as rabbits, sheep, goats, horses and cattle, as well as rodents and humans, hatch in the intestines. The larvae travel through the bloodstream to internal organs such as mesenteric lymph nodes, lung, and liver, and less frequently to other organs such as brain, intestine and prostate, where they undergo several changes until they develop into the infective third-stage larvae (nymphs). Thus, the visceral linguatulosis form of the parasite occurs in intermediate hosts.[2,3] In the visceral form, infective nymphs with the size of 3.00 - 5.00 mm are found in small cystic structures surrounded by inflammatory fibrous tissue. When tissues and organs infected with nymphs are eaten by the final hosts, the infective larvae migrate to the nasal cavities, where they mature; thus, completing the parasite's biological cycle.[4] Humans develop naso-pharyngeal linguatulosis or Halzoun syndrome when they ingest stage parasite larvae; however, asymptomatic visceral linguatulosis occurs when they ingest eggs.[1]

It has been reported that *L. serrata* infestations occur mostly in mesenteric lymph nodes of ruminants (sheep, goats, cattle and buffaloes)[5,6] and dogs, lung of cats[7] and eye[8] and nasopharyngeal region of humans.[9] However, to our knowledge, there are no reports of *L. serrata* infestations in rabbits. In addition, there is no comprehensive information about the lung lesions of the parasite in the studies reported so far. This study presents clinical and pathological findings regarding a case of pulmonary linguatulosis in a rabbit with severe lung lesions.

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Case Description

The material for this study was an 18-month-old male rabbit brought by a hobby rabbit farmer. The farmer reported that his rabbits died at different times within 20 days. The most striking clinical findings in the deceased rabbits were wheezing and labored breathing. In the Department of Pathology, Faculty of Veterinary Medicine, Van Yüzüncü Yil University, Van, Türkiye, a necropsy of one of the dead rabbits was performed, the morphological changes were recorded, and the tissue samples were fixed in 10.00% neutral buffered formalin. Sections obtained at the end of routine tissue follow-up were stained with Hematoxylin and Eosin and examined under a light microscope (80i DS-RI2; Nikon, Tokyo, Japan).

Macroscopical findings. At necropsy, it was noted that the most prominent morphological changes were occurred especially in the lungs, and to a lesser extent, in the liver, kidneys, stomach and intestines. The lungs were hyperemic, slightly swollen, partially hard, consistently elastic and poorly collapsed. Dark or light-colored consolidated areas were seen, being more prominent especially in the caudal lobes. In addition, well-circumscribed, flat or slightly raised, solitary grayish-white nodules, usually 2.00 - 3.00 mm in diameter, were detected (Fig. 1A). On the cross-sectional surface of the lung, whitish-colored developmental forms of the parasite were seen, some of which were characteristically tongue-shaped. Diffuse emphysematous alveoli were also observed as caverns or cystic cavities (Fig. 1B). The liver showed mildly swollen and pale areas with focal disseminated small collapsed foci (Fig. 1C). Mesenterial, portal and bronchial-mediastinal lymph nodes showed hyperemia and edema, and multifocal petechial areas and erosive-ulcerative lesions were found in the stomach (Fig. 1D) and small intestine.

