Pathogenicity of *Mycobacterium avium* Serovar 1 Isolated from Swine in Japan for the First Time

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**ABSTRACT.** A total of 116 strains of *Mycobacterium avium-intracellulare* complex were isolated from swine with tuberculous lesions at meat inspection, during 1982–1983. Of them, one strain isolated from the lesion in the mesenteric lymph nodes was identified as *M. avium* serovar 1 (swine strain). The pathogenicity of the swine strain in chickens was compared with that of a reference strain (*M. avium* serovar 1). Chickens were inoculated intravenously with each strain (2×10^6 colony forming units: CFU) and they were autopsied at 30 days after inoculation. Enlargement of the liver, spleen and kidney was observed in the inoculated chickens. In histopathological examination, many granulomatous lesions were observed in the lung, liver and spleen. Infiltration of lymphocytes was found in the kidney and pancreas. Both inoculated organs were recovered from the liver (10^2–10^6 CFU/g) and spleen (10^2–10^3 CFU/g) of each inoculated chicken. The reference strain (<3×10^5 CFU/ml) was also recovered from the liver, kidney, pancreas and bile of the inoculated chickens. All the isolates were identified as serovar 1. It was shown that the swine strain had pathogenicity in chickens comparable to that of the reference strain.—**KEY WORDS:** atypical mycobacteriosis, *Mycobacterium avium* serovar 1, swine.

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*Mycobacterium avium-intracellulare* complex (MAI complex) is one of the agents causing human and animal atypical mycobacteriosis. At meat inspection, tuberculous lesions suspected of being mycobacteriosis were frequently observed in pigs, and mainly MAI complex was isolated from the lesions [5, 6, 10, 12, 18, 20]. Infection by the MAI complex causes serious economic losses in many parts of the world because of condemnation of the animals at meat inspection.

The MAI complex is composed of 28 serovars [13, 14], from 1 to 3 (*M. avium*) and from 4 to 28 (*M. intracellulare*). Serovars 1, 2 and 3 are distinguished from other serovars because of their high virulence in chickens [1, 2, 6, 15]. The serovars of MAI complex are valuable for epidemiological studies of human and animal atypical mycobacteriosis.

The authors examined the serovars of 116 MAI complex strains isolated from tuberculous lesions in slaughtered swine. As the result of serological examination, one strain isolated from lesions in the mesenteric lymph nodes (swine strain) was identified as serovar 1. Until now no paper has been published on the isolation of *M. avium* serovar 1 from swine in Japan.

In this investigation, the virulence of the swine strain in chickens was studied in comparison with that of the reference strain *M. avium* serovar 1.

**MATERIALS AND METHODS**

**Strains:** Two strains of *M. avium* were used in the present series of experiments. One was swine strain HS9–116 isolated from lesions in the mesenteric lymph nodes. The strain came from 116 MAI complex strains isolated from swine tuberculous lesions during 1982–1983. Another was the reference strain of *M. avium* serovar 1 (11907–300) for Schaefer's serovars. The reference strain was isolated from cattle and used in virulence tests on chickens by Schaefer et al. [15]. It was kindly provided by Drs. J. K. McClatchy and A. Y. Tsang of the National Jewish Hospital, Denver, Colorado, U.S.A.

**Virulence Test:** Six 6-week-old female White Leghorn chickens weighing from 300 g to 400 g were used. Two birds were inoculated intravenously with 2.2×10^6 colony forming units (CFU) of the swine strain and two with 2.0×10^6 CFU of the reference strain. The other two birds inoculated with 1 ml of PBS were used as the control. Before inoculation it was confirmed that none of the birds had any mycobacterial organisms in their feces. After inoculation, all the birds were kept individually in polycarbonate isolators for animal experiments placed in an air-conditioned (25°C) isolated unit maintained at 14L:10D in an animal quarter and they were clinically observed for 30 days. After the observation period, they were sacrificed for pathological and bacteriological examination.

**Pathological examination:** The liver, spleen, lung, kidney and pancreas were submitted to histopathological examination after macroscopical observation. A small piece of each organ was fixed in 10% formalin saline for 4 to 5 days and embedded in paraffin. The sections were stained with hematoxylin and eosin (HE).

**Bacterial examination:** The specimens such as the liver, spleen, lung, kidney, pancreas, blood and bile were submitted to bacteriological examination. Ten-fold serial dilutions of each specimen were made with 1% NaOH. Ogawa's egg medium (Eiken, Tokyo) was inoculated with one-tenth ml of each dilution. The inoculated medium was incubated at 37°C for 10 weeks. The typical colonies of MAI complex grown on the medium were counted and the numbers of the inoculated organisms per g or ml of each...
specimen were expressed as colony forming units (CFU).

