J. Vet. Sci. (2002), 3(3), 163-166

JOURNAL OF
Veterinary
Science

Mycobacterium bovis Infection in a Farmed Elk in Korea

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Received June 3, 2002 / Accepted August 2, 2002

Abstract

A case of tuberculosis is reported in an eight-year-old, male, elk (*Cervus elaphus nelsoni*). The elk showed severe coughing, respiratory distress, abdominal breathing, anorexia, and severe progressive emaciation in the elk farm. At necropsy, the elk appeared in poor body condition. Mild enlargement of retropharyngeal and submandibular lymph node was observed in the head. Diffuse fibrinous pleuritis and purple red lobar pneumonia were found in the thorax. Well demarcated numerous dark yellow discrete or confluent nodules from 0.3 to 2 cm in diameter were scattered in the whole lung. Bronchial and mediastinal lymph nodes were also enlarged. Histopathologically, lungs had typical classical tuberculous granulomas, multiple abscesses, and numerous macrophages and Langhans giant cells infiltration in alveolar lumen. In the lymph nodes, there were small clusters of necrosis and infiltration of numerous macrophages, epithelioid cells, and Langhans giant cells. With the acid-fast staining, numerous mycobacteria were revealed in the lung and lymph nodes. According to this study, there are differences of the histopathologic lesions and the numbers of acid-fast bacilli in the lesions between this elk and cattle. *Mycobacterium bovis* was confirmed as a causative agent in this elk using bacterial isolation, biochemical characteristics, and PCR technique. The isolate was negative for niacin test, nitrate reductase, and pyrazinamidase. This is a first report for bovine tuberculosis of farmed elk in Asia.

Key words: Elk (*Cervus elaphus nelsoni*), *Mycobacterium bovis*, tuberculosis, Langhans giant cell

Introduction

Tuberculosis (TB) caused by *Mycobacterium* spp. is a chronic progressive disease and have been documented in a wide variety of mammalian species including cattle, man, and bird. *M. bovis* infections have been reported mainly in bovidae, cervidae, and occasionally carnivores [14]. Tuberculosis caused by *M. bovis* in human is well known, and it was a common cause of TB transmitted by infected dairy products. As a result of the pasteurization of milk and TB eradication programs in many countries, zoonotic transmission of *M. bovis* through domestic animals have been rapidly decreased.

In deer, TB is most commonly caused by *M. bovis* [5]. The disease has been found sporadically in wild deer population [13] and more frequently occurred in farmed deer herds. Species that has been known to infect with *M. bovis* include white tailed deer, axis deer, fallow deer, sika deer, red deer, and elk [5, 9]. Infected wild deer are a possible source of contamination for domestic livestock [18]. Captive deer in zoos and parks also act as a significant reservoir of TB [19]. From 1970's, intensive farm management practices for deer have been common in worldwide. In recent years tuberculosis in deer caused by *M. bovis* has become a disease of economic and public health importance to the deer farming industries of several countries, especially in Canada, Denmark, New Zealand, and the United Kingdom [5, 9].

In Asia, there were few cases of TB reported; axis deer in India [1], farmed sika deer in China [17], and captive sika deer in Japan [10]. Although the occurrences of TB gradually decreased, TB of dairy cattle has not been eradicated in Korea. Herein, we described the first case of bovine tuberculosis in a farmed elk (*Cervus elaphus nelsoni*) in Korea.

Materials and Methods

Case histories

An eight-year-old male elk kept in an elk farm has been suffered from chronic coughing, respiratory distress, abdominal breathing, anorexia, and anorexia for 2 months duration. The elk had been treated with antibiotic therapy but was unresponsive to it. Due to a poor prognosis, the elk was admitted to the Pathology Division, National Veterinary Research and Quarantine Service for a diagnosis after euthanasia. Due to recent confirmation of chronic wasting disease (CWD) in Korea, the elk was also tested for CWD.