Histopathological findings. The most striking morphological changes in the lung parenchyma were diffuse thickening of the inter-alveolar septum, fibrinoid necrotic vasculitis, medial smooth muscle cells hypertrophy of the arteries, alveolar emphysema and nymphal causative agents (Fig. 2A). The numerous nymphs observed in the lung parenchyma were localized in thin-walled cystic alveolar cavities. The lung parenchyma surrounding these infective larvae (nymphs) was found to be compressive atelectatic, but there were no obvious fibrotic capsule and macrophage proliferations or giant cells; only a cellular reaction consisting of eosinophilic-lymphoid cells and hyperplasic alveolar epithelium were seen (Fig. 2A). Inter-alveolar septa were diffusely thickened (diffuse interstitial pneumonia) with marked capillary hyperemia, edema and inflammatory cells infiltration (lymphoid cells, macrophages and eosinophils; Figs. 2A to 2C). Irregularly shaped, hyper-chromatic, multi-nucleated cells (mega-karyocytes) were observed in the lumen or adherent to the wall of capillaries, arterioles and arteries, being thought to be extra-medullary hematopoietic foci (Fig. 2B). Hemosiderin pigment deposition in the inter-alveolar septum and hemorrhage were also seen. Vasculitis was characterized by the accumulation of eosinophils, erythrocytes and inflammatory cells in the vessel lumen, and the presence of hyalinized-fibrinoid necrotic changes in the vessel walls (Figs. 3A and B). There was also marked thickening of the vessel walls due to the smooth muscle cells hypertrophy in medium and large arteries (Fig. 3A). Eosinophilic-hyalinized fibrin deposits were detected in the lumen of alveoli, capillaries and arterioles (Fig. 3B). In liver, hyperemia and accumulation of inflammatory cells including a small number of eosinophils in sinusoids, venules, portal veins and arteries and portal spaces (Fig. 3C) were detected. Moreover, bile ducts hyperplasia, hemorrhage in the parenchyma, and rarely fibrinoid necrotic changes in the vessel walls were observed (Fig. 3C).

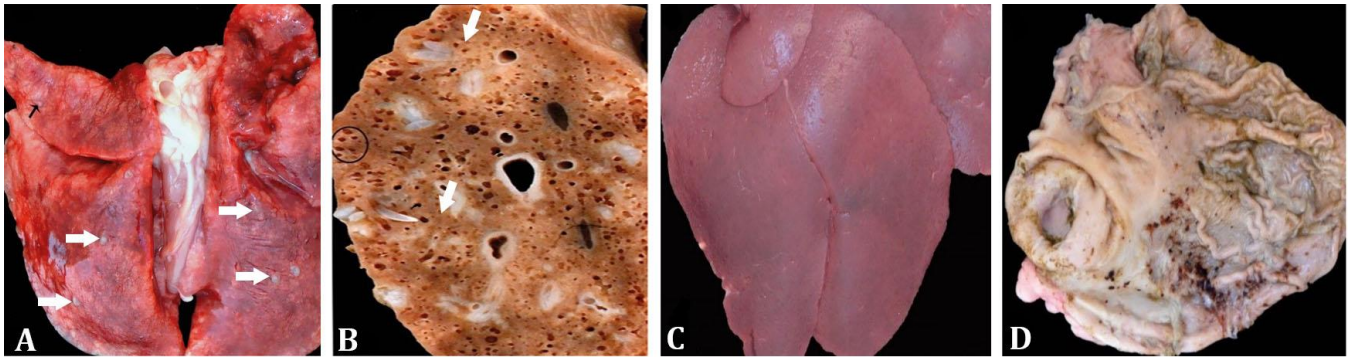

Fig. 1. Gross appearances of the lung, liver and stomach in an 18-month-old male rabbit with pulmonary linguatulosis. **A)** The lung is slightly swollen and poorly collapsed; there are well-circumscribed, flat or slightly raised, solitary grayish-whitish nodules usually 2.00 – 3.00 mm in diameter (arrows); **B)** The cross-sectional surface of the lung showing whitish-colored developmental forms of the parasite (arrows); some of which were characteristically tongue-shaped. Emphysematous alveoli as caverns or cystic cavities (circular shape) were also obvious; **C)** The liver is mildly swollen and there are pale areas with focal disseminated small collapsed foci; **D)** Multi-focal petechies in the stomach. Note: The cross-sectional surface of the lung was photographed after fixation of the tissue in formalin solution.

Tubular necrosis, vasculitis, hyperemia and fibrin plugs were observed in the kidneys, especially in the glomerular capillaries (Fig. 3D). Follicular necrosis, interstitial edema and dense accumulation of inflammatory cells, including eosinophils in and around the vessels, were found in the lymph nodes. The morphological appearance of the nymphs observed in tissue sections generally showed multiple transverse grooves, saw-like cuticles, peribuccal hooks and acidophilic glands (Figs. 2A and 2C).

