

Pathogenicity of *Corynebacterium kutscheri* in the Syrian Hamster

Hiromi AMAO, Tougaku KANAMOTO, Yumi KOMUKAI¹⁾, Kazuaki W. TAKAHASHI, Takuo SAWADA²⁾, Manabu SAITO³⁾, and Masahiro SUGIYAMA⁴⁾

Departments of Laboratory Animal Science, ²⁾Veterinary Microbiology and ⁴⁾Veterinary Pathology, Nippon Veterinary and Animal Science University, 7-1, Kyonan-cho 1-chome, Musashino-shi, Tokyo 180, ¹⁾Mercian Cleantec, 5-8, Kyobashi 1-chome, Chuo-ku, Tokyo 104, and ³⁾Division of Experimental Animal Research, National Institute of Health, 7-1, 4-chome, Gakuen, Musashimurayama-shi, Tokyo 190-12, Japan

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ABSTRACT. The pathogenicity of *Corynebacterium kutscheri* isolated for the first time from Syrian hamster was experimentally studied in hamsters. In hamsters given intramuscular (i.m.) or subcutaneous (s.c.) inoculation with 10 or 10^3 bacteria, neither clinical signs nor gross lesions were found. In those given 10^5 bacteria i.m., moderate proliferation of granulation tissue was found in the muscle of the inoculation region at necropsy. In the animals given 10^5 bacteria s.c., a nodular lesion was observed at the inoculation site 2 days post-inoculation (p.i.), but the nodules subsided gradually from 6 days p.i. and were unclear 10 days p.i. At necropsy, small abscesses were found in all the animals in this group. In those given 10^7 bacteria either i.m. or s.c., lesions were clearly observed at the inoculation site 1 to 10 days p.i., and a large abscess was noted at necropsy. The organisms were isolated only from the lesions in the groups. Agglutinating antibody in the sera was detected only in the animals given 10^5 or 10^7 bacteria. This suggests that 10^5 of *C. kutscheri* are needed to form localized nodular abscesses in Syrian hamsters.—**KEY WORDS:** *Corynebacterium kutscheri*, pathogenicity, Syrian hamster.

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Corynebacterium kutscheri is known to cause pseudo-tuberculosis with the formation of granulomatous lesions in laboratory mice and rats [4, 7, 9–11, 16, 21, 22]. The organism has been also isolated from naturally infected guinea pigs [20] and wild voles [3] with gross lesions. The authors reported the first isolation of *C. kutscheri* from subclinically infected Syrian hamsters and revealed that colonization of the organisms was most frequently found in the oral cavity, esophagus, cecum and, colon and rectum [1]. However, the pathogenicity of *C. kutscheri* in Syrian hamsters still remains uncertain. In this report, the authors describe the pathogenicity of the organism isolated from naturally infected Syrian hamsters by intramuscular (i.m.) or subcutaneous (s.c.) inoculation.

MATERIALS AND METHODS

Animals: Male Syrian hamsters (Std: Syrian), 3 weeks of age, were purchased from Japan SLC Inc., Shizuoka, Japan. Following one week quarantine and acclimation, three or four animals were randomly assigned to each treatment group. All the animals were maintained in aluminum-metal cages (W30 × D40 × H25 cm) with 3 to 4 in each cage. They were kept in a room under conditions of 20–25°C temperature, 40–70% relative humidity and a 14 hr/day light cycle. Commercial diet (MB-1, Funabashi Farm Co., Ltd., Chiba, Japan) and water were given *ad libitum*.

Inoculation of bacteria: The strain HO of *C. kutscheri* isolated from the oral cavity of a hamster [1] was used. Frozen stock culture of the strain was thawed and cultured on heart infusion agar (HIA: Eiken Chemical Co., Ltd., Tokyo, Japan) for 48 hr at 37°C. Serial 10-fold dilutions of the bacterial suspension were then made with heart

infusion broth (HIB: Eiken Chemical Co., Ltd., Tokyo, Japan). The hamsters were inoculated with 6.6×10 , 6.6×10^3 , 6.6×10^5 or 6.6×10^7 CFU bacteria i.m. into the right gastrocnemius muscle or s.c. in the right inguinal region.