During the experimental period, about 2 g fecal specimens from the inoculated chickens were also submitted to bacterial examination once a week.

Identification of serovars: Serovars of the isolates were identified by the tube agglutination method [13, 14] and by the specific rate of flow (Rf) of diacetylated peptidoglycolipid (dPGL) on thin-layer chromatography (TLC) according to the method established by Brennan et al. [3].

Tube agglutination was done by the method described by Schaefer et al. [14, 15]. Formalin-killed bacteria were used as the antigens. For the agglutination reaction, 0.25 ml of diluted antisera at a titer of 1/80 or 1/160 were mixed with equal volumes of the antigen in tubes. The mixtures were incubated at 37°C for 5 hr.

The TLC was done by the method described by Brennan et al. [3]. The extracted lipid including dPGL was applied on a silica gel 60 sheet pre-coated on a glass plate without fluorescent indicator (Merck, Germany) for TLC. The dPGL was developed by chloroform-methanol-water (65:25:4). Forty % H₂SO₄ was sprayed on the TLC plate and the plate was heated at 125°C for 30 min. The developed dPGLs were visualized as yellow-black spots. The results of TLC were compared with the results of the tube agglutination method.

Table 1. Pathological changes in chickens inoculated with M. avium serovar 1

<table>
<thead>
<tr>
<th>Chicken Number</th>
<th>Inoculated Strain (CFU)</th>
<th>Gross changes in the:</th>
<th>Histopathological changes in the:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lung</td>
<td>Liver</td>
</tr>
<tr>
<td>1</td>
<td>Reference Strainª (2.0×10⁹)</td>
<td>NC</td>
<td>Enlargement</td>
</tr>
<tr>
<td>2</td>
<td>Reference Strainª (2.0×10⁹)</td>
<td>NC</td>
<td>Enlargement</td>
</tr>
<tr>
<td>3</td>
<td>Swine Strainª (2.2×10⁹)</td>
<td>NC</td>
<td>Enlargement</td>
</tr>
<tr>
<td>4</td>
<td>Swine Strainª (2.2×10⁹)</td>
<td>NC</td>
<td>Enlargement</td>
</tr>
</tbody>
</table>

NC: No change, GL: Granulomatous lesion, LI: Lymphocytic infiltration.
*ª: Figures indicate the number (colony forming units) of organisms used in inoculation.
a) M. avium (11907–300) was isolated from cattle, and it was contributed by Drs. J. K. McClatchy and A. Y. Tsang.
b) M. avium serovar 1 (H59–116) isolated from swine.

Fig. 1. Histopathological change in the lung of chicken 1 inoculated with reference strain of M. avium serovar 1 showing the extensive tissue damage. A granulomatous tissue with centrally located necrosis surrounded by epitheloid cells characterizes pulmonary involvement. (Hematoxylin and eosin stain, × 400).
RESULTS

None of the chickens inoculated with the swine strain or the reference strain died during the 30 day experimental period. Slight exhaustion was observed in all the inoculated chickens, but not in the PBS-control group.

Gross and histopathological changes in chickens inoculated with swine or reference strain are given in Table 1. Enlargement of the liver, spleen and kidney was observed in all the inoculated chickens. In chicken No. 1, inoculated with the reference strain, enlargement of the pancreas was also found. On histopathological examination, many granulomatous lesions with central necrosis surrounded by epitheloid cells were observed in the lung, liver and spleen of all the inoculated chickens (Figs. 1 and 2). Lymphocytic infiltrations were found in the kidneys and pancreas in all inoculated chickens (Fig. 3). No pathological change was observed in the control group.

In Table 2, the recovery of M. avium from inoculated chickens is shown. The numbers of organisms recovered varied from $10^2$ to $10^6$ CFU/g in the liver and $10^3$ to $10^5$ CFU/g in the spleen, respectively. In the chickens inoculated with the reference strain, the numbers of the organisms in the lung, kidney, pancreas or bile were smaller than $3.0 \times 10^2$.

Fig. 2. Histopathological change in the liver of chicken 1 showing the extensive tissue damage. A granulomatous tissue with centrally located necrosis surrounded by epitheloid cell characterizes hepatic involvement. (Hematoxylin and eosin stain, $\times 100$).