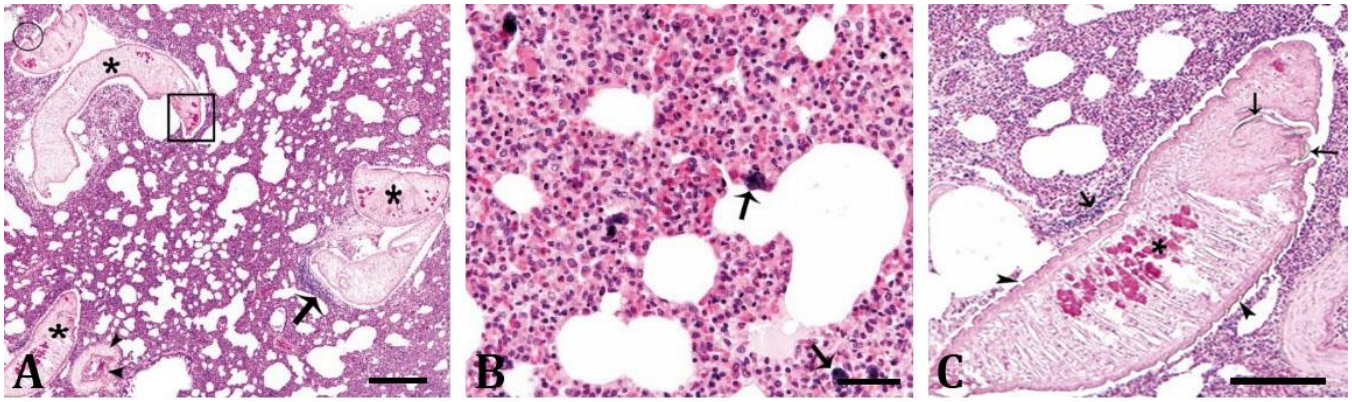

Fig. 2. Histopathological findings in the lung tissue sections of an 18-month-old male rabbit with pulmonary linguatulosis (Hematoxylin and Eosin staining). **A)** The lung parenchyma showing diffuse thickening of the inter-alveolar septum, alveolar emphysema and numerous nymphs (*) being localized in thin-walled cystic alveolar cavities. The anterior portion of the nymph shows acidophilic glands (square shape); (bar = 500 µm); **B)** Inter-alveolar septa showing diffusely thickened (diffuse interstitial pneumonia) with marked capillary hyperemia, edema and inflammatory cells infiltration (lymphoid cells, macrophages and eosinophils). Irregularly shaped, hyperchromatic, multi-nucleated cells (megakaryocytes, extra-medullary hematopoietic foci; arrows) can be seen (bar = 50 µm); **C)** Close-up view of the nymph in Fig. 2A showing multiple transverse grooves and saw-like cuticular spines (arrowheads), peri-buccal hooks (arrows) and acidophilic glands as indicated by * (bar = 250 µm). Thick arrows indicate inflammatory cells (lymphoid cells) infiltration around the nymphs in Fig. 2A and 2C.

