Pathogenicity of Mycobacterium avium Complex Serovar 9 Isolated from Painted Quail (Excalfactoria chinensis)

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ABSTRACT. Avian tuberculosis accompanied with many tubercular lesions in the liver and spleen was found in a painted quail at a zoological garden in Japan. Mycobacterium avium complex (MAC) serovar 9 without insertion sequence of IS901 was isolated from the liver (1.3 × 10⁸ CFU/g), oviduct (9.4 × 10⁷ CFU/g), and intestine (1.5 × 10⁵ CFU/g). The isolates were inoculated intravenously to chickens. The inoculated chickens showed clinical symptoms of avian tuberculosis. Birds are susceptible to MAC serovar 9 without IS901.—KEY WORDS: avian tuberculosis, Mycobacterium avium complex serovar 9, painted quail.


Mycobacterium avium complex (MAC) is the agent of atypical mycobacteriosis in man and animals [1, 2, 6, 13, 18, 19, 28]. MAC is composed of 28 serovars [23, 24, 29], from 1 to 3 (as M. avium) and 4 to 28 (as M. intracellulare). It is known that serovars 1, 2 and 3 of the species have high virulence for chickens causing tuberculosis [1, 2, 25]. Avian tuberculosis is a common disease in birds in several countries [4, 10, 13, 14, 21, 23, 24], although it is a rather rare disease among birds in Japan and was sometimes found in birds at zoological gardens [8, 9, 30]. The authors have detected the disease caused by serovar 2 in a racing-pigeon imported from Belgium [15]. Avian tuberculosis has been caused by serovar 1 or 2, but not by other serovars in Japan [8, 9, 15, 30].

In 1994, a case of avian tuberculosis was found in a painted quail (Excalfactoria chinensis) at Ueno Zoological Gardens, and a frozen carcass was sent to our laboratory for examination within 48 hr after death. At autopsy, enlargement of the liver was observed with small white tubercles on the surface of the organ (Fig. 1). Acid-fast bacilli were detected microscopically in smears of the liver, intestines and oviduct. The liver, lung, kidney, spleen, intestine and oviduct of the dead quail were submitted for qualitative and quantitative culture for mycobacteria by the same methods reported previously [5, 17]. The isolates were identified from their biochemical characteristics [29] and by polymerase chain reaction (PCR) [12, 26] and classified by the tube agglutination test [23, 24] and thin-layer chromatography with deacetylated peptidoglycolipid (dPGL) [5]. The virulence of the isolates was examined also by inoculation to chickens.

A considerable number of Mycobacterium was isolated from the liver (1.3 × 10⁶ CFU/g), oviduct (9.4 × 10⁷ CFU/g) and intestines (1.5 × 10⁵ CFU/g). All the isolates were identified as MAC serovar 9. Although an insertion sequence of IS901 in chromosomal DNA was examined also by PCR [11], none of the isolates amplified the sequence.

A smooth and dome (SmD) colony variant and a rough and granular (RG) colony variant were observed on Middlebrook 7H10 agar plates (Difco, Detroit, MI). Both variants were isolated as many as possible. To investigate the pathogenicity to chickens, each variant was inoculated intravenously to birds by the same procedure reported previously [15, 18]. Six female White Leghorn chickens weighing from 300 to 400 g were used for the examination. The birds were confirmed to have no mycobacterial organisms in their feces before inoculation. Each of two birds was inoculated intravenously with 1 ml of 3.1 × 10⁷ colony forming unit (CFU) of the SmD variant and two other birds with 1 ml of 4.8 × 10⁷ CFU of the RG variant. The two other birds were inoculated with 1 ml of phosphate buffered saline (PBS) as control.

After inoculation, each bird was kept individually in a polycarbonate isolator placed in an isolation unit of the animal quarter. Clinical conditions of the inoculated chickens were observed for 44 days or longer. During the experimental period, fecal specimens were obtained from all chickens once a week and submitted for isolation of mycobacterium. Alkaline treatment of fecal specimens was
made with a sterilized 4% NaOH solution. The alkaline-treated samples were inoculated onto 3% Ogawa’s egg media, which were incubated at 37°C for 10 weeks.

