Mycobacterium Tuberculosis Infection in an Orangutan (*Pongo pygmaeus*)

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ABSTRACT. A respiratory disorder was noted in a 5-year-old female orangutan kept in the Yongin Farmland. Radiographically, multiple radiodense foci ranging from 2 to 6 mm diameter were seen throughout the lung lobes. Grossly, the thoracic cavity revealed a firm texture and grayish-pink discoloration of the left apical lung lobe. Histopathologically, multifocal areas of granulomatous pneumonia present the right and left apical lung lobes. Both primers from IS1081 and IS6110 targeting 196 bp and 245 bp respectively were used in polymerase chain reaction, *Mycobacterium tuberculosis* was isolated from liver and confirmed by polymerase chain reaction.—KEY WORDS: *Mycobacterium tuberculosis*, orangutan, polymerase chain reaction.

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Tuberculosis is frequent in nonhuman primates and is usually caused by the human strain of tubercle bacillus. It has been reported in pig-tailed macaque (*Macaca nemestrina*) [6], stumptailed macaque (*M. speciosa*) [7], squirrel monkey (*Saimiri sciureus*) [2], rhesus monkey (*M. mulatta*), and cynomolgus monkey (*M. philippinensis*) [4]. An incidence of tuberculosis of 16.4% in 708 monkeys necropsied at the National Institute of Health has been reported [4]. Published reports of tuberculosis in the orangutan (*Pongo pygmaeus*) appear to be rare [3]. This is a report of spontaneous disseminated tuberculosis in this species.

The orangutan was caged indoors in a family group, and fed commercial monkey food and fresh fruits in the Yongin Farmland. The animal's environment was maintained at an ambient temperature of 17–27°C, a relative humidity of 40–60% with a minimum of 10 complete changes of 100% conditioned fresh air per hour, and a light/dark cycle of 12 hr light and 12 hr dark with no twilight. The orangutan had tested tuberculin one time in her life and negative.

On 22 January 1995, a 5-year-old female orangutan kept in the Yongin Farmland was examined because of anorexia, dyspnea and weight loss. Radiographically, multiple radiodense foci ranging from 2 to 6 mm diameter were seen throughout the lung lobes. An area of increased radiodensity approximately 10 mm in diameter was noted in the right apical lung lobe (Fig. 1). Atelectasis of the diaphragmatic lobe of the left lung was also found.

Her physical condition did not improve with treatment, and she became rapidly depressed and died. Necropsy was performed after death. Both left and right lung were severely affected and contained a great amount of caseous material with effusion. Tuberculous lesions were disseminated throughout the body, but there was no calcification. Organs were fixed in 10% neutral buffered formalin. Tissues were embedded in paraffin, cut in 5-μm sections, and stained with hematoxylin and eosin (HE).

Examination of the thoracic cavity revealed a firm texture and grayish-pink discoloration of the left apical lung lobe. There were numerous tan purulent gray areas on the surface of pleura. The liver and spleen were covered with white circular, raised spots, pinpoint to 2.0 cm in diameter. The renal cortex had numerous small nodules on the surface. The pericardium had numerous grayish raised spots. Other organs appeared normal.

Histopathologically, multifocal areas of granulomatous

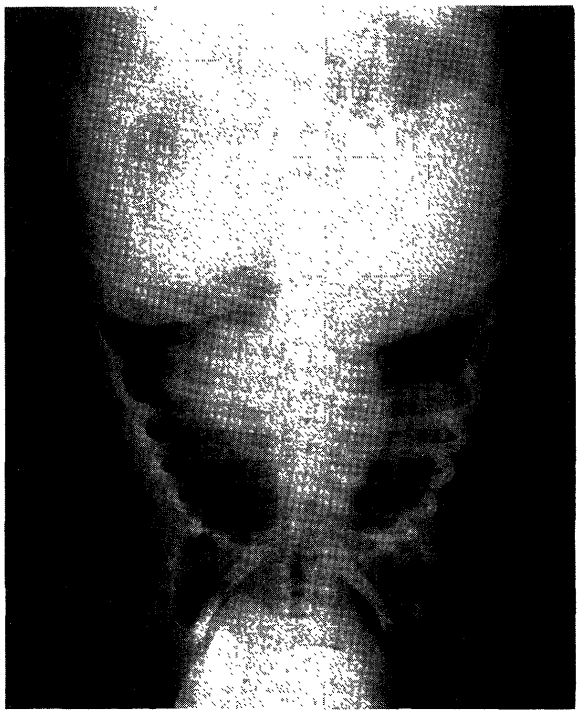

Fig. 1. Ventrodorsal radiograph of the thorax. An area of increased density approximately 10 mm in diameter was noted in the right apical lobe.

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pneumonia present the right and left apical lung lobes in association with the lung parenchyma surrounding the grossly visible abscesses. The centers of the granulomatous areas consisted of massive caseous necrosis. The margins were formed by proliferating epithelioid cells, mononuclear cells, fibroblasts, and a few Langhans' giant cells (Fig. 2). The abscesses contained caseous necrosis with infrequent acid-fast bacilli. Calcification was not noticed in any of the tubercles. The spleen, liver, kidney and pericardium contained numerous miliary and conglomerate necrotizing granulomas.