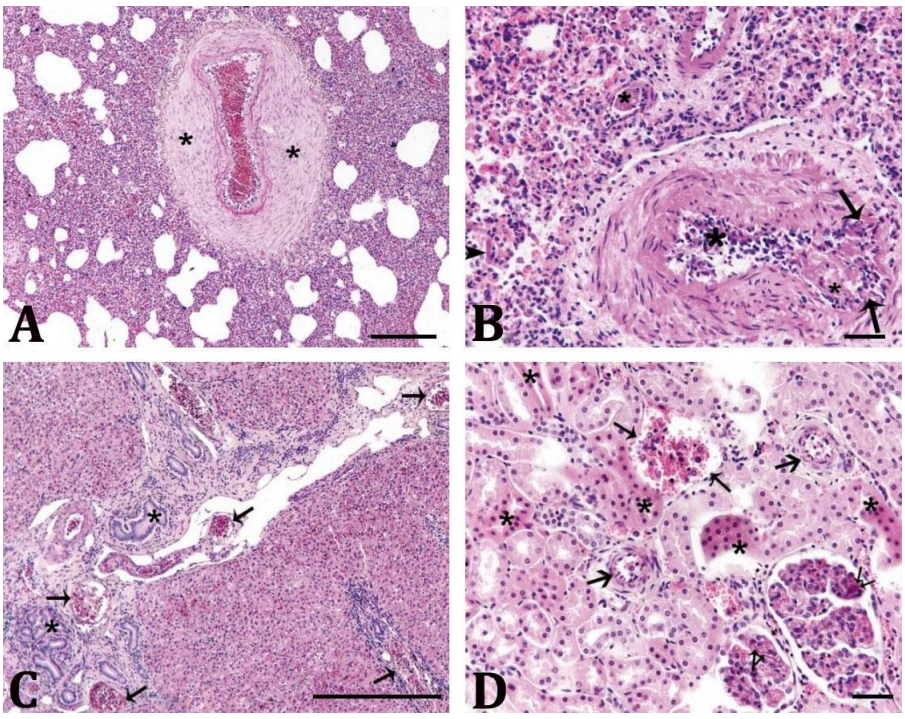

Fig. 3. Histopathological findings in the lung, liver and kidney tissues sections of an 18-month-old male rabbit with pulmonary linguatulosis (Hematoxylin and Eosin staining). **A)** The arterial vessel wall showing the marked thickening as a result of smooth muscle cells hypertrophy as indicated by * (bar = 250 µm); **B)** The vessel showing fibrinoid necrotic vasculitis characterized by eosinophilic-hyalinized fibrin deposits (*), necrosis and inflammatory cells accumulation (arrows; bar = 50 µm); **C)** The portal areas showing hyperemia and vasculitis (arrows) in portal veins and arteries, proliferation in the bile ducts and hyperplasia in the bile ducts cells (*) and inflammatory cells accumulation (bar = 500 µm); **D)** The kidneys showing tubular necrosis (*), hyperemia and vasculitis (arrows) and fibrin plugs (double arrows) in the glomerular capillaries (bar = 50.00 µm).

Discussion

Nasopharyngeal and visceral forms of linguatulosis have been reported in humans in several countries; the ocular form has also been reported very rarely. The nasopharyngeal form (Halzoun's syndrome is caused by the ingestion of raw or under-cooked liver or lymph nodes of intermediate ruminants infected with nymphs or 3rd instar larvae by humans. The infestation's visceral form is caused by ingesting of water and food contaminated with parasite eggs in feces and pulmonary excretions of infected carnivores.[1,9]

It has been emphasized that visceral linguatulosis is diagnosed by observing appropriate macroscopical lesions and nymphs in the target organs. Liver and lymph nodes (mesenteric, portal and mediastinal) have been reported as target organs of the parasite.[10,11] However, in the present study, the case was named pulmonary linguatulosis because the lesions detected in the liver and lymph nodes were milder and no causative agents were observed; whereas, the characteristic macroscopical-microscopical lesions in the lungs were much more severe and many causative agents were found.

Reportedly, morphological changes such as macroscopically sub-capsular nymphs, hemorrhage, necrosis, fibrosis, microscopically nymphs being surrounded by a thin fibrous band and a wall of lymphocytes; granulomatous reaction consisting of macrophages, lymphocytes and giant cells around degenerated-necrotic parasites and hemosiderin pigment accumulation in macrophages were recorded in the liver of ruminants.[12-14] However, no study on rabbits has been found. In our case, although several sections of the liver, related lymph nodes and kidneys were examined, nymphs were not seen, but findings similar to the lesions mentioned above were found. Unlike the above-noted lesions, vasculitis was the most striking lesion in the liver, kidneys and lymph nodes. These morphological changes observed in the lymph nodes, liver and kidneys are thought to be caused by the passage of toxic metabolites being produced during the widespread development and migration of the parasite in the lungs into the bloodstream. Morphologically, nymphs are characterized by the presence of a saw-like cuticular spine with multiple transverse grooves, two pairs of peri-buccal (oral) hooks, a ventral buccal apparatus with acidophilic glands, a complete digestive tract and primordial genitalia; in degraded nymphs, the hooks and remnants of the cuticle are the only diagnostic findings of the parasite.[1,15] To identify nymphs in tissue sections, the detection of typical acidophilic glands in the anterior portion of the parasite was considered to be characteristic and pathognomonic. The etiopathological diagnosis is made by the presence of at least one characteristic structure in pentastomids or nymphs with macroscopically and microscopically compatible lesions regarding *L. serrata*. In the present case, it was noteworthy that the morphological appearance of the nymphs observed in the lung sections had similarity with appearances reported previously.[15]