All hamsters were observed for clinical signs and inoculation sites every day for 10 days post-inoculation (p.i.), and body weight was measured at 0, 5 and 10 days p.i.

Necropsy: All hamsters were humanely killed with ether, bled by cardiac puncture and necropsied at 10 days after inoculation. The inoculation regions were carefully inspected. For histopathology, tissues were fixed in 10% neutral phosphate-buffered formalin, processed routinely and embedded in paraffin. Five micrometer sections were stained with hematoxylin and eosin (HE). Histopathological examination was carried out only for the inoculated region.

Bacteriology: Samples from gross lesions were stamped on 5% horse blood agar plates and streaked with a platinum loop. As a selective medium, furazolidone-nalidixic acid-colimycin (FNC) agar [2] was used for the recovery of *C. kutscheri*. The mucous membranes of the oral and nasal cavities and trachea, and the right inguinal region were wiped with a fine cotton swab moistened with sterile HIB and the swabs were stamped onto FNC agar. Samples from the lung, liver, kidney and right gastrocnemius muscle were also stamped on FNC agar in the same way. The cecal contents (200 to 300 mg) were homogenized in 10 volumes of sterile HIB. Serial 10-fold dilutions of the homogenates were made with HIB, and 0.05 ml of each dilution was placed on FNC agar. After incubation at 37°C for 72 hr, *C. kutscheri* colonies were counted. The average of triplicated values was used for

calculation of the number of bacteria (CFU/g). The identification of the isolates was carried out with Gram's stain and agglutination test with rabbit antiserum prepared against the strain S5L [2].

Agglutination test for antibody to C. kutscheri: Blood samples were collected from the animals by heart puncture at necropsy. Agglutination tests for serum antibody to *C. kutscheri* were performed by means of a microtiter system as described previously [17, 18] with a commercial antigen (Denka Seiken Co., Ltd., Tokyo, Japan). Sera showing agglutination at more than 1:5 dilution were considered as antibody positive.

RESULTS

The presence of gross lesions is shown in Table 1. In the groups given 10 or 10^3 bacteria either i.m. or s.c., neither clinical signs nor gross lesions were found during the observation period or at necropsy. One of four animals given 10^5 bacteria i.m. had a slight swelling in the region 2 to 7 days p.i. but it had disappeared by the time of necropsy. In all the animals given 10^7 bacteria i.m., clear swelling of the inoculation region was observed 1 to 10 days p.i. All the animals had nodules which were yellowish-white and approximately 10×6 mm in size in the inoculation region (Fig. 1A).

In all the animals given 10^5 bacteria s.c., a nodule 3 to 6 mm in diameter was observed in the inoculation region from 2 days p.i. The nodule subsided gradually from 6 days p.i. At necropsy, there were yellowish-white nodules, 5×3 mm in mean size in the subcutaneous tissue in the inoculation region. In all the animals given 10^7 bacteria s.c., a nodule in the inoculation region was clearly seen from 1 day p.i. The diameter of the nodule was 5 mm

on day 1, 6 to 12 mm on day 2, and 5 to 10 mm on day 3. The nodule subsided gradually from 6 days p.i. in one animal and from 9 days p.i. in 2 animals, but did not disappear completely at 10 days p.i. At necropsy, 2 to 3 nodules which were yellowish-white and 5×4 mm in mean size were seen in the muscle in the inoculation site in 3 animals (Fig. 1B). The remaining one animal had two nodules, 6×4 mm in the subcutaneous tissue and the muscle in the inoculation site, respectively. No gross lesions were noted in regions other than the inoculation site.

The mean body weight after inoculation is shown in Table 2. The weight of the animals given 10^7 bacteria i.m. was significantly lower than that of the animals given 10 or 10^3 bacteria ($P < 0.05$).

