Short Communication

Disseminated Tuberculosis in Captive Malayan Tapir (*Tapirus indicus*)

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Abstract

Tuberculosis caused by *Mycobacterium tuberculosis* is an important zoonotic disease that concerns the survival of endangered species and public health aspects. A 17-year-old, female Malayan tapir from a zoo in Thailand died with a sign of panting after anesthesia for the treatment of her oral problems. Grossly, multiple caseous white nodules were scattered throughout the parenchyma and bulged from the capsular surface in affected organs such as lung, lymph node and liver. Histologic examination revealed multifocal to coalescing granulomas characterized by caseous necrotic center, loosely organized inflammatory zone with low numbers of Langhans's giant cells and thin fibrotic zone. A few acid fast bacilli were found within the multinucleated giant cells at the periphery of the granulomas. *Mycobacterium tuberculosis* was identified from affected organs using specific Rv1970 PCR primer. The aim of this study is to report a rare case of disseminated tuberculosis caused by *M. tuberculosis* in a captive Malayan tapir.

Keywords: Disseminated tuberculosis, Malayan tapir, *Mycobacterium tuberculosis*, PCR

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บทคัดย่อ

รายงานสัตว์ป่วย: วัณโรคแบบแพร่กระจายในสมเสร็จเลี้ยง

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วัณโรคเป็นโรคติดต่อเรื้อรังที่สำคัญสามารถติดต่อระหว่างคนกับสัตว์ได้ ซึ่งส่งผลกระทบทางด้านสาธารณะสุขของทั้งคนและสัตว์ โดยเฉพาะอย่างยิ่งสัตว์ป่าที่ใกล้จะสูญพันธุ์ สมเสร็จเพศเมียอายุ 17 ปี ในสวนสัตว์แห่งหนึ่งในประเทศไทย ตายในขณะวางยาเพื่อทำการรักษาโรคทางช่องปาก จากการชันสูตรซาก พบตุ่มนูนสีขาวคล้ายเนยแข็งขนาดต่างๆ แพร่กระจายในอวัยวะภายใน ได้แก่ ปอด ต่อมน้ำเหลือง และตับ เป็นต้น ผลทางจุลพยาธิวิทยาพบว่าการอักเสบแบบแกรนูโลมากระจายแทรกในหลายอวัยวะ โดยมีลักษณะเป็นเนื้อตายคล้ายเนยแข็งอยู่ส่วนกลางของก้อน ล้อมรอบด้วยชั้นที่พบเซลล์อักเสบแบบหลวมๆและชั้นเซลล์พังผืด โดยในชั้นของเซลล์อักเสบจะพบมีเซลล์เก็บกินขนาดใหญ่ค่อนข้างน้อย จากการย้อมด้วยสีพิเศษ Acid fast พบให้ผลบวกต่อเชื้อแบคทีเรียติดเป็นสายสีแดงในไซโตพลาสมของเซลล์เก็บกินขนาดใหญ่และขอบของก้อนแกรนูโลมา จากผลการจำแนกชนิดของเชื้อไมโครแบคทีเรียม ด้วยเทคนิคปฏิกิริยาลูกโซ่โพลิเมอเรสพบว่า เป็นเชื้อชนิด *ไมโครแบคทีเรียม ทูเบอร์คูโลซีส* จุดประสงค์ในการศึกษาครั้งนี้เพื่อรายงานการติดเชื้อวัณโรคแบบแพร่กระจายในสมเสร็จเลี้ยงซึ่งพบอุบัติการณ์ค่อนข้างน้อยและยังไม่มีรายงานในประเทศไทย

คำสำคัญ: วัณโรคแบบแพร่กระจาย ไมโครแบคทีเรียม ทูเบอร์คูโลซีส เทคนิคปฏิกิริยาลูกโซ่โพลิเมอเรส สมเสร็จ

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Introduction

Tuberculosis (TB) is one of the most widespread and important zoonotic infectious diseases caused by the rod-shaped, gram-positive, acid-fast pathogen from the genus Mycobacterium (M). The highly pathogenic *Mycobacterium tuberculosis* complex including *M. tuberculosis*, *M. bovis*, *M. 45 africanun*, *M. microti* and *M. canetti* are the main hazard pathogen, which can cause chronic progressive granulomatous infection in animals and human being (van Soolingen et al., 1997). WHO estimates that one-third of the World's current population is infected with tuberculosis. Mortality and morbidity statistics of TB were concentrated mostly in developing countries, particularly Asia and Africa (World Health Organization, 2007). Thailand is one of the high-burden countries on the World Health Organization's list for tuberculosis incidence. The rise of incidence rate is closely associated with the emergence of the HIV/AIDS epidemic (Palwatwichai, 2001).