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Necropsy and histopathology

Complete postmortem examination was performed on the elk. Tissue samples from the lung, heart, liver, spleen, kidney, lymph nodes (submandibular, retropharyngeal, bronchial, mediastinal, and mesenteric), stomach, intestine, brain, and spinal cord were fixed in 10% phosphate-buffered formalin, routinely processed, and stained with hematoxylin and eosin (HE) for light microscopic examination. Replicate sections of the lung and lymph nodes were also stained with Gram's stain, Periodic Acid-Schiff (PAS), and Ziehl-Neelsen (ZN) acid-fast stain to detect the causative agents. Histopathologic examination and immunohistochemistry (IHC) for CWD were carried out as previously described to rule out the possibility of prion infection [15].

Bacterial culture

Portions of the lung were aseptically removed and used for mycobacterial isolation. A pool of tissue (10g) was homogenized in 5ml sterile saline using tissue grinder. Equal volume of 2% NaOH was added to the tissue homogenate and was left for 10 min at room temperature. The sample was diluted ten times with phosphate buffered saline (pH 7.3), and then centrifuged at 6,000 X g for 30min; this process was repeated twice with sterile distilled water and the final pellet was suspended in saline before it was inoculated on to the solid culture media. The isolation media was BACTEC 12B medium that includes whole egg and 2% malachite green. The culture media were incubated at 37℃ for 10 weeks and examined every 2 weeks for mycobacterial growth. The organisms were confirmed as *M. bovis* by their cultural characteristics [11] and by Ziehl-Neelsen (ZN) staining. The isolate was negative for niacin test, nitrate reductase, and pyrazinamidase.

PCR methods

The mycobacterial DNA was extracted from the colony grown on solid agar using guanidinium thiocyanate (GuSCN) with silica particles and multiplex PCR were performed as previously described [3, 20]. The following primers were used to differentiate *Mycobacterium* species: spacer region 33 specific primer 5′-ACA CCG ACA TGA CGG CGG-3′ and spacer region 34 specific primer 5′-CGA CGG TGT GGG CGA GG-3′; IS6110, *Mycobacterium tuberculosis* complex (MTC) specific primers, TB284 5′-GGA CAA CGC CGA ATT GCG-3′ and TB850 5′-TAG GCG TCG GTG ACA AAG GCC AC-3′; and *Mycobacterium* genus specific (65kDa antigen gene) primers, TB11 5′-ACC AAC GAT GGT GTG TCC AT-3′ and TB12 5′-CTT GTC GAA CCG CAT ACC CT-3′. PCR were performed with PreMix (Bioneer, Korea) containing 1 ul of each primer at 15 pmol/ul, and 5 ul of extracted DNA. The PCR conditions were 95℃ for 5 min, 30 cycles of 95℃ for 30 s, 60℃ for 45 s, 72℃ for 30 sec; and 72℃ for 7 min. After PCR, the products were run on a 2% agarose for 1 hour. Standard *M. bovis* AN5, *M. bovis* BCG, and *M. paratuberculosis* (ATCC 19698) strain were used in this experiment.

Results

Gross pathology

At necropsy, mild to moderate enlargement of retropharyngeal and submandibular lymph node (LN) was observed. There was diffuse fibrinous adhesion between the pleural surface of the lung and the thoracic wall. Extensive purple red consolidations were noted in the apical and cardiac lung lobes. Well demarcated numerous dark yellow discrete or confluent nodules from 0.3 to 2 cm in diameter were scattered throughout the lung lobes (Fig. 1). Bronchial and mediastinal lymph nodes were also enlarged to 2 to 3 times of their normal sizes.

Histopathology

Most of the lung sections had classical tuberculous granulomas and multiple abscesses. However, the tendency in this elk to the formation of abscesses rather than the development of granulomas is apparent in the lung. Numerous thin-walled abscesses, which composed with fibrin, degenerated leukocytes, and mononuclear cells, were distributed in alveolar wall and bronchioles. Most granulomas consisted of central caseous necrosis with central and/or peripheral mineralization surrounded by moderate mantle of mixed population of inflammatory cells and thin connective tissue (Fig. 2). Inflammatory cell consisted of large numbers of neutrophils, moderate lymphocytes, plasma cells, macrophages, epithelioid cells and a few Langhans giant cells. Interlobular septa and pleura were thickened with proliferation of connective tissue and moderated infiltration of inflammatory cells. Sometimes calcification foci were scattered in bronchiole lumen, interlobular septa or alveolar lumen. With the acid-fast staining, numerous mycobacteria were revealed in the caseous foci of granulomas, abscess and inflammatory cells (Fig. 3).