Histopathological findings: Results of histopathological examination of the inoculation site are summarized in Table 3. In 3 of 4 animals given 10 or 10^3 bacteria i.m., only slight degeneration and necrosis were observed in the muscular tissue. In the animals given 10 or 10^3 bacteria s.c., no histopathological lesion were observed. On the other hand, in all the animals given 10^5 bacteria i.m., there was not only slight or moderate degeneration and necrosis but moderate granulation tissue was found in the muscle. In all the animals given 10^5 bacteria s.c., small abscesses with epithelioid cells, neutrophil and lymphoid cells were found in addition to the findings seen in i.m. animals. In all the animals given 10^7 bacteria either i.m. or s.c., large abscesses surrounded by granulation tissue were seen (Fig. 2).

Isolation of C. kutscheri and serum antibody: Isolations of *C. kutscheri* and serum antibody titers to *C. kutscheri* are shown in Table 4. *C. kutscheri* were detected in the lesions in 3 of 3 hamsters given 10^7 bacteria i.m., in 2 of 4

Table 1. Gross lesion of *C. kutscheri* inoculated Syrian hamsters

Route of inoculation	Dose	Number of animals	Days post-inoculation										At necropsy ^{a)}
			1	2	3	4	5	6	7	8	9	10	
i.m.	10	4	— ^{b)}	—	—	—	—	—	—	—	—	—	—
	10^3	4	—	—	—	—	—	—	—	—	—	—	—
	10^5	4	—	$\frac{1}{+^{c)}}$	$\frac{1}{+}$	$\frac{1}{+}$	$\frac{1}{+}$	$\frac{1}{+}$	$\frac{1}{+}$	—	—	—	—
	10^7	3	+	+	+	+	+	+	+	+	+	+	+
s.c.	10	4	—	—	—	—	—	—	—	—	—	—	—
	10^3	4	—	—	—	—	—	—	—	—	—	—	—
	10^5	4	—	+	+	+	+	$\frac{1}{+}$	$\frac{1}{+}$	$\frac{1}{+}$	—	—	+
	10^7	4	+	+	+	+	+	+	+	+	+	+	+

a) 10 days post-inoculation. b) —: No lesion. c) +: Presence of lesion.

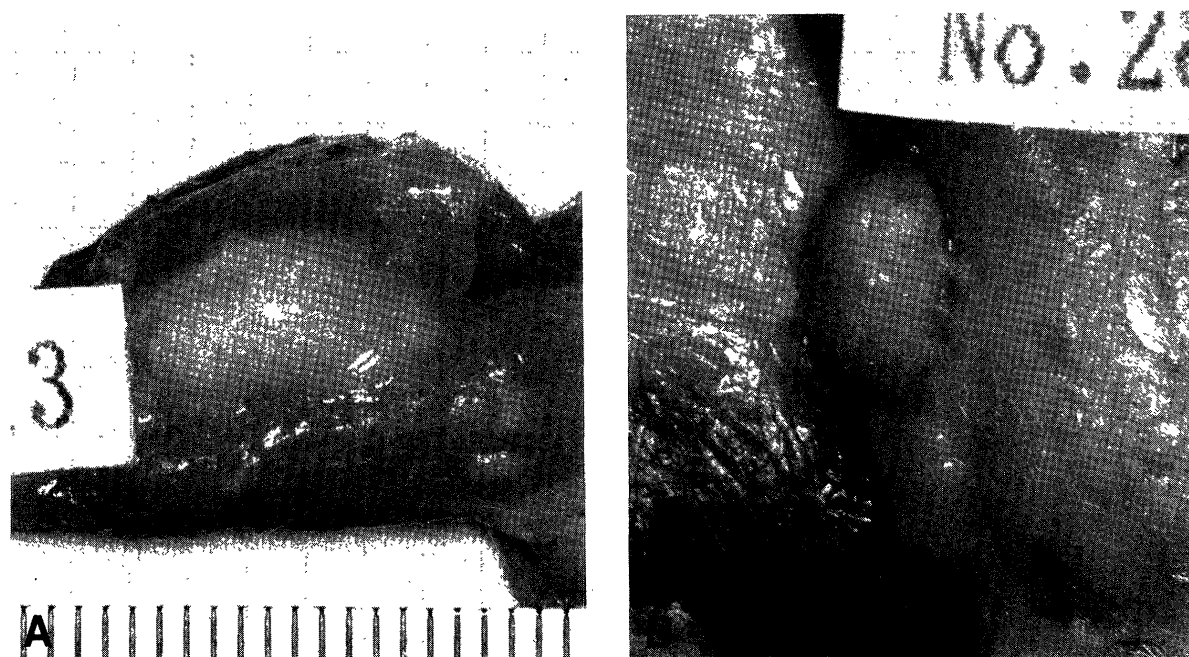


Fig. 1. Gross lesions at the inoculation site in hamsters inoculated i.m. and s.c. with 10^7 bacteria at necropsy. A: A nodular lesion in a hamster (i.m.). B: Two nodules in a hamster (s.c.).

