Avian Tuberculosis which Occurred in an Imported Pigeon and Pathogenicity of the Isolates

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ABSTRACT. A tubercular lesion (15 mm in diameter) was found on the skin of the caudal part of the cloaca in a 2-year-old male carrier-pigeon. *Mycobacterium avium* serovar 2 was isolated from the tubercular lesion on the skin and in the feces. The smooth and dome (SmD) variant (4.9×10^6 CFU) and rough and granular (RG) variant (3.7×10^7 CFU) of the isolates were injected into chickens intravenously. The chickens inoculated with the RG variant died on the 39th day and the one with the SmD variant died on the 77th day after inoculation. Enlargement of the liver and spleen was observed in all the inoculated chickens. Many white tubercular lesions were observed in the liver, spleen and lung of the chicken inoculated with the SmD variant, while no macroscopical change was observed in the bird inoculated with the RG variant. Inoculated organisms were recovered from the liver, spleen, kidneys, lungs and pancreas of each inoculated chicken.—**KEY WORDS:** avian tuberculosis, imported pigeon, *Mycobacterium avium* serovar 2.

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Avian tuberculosis due to *Mycobacterium avium* (serovars 1, 2 and 3) is a common infectious disease in birds in the United States [1, 8, 9, 11, 12], the United Kingdom [5] and West Germany [7]. In Japan, the occurrence of avian tuberculosis was rarely found in birds. In 1935 [13], the first case of the disease was found in a flamingo (*Phoenicopterus ruber*) kept at Ueno Zoo in Tokyo. Since this first occurrence, the disease has been found only in captive wild birds in zoological gardens such as a demoiselle (*Anthropoides virgo*) [13], so-called kingin-kei (*Chrysolophus* spp.) [3] and a couple of blue-eared pheasants (*Crossoptilon auritum*) [4]. However, no available report has been published on the disease in domestic fowls and pet birds in this country until now. In 1990 the authors encountered one case of avian tuberculosis in a carrier-pigeon imported for racing. In this paper, the results of tests on the biochemical and serological properties, and the pathogenicity in chickens of the isolated organisms are described.

The sick pigeon was a 2-year-old male carrier-pigeon imported from the Kingdom of Belgium to Japan. On September 25, 1990, the bird was sent to the Ushihama Pet Clinic due to weight-loss and lethargy, though it had a normal appetite. On examination at the clinic, a tubercle (15 mm in diameter) was found on the skin of the caudal part of the cloaca. The right forearm and tibia of the bird were unusually curved. Radiographic examination revealed the formation of a mass (15 × 20 mm) in one lung, enlargement of the liver, bone resorption and metaphysis in the right tibia, and congestion of the air sac. The tubercle removed surgically was sent for bacteriological examination. Though the bird was treated with antitrichomonal drug (Tinidazole®, Merck, Germany) and antibiotics (Tribissen®, Wellcome, U.K., or Tylosin®, Nihon Seiyaku, Japan), the bird died on 22nd, October, 1990. No post-mortem examination could be done.

The cutaneous tubercle and feces of the pigeon were examined for *Mycobacterium*. Smear preparations of both specimens were made, and stained by Ziehl-Neelsen's

procedure to demonstrate acid-fast bacteria. Quantitative culture was carried out on both specimens using the same media. The tubercle was cultured by the procedure reported by Kudou[6], using a combination of 1% NaOH and 1% Ogawa's egg media. The feces were inoculated on 3% Ogawa's egg media after treatment with 0.1% acriflavin solution and 4% NaOH. The media were incubated at 37°C for 10 weeks. The numbers of growing colonies were counted, and the number of *Mycobacteria* per g of each specimen was expressed as colony forming units (CFU/g). A specimen from each of the 5 colonies grown on the media was subcultured onto 1% Ogawa's egg medium. A total of 10 strains from both specimens were examined microscopically, biochemically, serologically and chromatographically in the manner described previously [2, 6, 10].

From the results of bacteriological examination, acid-fast bacilli were demonstrated microscopically in the smears of tubercle and feces. The organisms were isolated from the tubercle (7.0×10^4 CFU/g) and feces (2.4×10^4 CFU/g), respectively. The isolates were identified as *M. avium* serovar 2.

A smooth and dome (SmD) variant and a rough and granular (RG) variant isolated from the cutaneous lesion were selected from the colonies developed on 7H10 agar plates (Difco, U.S.A.). The virulence of the each variant in chickens was examined. Four 6-week-old female White Leghorn chickens weighing from 300 to 400 g were used. Before inoculation, it was confirmed that none of the birds had any mycobacterial organisms in their feces. One bird was inoculated intravenously with 3.7×10^7 CFU of the SmD variant and one with 4.9×10^6 CFU of the RG variant. The other 2 birds inoculated with 1 ml of PBS were used as the control. After inoculation, all the birds were kept individually in polycarbonate isolators for animal experiments that were placed in an air-conditioned (25°C) isolated unit maintained at 14L:10D in an animal quarter and they were observed for 39 to 77 days.