In the lymph nodes, most lesions consisted of small clusters of necrosis and massive infiltration of inflammatory cells. Inflammatory cells composed with diffuse accumulation of macrophages, epithelioid cells, and Langhans giant cells (Fig. 4). Histopathologic lesions were found in bronchial, mediastinal, and retropharyngeal lymph nodes.

Myriads of acid-fast positive bacilli were noted in the cytoplasm of infiltrated macrophages and giant cells. They were 3-5-um-long rods and were occasionally grouped in parallel sheaves. Gram and PAS method failed to demonstrate the organisms. No evidence of CWD was evident on histopathology and immunohistochemical staining for PrP^{Sc}.

PCR

Mycobacterium genus specific 65-kDa antigen gene was amplified from *M. bovis*, *M. bovis* BCG, *M. paratuberculosis* and elk isolate. *Mycobacterium tuberculosis* complex-specific IS6110 was amplified from *M. bovis*, *M bovis* BCG, and elk isolate. However, spacer region 33, produced 99-bp band, was amplified from *M. bovis* and elk isolate (Fig. 5). And *M. bovis* BCG produced two bands of 172 and 99-bp corresponding products both of the spacer region 33 in conjunction with spacer region 34.

Fig. 1. The lungs showing well demarcated yellowish white nodules in the cut surface.

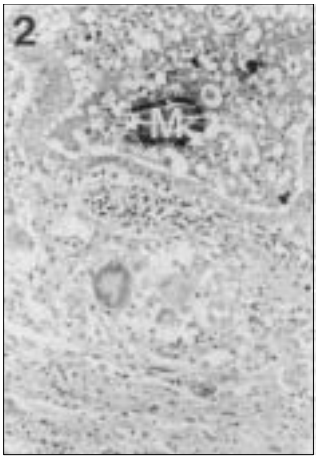

Fig. 2. Lung; Note the typical granuloma with mineralization (M) and surrounded inflammatory cells. HE. ×100.

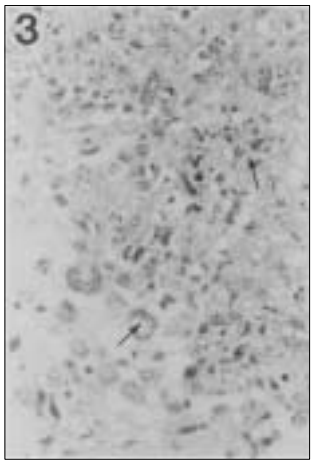

Fig. 3. Lung; Acid-fast bacilli (arrow) were presented in the abscess and inflammatory cells. Ziehl-Neelsen. ×400.

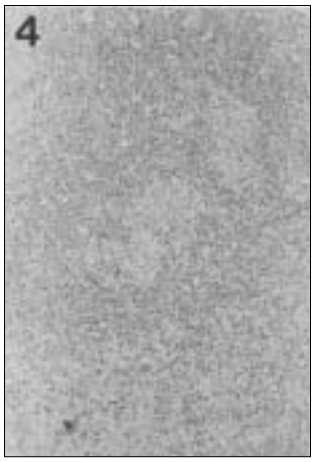

Fig. 4. Lymph node; Lots of macrphages and Langhans giant cells were accumulated in the subcapsular sinus of retropharyngeal lymph node. HE. ×100.