Tuberculosis caused by *M. tuberculosis* has been recognized within a wide range of species including humans, non-human primates, elephants, exotic ungulates, carnivores, fish, reptiles, marine mammals and birds (Michalak et al., 1998; Pavlik et al., 2003). The type of lesions and severity of TB varied depending on the species of animals and mycobacteria. Tuberculoculous lesions are classified into 3 forms including pulmonary, extrapulmonary and disseminated (miliary) tuberculosis. Pulmonary TB is the most common in human and animals; whereas disseminated TB is the most severe form, which frequently occurs in infants and elderly. Patients with disseminated form have almost 100% mortality rate if untreated (Palwatwichai, 2001).

Tuberculosis in zoo and captive wildlife animals concerns the survival of endangered species as well as public health aspects. The most common form of TB in wildlife or exotic animals is pulmonary forms. Tuberculous lesions are often found in lungs and lymph nodes, mainly bronchial and mediastinal parts. Some cases of disseminated TB have been reported, which were characterized by generalized miliary tubercles involving entire organs of both thoracic and abdominal cavities (Radostits, 1999). To our knowledge, there have not been any completed pathological reports of disseminated form of TB in tapir.

Tapirs are odd-toed ungulates in the family Tapiridae in the genus Tapirus (T), which inhabit jungle and forest regions of South America, Central

America, and Southeast Asia. Four species of tapir including Braid's tapir (*Tapirus bairdii*), Malayan tapir (*T. indicus*), Mountain tapir (*T. pinchaquei*) and Brazilian tapir (*T. terrestris*) are classified as the endangered species (Janssen, 2003). The purpose of this study is to report a rare case of disseminated tuberculosis in a captive Malayan tapir, which *M. tuberculosis* was identified as the pathogenic agent.

Case report

Physical examination: A 17-year-old, 320 kg, captived female Malayan tapir from a zoo in Bangkok, Thailand was referred for treatment of dental tartar and caries. She had inappetite, weight loss and hypersalivation. After anesthesia for the treatment, she died with sign of panting. The animal was submitted to Department of Veterinary Pathology, Chulalongkorn University for routine necropsy and histopathological evaluations. Affected tissues were fixed in 10% formalin solution. Fresh tissues were collected for pathogen specie identification using polymerase chain reaction (PCR) technique. The PCR reactions were performed in 25 µl mixture on a PCR Sprint Thermocycler® (Thermo Electron Corporation®, Cambridge, UK). The PCR products were identified by gel electrophoresis in a 2% agarose gel and were visualized by ethidium bromide staining under ultraviolet light. The positive result of PCR showed a single band of fragment compared with the 100-bp DNA ladder (GeneRuler®, Fermentas). *M. tuberculosis* H37Rv (KK11-20) and *M. bovis* (ATCC 19210) were included as positive and negative controls.

Macroscopic findings: At necropsy, the lungs consisted of 0.1 to 0.5 cm in diameter, soft to firm, white well-demarcated raised nodules that were distributed randomly throughout the lung parenchyma. On the cut surface, there were multiple and variably-size, multifocal to coalescing abscesses with caseous necrotic center. The mediastinal lymph nodes were enlarged approximately 2-3 times of their normal size. On cut surface, the affected lymph nodes displayed numerous, military white to yellowish foci scattered throughout the parenchyma (Figure 1). Lesions contained dozens of similar nodules were also found in liver, spleen, kidney, ovary and serosal areas of diaphragmatic muscle, abdominal mesentery, intestines and uterus.