Table 2. Body weight of Syrian hamsters inoculated with *C. kutscheri*

Route of inoculation	Dose	Days post-inoculation			
		0	5	10	
i.m.	10	64.0±3.7 ^{a)}	78.5±4.1	92.0±5.1	* ^{b)}
	10^3	66.5±4.9	80.5±4.9	93.6±6.5	
	10^5	63.5±3.1	77.5±2.7	86.1±3.0	
	10^7	62.3±3.2	73.3±2.1	83.2±1.6	
s.c.	10	62.5±3.8	76.3±6.7	88.3±8.3	
	10^3	63.5±3.1	78.8±2.5	91.4±5.4	
	10^5	61.5±2.7	76.8±4.6	84.9±5.3	
	10^7	64.5±4.1	73.8±5.2	84.4±8.9	

a) Mean±S.D. (g). b) Statistically significant ($P<0.05$, Student's t-test).

given 10^5 bacteria s.c. and in 3 of 4 given 10^7 bacteria s.c. No organisms were isolated from animals without a lesion. *C. kutscheri* was isolated from oral and nasal cavities and cecal contents (number of organism: 2×10^2 CFU/g) of only one animal given 10^7 bacteria s.c. In the hamsters given 10 or 10^3 bacteria, no antibody to *C. kutscheri* was detected. On the other hand, seroconversion was observed in 2 of 4 hamsters (titer: 1:40) given 10^5 bacteria i.m., in 3 of 3 (titer: 1:20 to 1:320) given 10^7 bacteria i.m., in 3 of 4 (titer: 1:10 and 1:20) given 10^5 bacteria s.c. and in 4 of 4 (titer: 1:20 and 1:40) given 10^7 bacteria s.c.

DISCUSSION

Although most reports until the present have described how murine corynebacteriosis occurred mainly in laboratory mice and rats, a few reports indicated pneumonia in guinea pigs [20] and wild voles [3] due to *Corynebacterium kutscheri*, and also chorioamnionitis and funisitis in human [8] due to the bacteria. Experimentally, intracardiac injection of the bacteria produced polyperi-arthritis in guinea pigs [6]. We previously reported the first isolation of *C. kutscheri* from the oral cavity of Syrian hamsters and frequent colonization of the organisms at this site [1]. As the Syrian hamster is unique because of the highly aggressive nature of both sexes [5, 14], a bite wound inflicted during a quarrel may lead to the infection and abscess formation. The present results of gross and histopathological observation indicated that less than 10^3 of *C. kutscheri* bacteria hardly produced lesions in Syrian hamsters. Animals given 10^5 bacteria either i.m. or s.c. showed inflammatory swelling and small abscess at the injection site and *C. kutscheri* was isolated from the sites followed by the disappearance of the gross lesions 7 or 8 days after inoculation. Animals given 10^7 , had prominent lesions at the injection site with isolation of the bacteria but no other gross lesions were observed. This indicated that 10^5 *C. kutscheri* were critical for forming the lesions. Because no remarkable weight loss was observed, Syrian hamsters were locally, not systemically, damaged by the bacteria. The presence of agglutinating antibody in the sera indicated that at least 10^5 to 10^7 bacteria would be needed to induce enough immune reaction in Syrian hamsters.