The primers used for PCR and their method have been reported elsewhere [1, 5]. An 196 base pairs (1081a: 5' CTGGGAGTATCCACTCGGCGG 3' and 1081b: 5' CCTCGCGCACCTTGAGCACCACCTGG 3') and 245 base pairs sequences (6110a: 5' GCATCGAGGTGGCCAGATGC 3' and 6110b: 5' CCTGCAAGGTAGGCGTCGG 3') located within IS1081 and IS6110, putative insertion sequences that occur multiple times in *Mycobacterium tuberculosis* chromosomes and are specific for the M. tuberculosis complex, are targeted. Briefly PCR amplification was carried out in 50 μl volume reactions containing final concentrations of 10 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.5 mM $MgCl_2$, 5 U Stoffel fragment, 0.5 U UNG, 200 μM dNTP, 20 nM of each primer, and 5μl of template from homogenized tissue with 1% NaOH treatment, boiling, and bead beating, (GeneAmp kit Perkin-Elmer Cetus, Norwalk, CT, U.S.A.). The samples treated with UNG at 75°C for 20 min were first denatured by beating at 95°C for 10 min and then incubated at 70°C for 2 min following 10 cycles of heating at 95°C for 1 min and 70°C for 2 min, and then 30 cycles of 95°C for 1 min, 65°C for 2 min, and 72°C for 2 min. After amplification 10 to 25 μl reaction mixtures were analysed on 1.2% agarose gel. *M. Tuberculosis* DNA was detected in only liver specimens by PCR after ethidium bromide staining and ultraviolet visualization (Fig. 3).

The definitive diagnosis depends on the isolation of the causative organisms from specimens of infected animals. But the conventional culture method takes more than 4 weeks to isolate mycobacteriae with less sensitivity incomparable to PCR. Thus PCR is recommendable to detect *M. tuberculosis*, providing a sensitive and specific means for the laboratory diagnosis of *M. tuberculosis* within 5 hr that is relatively simple to perform.

An absolute diagnosis of tuberculosis is to depend on isolation of *M. tuberculosis* from tissue of the infected animals. Although definitive identification is based on the morphologic and physiologic characteristics of the cultured organism, the long time required for isolation of *M. tuberculosis*. The reliable, rapid, and sensitive test should be used for diagnosis. Detection of *M. tuberculosis* by polymerase chain reaction (PCR) is the example. Amplification of target DNA using PCR provides a means for dramatically increasing the sensitivity of detection.

This orangutan was a showing animal and had a lot of exposure to human. In this case, transmission of the disease

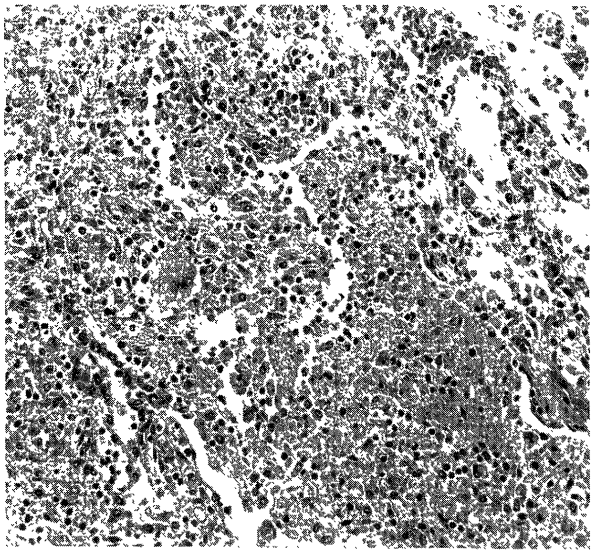

Fig. 2. Tubercle in the lung.

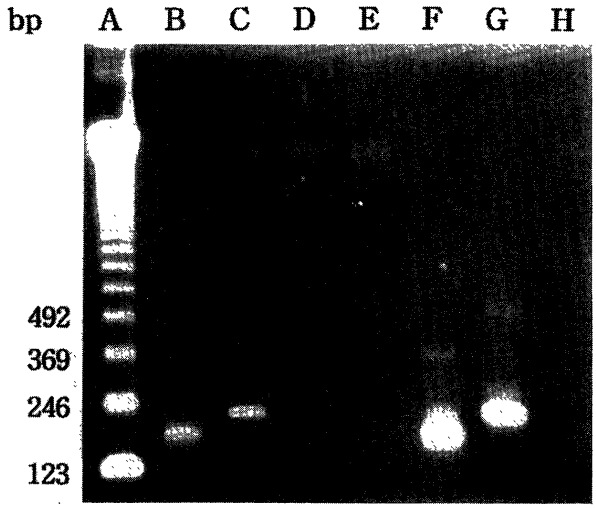

Fig. 3. An ethidium bromide-stained agarose gel electrophoresis of PCR amplification products. Lane A is a 123-base pair ladder marker. Lane B is a sample from liver with IS1081 primer. Lane C is a sample from liver with IS6110 primer. Lane D is a sample from ascites with IS1081 primer. Lane E is a sample from ascites with IS6110 primer. Lane F is a positive control for IS1081 primer. Lane G is a positive control for IS6110 primer. Lane H is a negative control.

was probably from man to monkey. The tubercle nodule found in the lungs suggested that the respiratory system was the site of entry to the tubercle bacilli. The wide-spread miliary granulomas indicated a secondary hematogenous dissemination to many organs including spleen, liver, heart and kidney. This orangutan had tuberculin test one time in three years ago and the result was negative. It is suggested that orangutan should be tested for tuberculin in every year. In this case, we make a

diagnosis of tuberculosis on the basis of clinical signs, radiographic and histopathologic examination, and polymerase chain reaction.

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