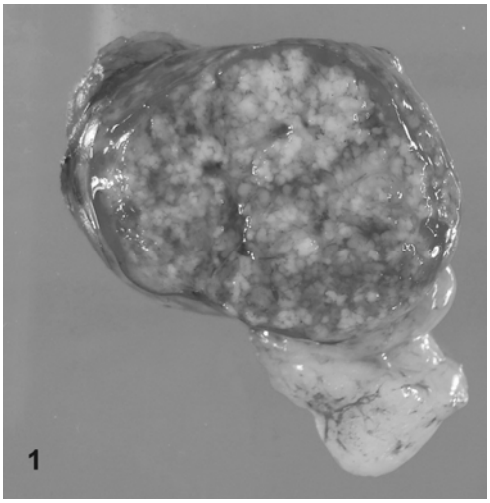

Figure 1. The enlarged mediastinal lymph node showed numerous miliary white to yellowish foci scattered throughout the parenchyma.

Histopathological findings: On microscopic examinations, the disseminated tubercles revealed the classical three zones of tuberculous granuloma. The center of the granuloma revealed large caseous necrosis with mild degree of calcification. The loosely organized inflammatory zone around the necrotic area contained various inflammatory cells including lymphocytes, plasma cells, macrophages, epithelioid cells and few Langhans's giant cells. Thin layers of fibrous connective tissue surrounding the inflammatory zone were observed sharply demarcated from the remaining normal tissue. A few acid fast bacilli were seen within active macrophages and Langhan's giant cells at the inflammatory zone of the tuberculous granuloma (Figure 2).

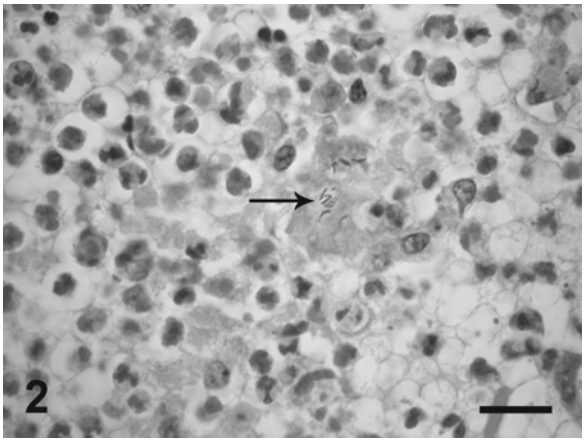

Figure 2. Acid-fast stain section revealed positive reddish acid-fast bacilli in active macrophages at the inflammatory zone of the tuberculous granuloma (bar = 50 µm).

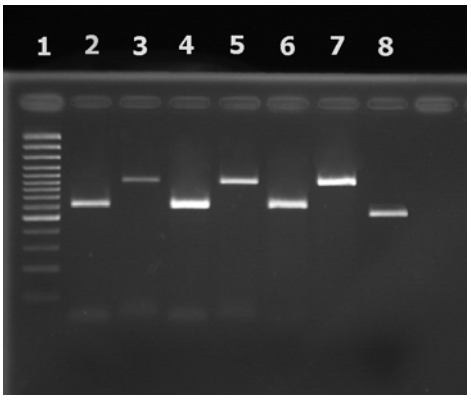

Figure 3. PCR results demonstrated the specific bands of M. tuberculosis in various affected organs. Lane 1: MW marker; Lane 2: 16SrRNA (lung tissues) 645 bp, Lane 3: M. tuberculosis (lung tissues) 925 bp, Lane 4: 16SrRNA (liver tissues) 645 bp, Lane 5: M. tuberculosis (liver tissues) 925 bp, Lane 6: 16SrRNA positive(645 bp), Lane 7: M. tuberculosis positive(925 bp), Lane 8: *M. bovis* positive 500 bp.

Molecular analysis: PCR detection of 16S rRNA gene was used to determine Mycobacterium species identity. A JB21/JB22 primer was used to distinguish *M. bovis* from the rest of the *Mycobacterium* spp, while *M. tuberculosis* was isolated by using specific Rv1970 primer. PCR results of affected organs and the lesions revealed a band specific to M. tuberculosis when the specific Rv1970 (925 bp) primer was used. No band was noted when the JB21/JB22 primer (M. bovis, 500 bp) was applied (Figure 3).