The present results indicate that more than 10^5 bacteria

Table 3. Histopathological lesions of *C. kutscheri* inoculation site at necropsy

Route of inoculation	Dose	Number of animals	Degeneration and necrosis	Abscess	Granulation tissue
i.m.	10	4	— ^{a)} { + ^{b)}	—	—
	10 ³	4	— { +	—	—
	10 ⁵	4	— { ++ ^{c)}	—	++
	10 ⁷	3	+++ ^{d)}	+++	+++
s.c.	10	4	—	—	—
	10 ³	4	—	—	—
	10 ⁵	4	+	+	++
	10 ⁷	4	+++	++ { +++	+++

a)–: No lesion. b)+: Slight lesion. c)++: Moderate lesion. d)+++ : Severe lesion.

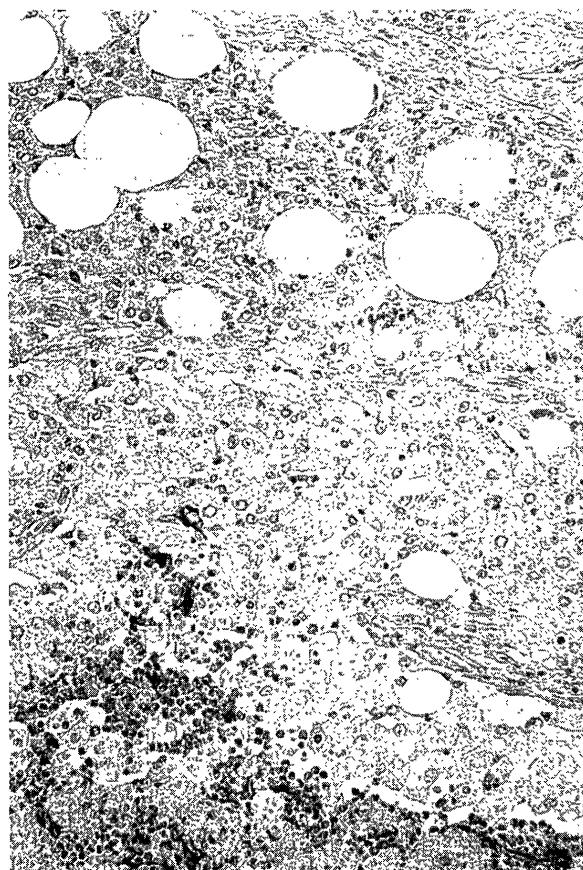


Fig. 2. Histopathological lesions of the inoculation site. Large granulomatous abscess in a hamster inoculated i.m. with 10⁷ bacteria. (H-E, × 380).

might be required to form lesions in Syrian hamsters. Infection of mice and rats with the organism is usually subclinical, and can be unmasked by transport stress [16], X-irradiation [15], malnutrition [24], coinfection [12] with other pathogens or treatment with cortisone [13, 19] or ACTH [23]. Further studies concerning such infection promoting factors are needed to clarify the pathogenicity of *C. kutscheri* in Syrian hamster.

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Table 4. Bacteriological and serological surveys in Syrian hamsters inoculated with *C. kutscheri*

Route of inoculation	Dose	Number of animals	Recovery of <i>C. kutscheri</i> ^{a)} from								Antibody ^{b)} titer at 10 day p.i.
			Lesions	Oral cavity	Nasal cavity	Trachea	Lung	Liver	Kidney	Cecal content	
i.m.	10	4	.	—	—	—	—	—	—	<10 ^{2c)}	<1:5
	10 ³	4	.	—	—	—	—	—	—	<10 ²	<1:5
	10 ⁵	4	.	—	—	—	—	—	—	<10 ²	<1:5 } 1:40
	10 ⁷	3	++++	—	—	—	—	—	—	<10 ²	1:20 } 1:320
s.c.	10	4	.	—	—	—	—	—	—	<10 ²	<1:5
	10 ³	4	.	—	—	—	—	—	—	<10 ²	<1:5
	10 ⁵	4	— } ++	—	—	—	—	—	—	<10 ²	<1:5 } 1:20
	10 ⁷	4	— } ++++	— } +	— } +	—	—	—	—	<10 ² } 2×10 ²	1:20 } 1:40

a) No. of colonies developed on isolation culture. ∴ No tubercle, —: Negative, +: 1–10, ++: 11–50, ++++: >100. b) Determined by a microtiter agglutination test. c) CFU/g.

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