The chicken (No.2) inoculated with the RG variant died on the 39th day, and the other (No.1) with the SmD variant died on the 77th day. Enlargement of the liver and spleen was observed in both inoculated chickens. Many

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macroscopical tubercular lesions were observed on the liver, spleen and lungs of chicken No.1 inoculated with the SmD variant (Fig. 1).

Fig. 1. Gross lesions in the spleen of chicken No. 1 inoculated with the SmD variant. Many white tubercles are observed.

Both SmD and RG variant caused similar histopathological changes in chicken organs. Many granulomatous lesions with central necrosis surrounded by epithelioid cells were observed in the liver, spleen, lungs and pancreas of all the inoculated chickens (Fig. 2). Infiltration of lymphocytes was found in the kidneys of the chickens inoculated with both variants (Table 1). No gross or histopathological changes were observed in the 2 control chickens sacrificed on the 52nd or 77th day.

Though the inoculum size of the SmD and RG variants was slightly different, the RG variant showed higher virulence than that of the SmD variant, as reported previously [12]. The chicken inoculated with the RG variant died, and enlargement of the liver and spleen without gross tubercle formation was observed. In the chicken inoculated with the SmD variant, the formation of small white tubercles in the lung, liver and spleen were observed.

The recovery of inoculated organisms from the chickens is shown in Table 2. In chicken No.1 inoculated with the SmD variant, the number of organisms recovered 10^7 CFU/g in the spleen, 10^6 CFU/g in the liver, 10^4 CFU/g in the lung and kidney, and less than 3.0×10^2 CFU/g in the pancreas. In chicken No. 2 inoculated with the RG variant, the number of organisms recovered 10^8 CFU/g in the liver, 10^7 CFU/g in the lung and spleen, and 10^5 CFU/g in the kidneys and pancreas. Both SmD and RG variants were isolated from the chicken inoculated with each variant. No acid-fast organism was isolated from the feces during the experimental period, or from the blood and bile at autopsy of any of the inoculated chickens. All the organisms recovered from the inoculated chickens were identified as *M. avium* serovar 2. No acid-fast organism was isolated from any specimen from control chickens examined.

Fig. 2. Histopathological changes in the lung of chicken No. 1 inoculated with the SmD variant. Granulomatous lesions with central necrosis and surrounded by epithelioid cells are observed. (Hematoxylin and eosin stain, $\times 100$).

Table 1. Pathological changes in chickens inoculated with *M. avium* serovar 2 isolated from the pigeon

Chicken number	Inoculated strain (CFU)*	Death (Day)	Gross changes in the:					Histopathological changes in the:				
			Lungs	Liver	Spleen	Kidneys	Pancreas	Lungs	Liver	Spleen	Kidneys	Pancreas
1	SmD variant ^{a)} (4.9×10 ⁶)	77th	TL	TL En	TL En	En	En	GL	GL	GL	LI	GL
2	RG variant ^{b)} (3.7×10 ⁷)	39th	NC	En	En	NC	NC	GL	GL	GL	LI	GL

TL: Tubercular lesions. En: Enlargement. NC: No change. GL: Granulomatous lesion. LI: Lymphocytic infiltration. *: Figures indicate the number (colony forming units) of organisms used in inoculation.

a) Smooth and dome colonies on 7H10 agar plate. b) Rough and granular colonies on 7H10 agar plate.

Table 2. Recovery of *M. avium* serovar 2 from inoculated chickens

Chicken number	Inoculated strain (CFU)*	Death (Day)	The number of organisms (CFU)* recovered from the:						
			Lungs	Liver	Spleen	Kidneys	Pancreas	Bile	Blood
1	SmD variant ^{a)} (4.9×10 ⁶)	77th	2.4×10 ⁴	2.2×10 ⁶	3.4×10 ⁷	1.5×10 ⁴	<3.0×10 ²	—	—
2	RG variant ^{b)} (3.7×10 ⁷)	39th	2.9×10 ⁷	3.6×10 ⁸	3.8×10 ⁷	3.2×10 ⁵	2.2×10 ⁵	—	—

*: Figures indicate the number of organisms recovered (inoculated) as CFU/g or CFU/ml.

—: No acid-fast organism was recovered.

a) Smooth and dome colonies on 7H10 agar plate.

b) Rough and granular colonies on 7H10 agar plate.

The present study reported a case of avian tuberculosis in an imported carrier-pigeon due to *M. avium* serovar 2. This is the first report of avian tuberculosis in a pigeon in Japan. Although the disease cause by *M. avium* (serovars 1, 2 and 3) in animals including birds is common in European countries [5, 7], it occurs rarely in Japan. It remains unclear when and where the pigeon became infected.

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