Discussion

This report describes the clinical and pathologic findings of TB caused by *M. tuberculosis* in a captive Malayan tapir. Tuberculosis in different species of tapirs has been reported by few authors. Tuberculosis caused by *Mycobacterium bovis* or *M. tuberculosis* in Brazilian tapirs in several zoos in Brazil was reported. Postmortem examination revealed pulmonary form of TB, characterized by multifocal abscesses and granulomas in the lungs, thoracic lymph nodes and thoracic cavity (Cubas, 1996). In Japan, TB caused by *M. Tuberculosis* was also reported in a family of captive Malayan tapirs. Unfortunately, the manifest clinical and necropsy detail was not available (Yoshikawa, 2006). In Thailand, tuberculosis in tapirs has never been reported. To our knowledge, this is the first case of the complete details of gross, histological studies and identification of the *Mycobacterium* species in Malayan tapir. The report case described here differs from previous reports of TB in tapirs, with respect to the breed, pathologic lesions and prevalence region. The lesions observed in this case were indicative of systemic form of TB characterized by multiple abscesses and granulomas with caseous necrotic center in most of the organs. The disseminated tubercles showed central necrosis and loosely organized inflammatory areas contained small aggregates of mononuclear inflammatory cells and epithelioid cells. Giant cells were rare and mild degree of calcification was seen. Scarce acid-fast bacilli were noted in the cytoplasm of macrophages and giant cells. The causative agent of TB in our case was *M. tuberculosis*. In other reports of *M. tuberculosis* infection in domestic and wild animals revealed that none or small visible lesions were observed in which the lesions were confined to the lymph nodes of respiratory and digestive systems. Cattle, sheep, goats and horses appear to be resistant to M. tuberculosis infection, but pigs may produce minor lesions in lymph nodes. The tubercle compose of foci of inspissated pus, necrosis and sometime calcified with much surrounding fibrous tissue (Francis, 1958; Thoen and Himes, 1981; Thoen, 1993). In wild bovines, cervines and antelopines, tuberculous lesions closely resemble those of domestic bovines. The tubercle comprises of caseous central necrosis, slight calcification, inflammatory zone of epithelioid cells and lymphocytes with a thick layer of fibrous connective tissue. In non-human primates, M. tuberculosis can produce extensive disease involving the parenchyma of the lungs as well as extrapulmonary tissues. Tuberculous lesions usually demonstrate microscopic similarities to those on other wild mammals (Thoen, 1994)

Infection of *M. tuberculosis* in captive wildlife animals especially in zoos might arise from transmission through close prolonged contact with a person or animal with active tuberculosis, and imported animals that were already infected with tuberculosis and develop the disease after being imported (Une and Mori, 2007). Several studies have been reported and reviewed tuberculosis prevalence in captive wildlife animals that related to human tuberculosis especially *M. tuberculosis* infection (Michalak et al., 1998; Alexander et al., 2002). Because Thailand is one of the high-burden countries for tuberculosis incidence (Palwatwichai, 2001), the possible sources of infection are either direct contact with the animals by the public or access to contaminated carcasses, animal carriers, or food and water containing the bacteria.

In animals, the affirmative diagnosis and identification of Mycobacterial infection is most likely to be found at postmortem examination. The validated ante-mortem diagnostic tests of TB infection are not currently available and difficult to interpret in some distance. The combination of ante-mortem diagnostic tests including the comparative intradermal skin test, biopsy, microbiological cultures and ELISA are required to confirm the infection (de Lisle et al., 2002; Parsons et al., 2002; Cousins and Florisson, 2005). In Thailand, tuberculosis was found occasionally at postmortem examination of zoo animals. Periodic tuberculosis screenings for TB in zoo and captive wildlife animals are performed but the practical and suitable diagnostic tests have not been established and done routinely. In this case report was also diagnosed by postmortem examination. Therefore, further ante-mortem screening tests for monitoring must be done to animals captive and raised in and around the effective area and also people who were closely in contact with this case in order to eliminate of other infected animals and prevent the spreading of the disease. In addition, investigation on the route of infection in the zoo should be considered to prevent disease transmission in the animals and humans. Good and energetic quarantine, screening, husbandry, decontamination and treatment system must be reviewed and executed continuously to completely prevent and control of TB infection in the zoo or wildlife herd.

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