It was reported that histopathological lesions in bovine livers infected with *L. serrata* nymphs were characterized by two types of inflammatory reactions; one of them was infiltration of eosinophils, lymphocytes, plasma cells and few macrophages and the other was granulomatous reaction with cellular infiltration of fibrous capsule, giant cells, lymphocytes, eosinophils and macrophages around degenerated nymphal structures.[16] In our case, no granulomatous reaction being characterized by a fibrous capsule, macrophage proliferation and giant cell reactions against infective larvae (nymphs) in the lung parenchyma was observed, being thought to be related to the fact that the nymphs in the lung parenchyma were not degenerated.

To the author's knowledge, there is no comprehensive information about the lung lesions related to the parasite. Only in one cat necropsy, it was reported that whitish nodules about 1 mm in diameter were incidentally observed in the diaphragmatic lobe of the lungs and sub-plural nymph surrounded by mononuclear cells infiltration, compressive atelectasis in the adjacent alveoli and acute diffuse interstitial pneumonia were also reported histopathologically. In our case, the findings were similar to those of Esmaeilzadeh *et al.* in the lungs.[7] Also, diffuse and severe fibrinoid necrotic vasculitis was observed in the lungs, being milder in the stomach, kidney, liver and lymph nodes. This finding was not found in the literature reviewed. In the lungs, marked thickening of the vessel walls and narrowing of the lumens were observed due to the medial smooth muscle cells hypertrophy, especially in the large arteries. These morphological changes in the arteries are thought to be related to the circulatory difficulties caused by interstitial pneumonia, numerous nymphs in the alveoli, emphysema and vasculitis in the pulmonary vessels.[10]

One of the most striking microscopical findings in the lungs of our case was the presence of megakaryocytes in the lumen of alveoli and pulmonary circulation, being thought to be extra-medullary hematopoietic foci. It has been recorded that extra-medullary hematopoiesis is a result of cytokine-induced increased cell production and depends on pluripotent hematopoietic stem cells availability.[2] It has been stated that during Schistosoma mansoni infection, involvement of various hematopoietic growth factors causes eosinophilic, neutrophilic, megakaryocytic and erythroid extra-medullary foci formation in the liver, lymph nodes and omental and mesenteric milky spots. It has been suggested that extra-medullary hematopoietic foci are locally regulated, and amplify and/or compensate the systemic hematopoietic response during the infection.[17] The extra-medullary

hematopoietic foci being seen only in the lungs in our case are thought to be formed to compensate for the erythrocyte and leukocyte losses occurring due to the acute diffuse interstitial pneumonia and hemorrhagic gastroenteritis.

In conclusion, the fact that *L. serrata* infestation caused severe pneumonic lesions in the lungs in the presented case and large numbers of causative agents were detected macroscopically and microscopically in the lungs suggests that the lungs may be a target organ in addition to the liver and lymph nodes (mesenteric, portal and mediastinal), being reported as target organs.[10,11] It has also been understood that the agent can cause death through causing acute diffuse interstitial pneumonia being characterized by diffuse hyperemia, edema, hemorrhage, alveolar emphysema, fibrinoid necrotic vasculitis in the vessels and medial smooth muscle cells hypertrophy of the arteries in the lung parenchyma. In this study, the pathological findings of *L. serrata* infestation in a rabbit herd, which primarily affected the lungs, were evaluated comprehensively for the first time.

Conflict of interest

The author declare that he has no conflict of interest.

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