Fig. 3. Histopathological changes in the kidney of chicken 1. Infiltration of lymphocytes was observed. (Hematoxylin and eosin stain, $\times 100$).
CFU/g or ml. No organism was isolated from the feces during the experimental period, or from the blood at the end of examination in all the inoculated chickens. The organisms recovered from inoculated chickens were identified as serovar 1 from the results of TLC (Fig. 4) and the tube agglutination method.

**DISCUSSION**

It is known that *M. avium* Schafer's serovars 1, 2 and 3 of MAI complex are highly virulent in chicken [1, 2, 6, 15]. Although a few cases of swine infection with *M. avium* serovar 2 or 3 have been reported in Japan [6, 20], serovar 1 has never been isolated from swine in Japan. In this study, the authors isolated *M. avium* from the tuberculous lesions of the swine mesenteric lymph nodes, and identified it as serovar 1 by the specific rate of flow of dPGF on TLC and the tube agglutination method. This is the first case of swine infected with *M. avium* serovar 1 in Japan.

*M. avium* serovar 1 is a common etiological agent in animal and human atypical mycobacteriosis throughout the world. It was reported that the infection rate for swine with serovar 1 of *M. avium* was 18.1% and 46.0% in the United States [9, 14], 2.5% in West Germany [8], and 18.3% in South Africa [11], respectively. However, the prevalence of swine infected with *M. avium* regardless of the serovar was fairly low in Japan. In view of the results mentioned above, it seems that there might be differences in the geographical distribution of *M. avium* serovar 1 in swine.

It was reported that *M. avium* had highly virulent effect on chickens [1, 4, 16, 17, 19]. However, the results of virulence tests varied according to the age of chicken, the inoculum size of the organisms, and the inoculation route [1, 2, 4, 6, 7, 15]. Kawashima and Okunuma [7] reported 'vilemin type tuberculosis' with enlargement of the liver and spleen with gross tubercle formation was observed 5 weeks after intravenous inoculation of about 10^6 CFU of the organisms. In this study, the inoculum size

<table>
<thead>
<tr>
<th>Chicken Number</th>
<th>Inoculated Strain</th>
<th>The number of organisms (CFU) recovered from the:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reference Strain^3</td>
<td>1.5×10^6 3.4×10^6 3.0×10^7 3.0×10^7 3.0×10^7 3.0×10^7</td>
</tr>
<tr>
<td>2</td>
<td>Reference Strain</td>
<td>&lt;3.0×10^2 6.0×10^2 3.3×10^2 3.3×10^2 3.3×10^2 3.3×10^2</td>
</tr>
<tr>
<td>3</td>
<td>Swine Strain^3</td>
<td>5.9×10^6 6.6×10^6 6.6×10^6 6.6×10^6 6.6×10^6 6.6×10^6</td>
</tr>
<tr>
<td>4</td>
<td>Swine Strain</td>
<td>8.3×10^6 6.6×10^6 6.6×10^6 6.6×10^6 6.6×10^6 6.6×10^6</td>
</tr>
</tbody>
</table>

*: Figures indicate the number of organisms recovered as CFU/g or CFU/ml.
—: No acid-fast organism was recovered.
a), b) See the footnote to Table 1.

Fig. 4. TLC of the extracted lipid from inoculated or recovered organisms, demonstrating the relative mobilities of the specific dPGFs. The arrows point to the type-specific dPGFs. a) Chicken number b) Recovered organs c) Recovered strain number
of the organisms was about same as in previous studies [7], and no gross tuberculous lesions were observed in the inoculated chickens. The results show that more than a 30 day period may be necessary to cause vilmann type tuberculosis in this swine strain.

In this experiment, typical tuberculous lesions were observed histopathologically in the lung, liver and spleen of chickens inoculated with the swine strain as well as the reference strain. Numerous organisms (10^5–10^6 CFU/g) were recovered from the liver and spleen of all the inoculated chickens. In the chickens inoculated with reference strain, numbers of organisms smaller than 3.0 × 10^2 CFU/g or ml were recovered from the lung, kidney, pancreas and bile as in previous reports [1, 4, 16, 17, 19]. The organisms recovered were confirmed to be serovar 1. It was also found that the swine strain of M. avium serovar 1 had a virulent effect on chickens comparable to that of the reference strain.

It was confirmed that M. avium serovar 1 has been disseminated in swine as a cause of atypical mycobacteriosis in Japan though the occurrence rate is rather low. The authors therefore consider that it is necessary to pay much attention to swine as one of the possible sources of atypical mycobacteriosis in man.

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REFERENCES