The two chickens each inoculated with RG (No.1) or SmD variant (No.3) were sacrificed on the 44th days, since both showed such critical conditions as remarkable weight-loss and lethargy. The other two chickens each inoculated with RG (No.2) or SmD variant (No.4) showed similar clinical signs. The former was sacrificed on the 50th day and the latter on the 72nd day after inoculation. Control birds were sacrificed on the 44th and 72nd days. They showed no symptoms during the experimental period and no gross pathological lesions were observed at necropsy.

Pathological changes of the inoculated chickens are shown in Table 1. Enlargement of the liver and spleen was observed in all inoculated chickens. Enlargement of the kidneys was also found in the chickens inoculated with RG variant. No tubercle lesions were observed macroscopically in any organs of all inoculated chickens; however, histopathologically many granulomatous lesions with central necrosis surrounded by epithelioid cells were found in the liver, spleen, lungs of all inoculated birds. Infiltration of lymphocytes was observed in the pancreas and kidneys of the chickens inoculated with either variant. Neither gross nor histopathological lesion was observed in the two control chickens. Prominent weight loss and lethargy were found in an earlier stage in the chickens inoculated with the RG variant than in those with the SmD variant.

The recovery of the organisms from the inoculated chickens is shown in Table 2. The number of the RG variants recovered from each organ was slightly higher than that of SmD variant. The organisms were also isolated from the blood of two chickens (Nos. 1 and 2) which received the RG variant.

The number of organisms recovered from the feces is given in Table 3. The organisms were isolated from the feces of the chickens inoculated with the RG variant from the 28th (No. 1) to 35th (No. 2) days after inoculation. In the chickens inoculated with the SmD variant (Nos. 3 and 4), the organisms in the feces were isolated from the 42nd

Table 1. Pathological changes in chickens inoculated with MAC serovar 9

<table>
<thead>
<tr>
<th>Chicken number</th>
<th>Variant inoculated (CFU)</th>
<th>Day examined</th>
<th>Gross changes in: Lungs</th>
<th>Liver</th>
<th>Spleen</th>
<th>Kidneys</th>
<th>Pancreas</th>
<th>Histopathological changes in: Lungs</th>
<th>Liver</th>
<th>Spleen</th>
<th>Kidneys</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RG variant&lt;sup&gt;a&lt;/sup&gt; (4.8 × 10&lt;sup&gt;7&lt;/sup&gt;)</td>
<td>44th</td>
<td>NC</td>
<td>En</td>
<td>En</td>
<td>En</td>
<td>En</td>
<td>GL</td>
<td>GL</td>
<td>GL</td>
<td>LI</td>
<td>GL</td>
</tr>
<tr>
<td>2</td>
<td>RG variant&lt;sup&gt;a&lt;/sup&gt; (4.8 × 10&lt;sup&gt;7&lt;/sup&gt;)</td>
<td>50th</td>
<td>En</td>
<td>En</td>
<td>En</td>
<td>En</td>
<td>En</td>
<td>GL</td>
<td>GL</td>
<td>GL</td>
<td>LI</td>
<td>GL</td>
</tr>
<tr>
<td>3</td>
<td>SmD variant&lt;sup&gt;b&lt;/sup&gt; (3.1 × 10&lt;sup&gt;7&lt;/sup&gt;)</td>
<td>44th</td>
<td>NC</td>
<td>En</td>
<td>En</td>
<td>NC</td>
<td>NC</td>
<td>GL</td>
<td>GL</td>
<td>GL</td>
<td>LI</td>
<td>GL</td>
</tr>
<tr>
<td>4</td>
<td>SmD variant&lt;sup&gt;b&lt;/sup&gt; (3.1 × 10&lt;sup&gt;7&lt;/sup&gt;)</td>
<td>72nd</td>
<td>NC</td>
<td>En</td>
<td>En</td>
<td>NC</td>
<td>NC</td>
<td>GL</td>
<td>GL</td>
<td>GL</td>
<td>LI</td>
<td>GL</td>
</tr>
</tbody>
</table>

<sup>a</sup>Rough and granular colonies on 7H10 agar plate.
<sup>b</sup>Smooth and dome colonies on 7H10 agar plate.