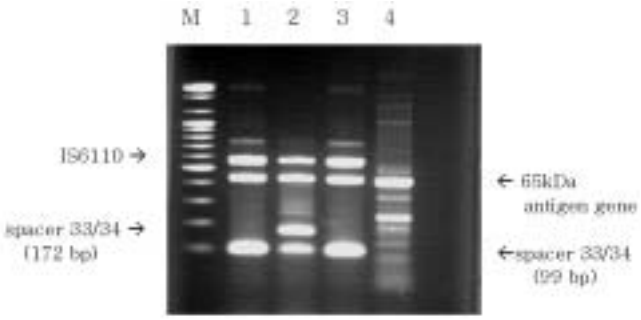

Fig. 5. Agarose gel electrophoresis of products of multiplex PCR assay using the IS6110, 65-kDa antigen gene, and spacer 33/34 specific primers. M = 100-bp size marker; lane 1 = *M. bovis* AN5; lane 2 = *M. bovis* BCG; lane 3 = Elk isolate; lane 4 = *M. paratuberculosis*

Discussion

Based on the results of gross pathology, histopathology, bacterial culture, and PCR, the elk was diagnosed to be infected by *M. bovis*. Lymphadenopathy is the most prominent gross lesion seen in elk infected with *M. bovis* [8]. In feral deer, lesions are found most commonly in the thorax (75%), or as generalized tuberculosis (13%) [6]. This may indicate the mode of mycobacterial transmission, which can occur either through respiratory route or by oral ingestion. Retorpharyngeal lymph node is the most commonly affected site in cervidae, while abscesses or pleuritis may also be found in the thorax or the mesenteric lymph node [2]. In this study, the lesions were distributed in the lung, pleura, retropharyngeal, bronchial, and mediastinal lymph nodes but not submandibular and mesenteric lymph nodes. Parenchymal organs in the abdominal cavity were not involved, suggesting that mycobacteria may be transmitted through the respiratory route in this elk.

The histopathologic lesions of this elk were compatible with previous studies [4, 16]. Histologically, the pulmonary lesions were classified into three types: classical tuberculous granulomas, multifocal abscesses, and macrophages and giant

cell rich alveolar lumen. More abundant neutrophils and fewer giant cells were observed in granulomas and abscesses. There were numerous macrophages and giant cells presented in alveolar lumen located peripheral area of granulomas or abscesses. Mineral deposit foci in the lesions frequently observed in interlobular septa and alveolar lumen beyond granulomas, whereas in bovine lesions they usually did not. In addition, mineralization had a tendency to locate in peripheral area than in central area of caseous necrosis.

Multifocal coagulative necrosis and infiltration of macrophages and giant cells were presented in the lymph nodes. Characteristic liquefaction, calcification, and laminated, caseous abscess were not observed in this case. The numbers of acid-fast bacteria were extremely abundant in lung and lymph nodes than in cattle. These findings are consistent with previous reports [4, 16].

M. bovis transmission and susceptibility of individual animals to the organism are influenced by host genotype and behavioral and environmental factors that influence immunocompetence at a phenotypic level [12]. Environmental factors include stressors associated with climate, nutrition, stocking density, and sexual behavior. However, deer in farm conditions do appear to be more susceptible to *M. bovis* than cattle, thus increasing the risks of spreading *M. bovis* to other deer and other species, including man [5]. There had been the evidence of spread to man from the *M. bovis* infected farmed deer. In Canada, 20.6% human were tuberculin skin test positive among 394 persons contacted domesticated elk herds. One case of active *M. bovis* infection was diagnosed by sputum culture [7].

Bovine tuberculosis in cattle has been reported since 1940 in Korea. From 1940, control measure for tuberculosis has been focused mainly on cattle. The number of deer farm has been increasing under intensive management systems in Korea. We successfully isolated *M. bovis* from the lungs of the elk. The results of this study strongly suggest that the transmission of tuberculosis is possible to domestic animals and man from farmed wild animals. Although uncommon, human tuberculosis caused by *M. bovis* still possible in Korea. Adequate surveillance program and control scheme should be performed skillfully on tuberculosis not only stock farms of deer but animals kept in zoological gardens. To our best knowledge, this study is the first report of *M. bovis* infection in farmed elk in Asia.

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