Table 2. The recovery of viable bacteria from chickens inoculated with the two variants of MAC serovar 9

<table>
<thead>
<tr>
<th>Chicken number</th>
<th>Variant inoculated (CFU)&lt;sup&gt;*&lt;/sup&gt;</th>
<th>Day examined</th>
<th>The number of organisms (CFU)&lt;sup&gt;+&lt;/sup&gt; recovered from:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RG&lt;sup&gt;a&lt;/sup&gt; (4.8 × 10&lt;sup&gt;7&lt;/sup&gt;)</td>
<td>44th</td>
<td>Lungs: 1.1 × 10&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>RG&lt;sub&gt;S&lt;/sub&gt; (4.8 × 10&lt;sup&gt;7&lt;/sup&gt;)</td>
<td>50th</td>
<td>Lungs: 2.2 × 10&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>SmD&lt;sup&gt;a&lt;/sup&gt; (3.1 × 10&lt;sup&gt;7&lt;/sup&gt;)</td>
<td>44th</td>
<td>Lungs: 8.6 × 10&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>SmD&lt;sub&gt;S&lt;/sub&gt; (3.1 × 10&lt;sup&gt;7&lt;/sup&gt;)</td>
<td>72nd</td>
<td>Lungs: 5.3 × 10&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>*</sup>Figures indicate the number of organisms recovered (or inoculated) as colony forming units per gram.
<sup>+</sup>Culture positive.
<sup>––</sup>Culture negative.
<sup>a</sup>Rough and granular colonies on 7H10 agar plate.
<sup>b</sup>Smooth and dome colonies on 7H10 agar plate.
day after inoculation. Both SmD and RG variants were isolated from the feces of the chickens inoculated with either variant. All the isolates from the chickens were identified as MAC. No acid-fast organism was isolated from any of the control chickens examined.

Atypical mycobacteriosis caused by serovar 9 occurs mainly in human and swine. It was reported that M. avium serovar 9 was isolated from AIDS patients at a rate of 7.3% during 1980 and 1985 in the United States [31]. In Japan, the isolation rate of serovar 9 was shown to be 13.0% in patients with atypical mycobacteriosis between 1972 and 1990 [19]. Serovar 9 was also detected at a rate of 13.8% among swine strains derived from a nation-wide scale survey by the Japan Association of Meat Inspection Laboratories in 1983 (Katsube Y., unpublished). However, no case of avian tuberculosis caused by serovar 9 has been reported in Japan. This seems to be the first case of naturally occurring avian tuberculosis caused by MAC serovar 9 in Japan. The results of the present investigation suggest that MAC serovar 9 may serve as a pathogen not only for human and swine, but also for birds.

Recently, it was reported that serovars 1 to 6, 8 to 11, and 21 were classified as M. avium and serovar 7 and 12 to 20 as M. intracellulare from the DNA homology [3, 22]. Further molecular biological analysis showed that the presence of IS901 might closely be related to the pathogenicity in animals [11, 20, 27]. The strains having IS901 (M. avium type A/I) were isolated mainly from cases of avian tuberculosis in birds. On the contrary, strains without IS901 (M. avium type A) were primarily isolated from the environment, swine and human cases of MAC infection such as AIDS patients [11]. The infection that frequently occurred in AIDS patients was considered as opportunistic infection [30]. It seems that the occurrence of atypical mycobacteriosis is related to immunocompromise of the hosts and the prevalence of mycobacteria in the environment. The isolates from the painted quail did not have IS901 and no avian tuberculosis has been found in birds of the same flock. Therefore, when birds have some immunological problems, MAC serovar 9 may be one of the causes of avian tuberculosis.

The organism was isolated from the intestines of the painted quail and feces of the inoculated chickens. The authors, therefore, consider it is necessary to pay attention to infected birds as one of the possible sources of environmental contamination or atypical mycobacteriosis in immunocompromised patients and animals.

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REFERENCES