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Serological Surveys of *Corynebacterium kutscheri* Infection in Mice and Rats

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Serological surveys of mice and rats naturally infected with *Corynebacterium kutscheri* were performed by examining serum samples collected from breeder and laboratory colonies between 1981 and 1983. Among 756 mice from 73 conventional colonies, only 4 animals (0.5%) from 3 colonies (4.1%) developed *C. kutscheri* antibody of 1:40 to 1:2,560 titers. Three of them suffered from abscess caused by the organism. Regarding a titer of 1:40 or higher as reliably positive, 87 (13.0%) of 669 conventional rats or 20 (32.8%) of 61 colonies were found to be infected with the organism. The antibodies were detected in both types of animals older than 6 months of age. No lesions caused by *C. kutscheri* were found in almost all the rats examined. Germ-free and SPF mice and rats were all negative for antibody at 1:5 serum dilution.

Corynebacterium kutscheri infection in laboratory mice has been reported by numerous investigators [1, 2, 10, 11, 12, 14, 15, 17], since it was first reported by Kutscher in 1894 [8], and in Japan since it was described by Shimizu *et al.* in 1932 [11]. The organism has been isolated from spontaneously infected rats [3] and from those administered with steroids [13] and ACTH [16]. Therefore, *C. Kutscheri* is commonly recognized as an important pathogen of mice and rats.

On the other hand, a serological checking method using the agglutination test has been established for *C. kutscheri* infection in mice by Fujiwara [4, 5] using a non-autoagglutinable strain CK1, isolated from a liver abscess of a mouse [17]. This method has been the prevalent one in this country for the seromonitoring of *C. kutscheri* infection and many serological

surveys were made in both mice and rats in the 1970 [6, 7]. However, no recent information on this infection has been available over the last several years. In the present study, seromonitoring of *C. kutscheri* infection was performed to elucidate the recent status of the pathogen in breeder and laboratory mouse and rat colonies.

Mice and rats, germ-free, specific pathogen-free (SPF) and conventional, were collected from commercial breeder and laboratory colonies during the period from 1981 through 1983. They included dd, C3H/He, C57BL/6, C57BL/10, DBA/2, CBA, BALB/c and A/J mice, and Wistar, SD, Brown Norway, Fisher, Donryu, SHR and Long-Evans rats. Their ages ranged from 8 weeks to 6 months or older (retired). Blood samples were taken by heart puncture. The serum was diluted to 1:5 with

phosphate-buffered saline (PBS, pH 7.2) and heated at 56°C for 30 min. The agglutination test was performed in V-typed microplates (Cooke Engineering Co.) by the use of commercial *C. kutscheri* antigen (Denka Co.), which had been prepared from Todd-Hewitt liquid medium culture of the CK1 strain by killing with 0.5% formaldehyde and adjusting to a density of 0.4 at OD 650. A mixture of serum and antigen, 0.025 ml of each per well, was incubated at 37°C for 2 h and then at 4°C overnight for reading.

Prior to the adoption of a microtitration method, it was confirmed that this method was almost equivalent to the tube method [15] for

the reading of antibody titers.

A total of 1,160 mice were examined for *C. kutscheri* antibody. Five of them were derived from 1 germ-free colony, 399 from 21 SPF colonies and 756 from 73 conventional colonies. The antibodies were detected in only 4 mice obtained from 3 conventional colonies. Three of these seropositive mice were suffering from subcutaneous or visceral abscesses from which *C. kutscheri* was isolated in pure culture by the method described previously [9], but one seropositive mouse appeared healthy. Antibody titers of the 3 diseased mice were from 1:1,280 to 1:2,560 and that of the healthy mouse was 1:40. These seropositive mice were

Table 1. Detection and distribution of antibody titers to *Corynebacterium kutscheri* in some conventional rat colonies.

Colony No.	No. of Rats		Distribution of antibody titer											
	Examined	Positive	%	1:5	10	20	40	80	160	320	640	1280	2560	5120
1	20	19	95							1	4	2	9	3
2	2	2	100						1		1			
3	11	11	100			1			1	6	2			1
4	7	5	75			2	3							
5	10	9	90	2	2	1	1		1		1	1		
6	20	13	65	3	3	2	3	2						
7	12	7	58		2		2		1	2				
8	5	5	100							1	1	2	1	
9	10	8	80		5	2					1			
10	8	8	100		4	1	2		1					
11	5	5	100			1			2		2			
12	10	8	80	2	1	1		1	1	2				
13	3	3	100						2		1			
14	20	10	50	4	5	1								
15	11	4	37	2	1	1								
16	25	14	56	3	10	1								
17	7	6	86		5	1								
18	20	18	90	12	6									
19	3	2	67		2									
20	10	9	90	7	2									
21	7	5	71	1	4									
22	14	3	21	2	1									
23	10	2	20		2									
24	10	1	10		1									
25	7	7	100	1	6									
26	11	4	36		4									

Rats older than 6 months of age, were tested.

Antibody titers of 1:5 or higher were preliminarily regarded as positive

older than 1 year of age. On the other hand, no antibody was detected at a serum dilution of 1:5 in all the mice from the germ-free and SPF colonies.

As for rats, 7, 439 and 669 animals were obtained from 2 germ-free, 33 SPF and 61 conventional colonies, respectively. Although no antibody was detected in any of the germ-free and SPF rats, many conventional rats developed antibodies with various titers. In many colonies, however, the antibody titers of seropositive rats did not vary, showing low levels such as 1:5 or 1:10. Some examples of the distribution of antibody titers in rat, especially older than 6 months of age, are shown in Table 1. Antibodies with significantly high titers were detected in many seropositive rats obtained from colony nos. 1 to 13, showing a maximum titer of 1:5,120. The frequency of the antibody was more than 58% in these colonies, when titers of 1:5 or higher were regarded as positive. Although the antibody was found as frequently as in 10 to 100% of animals from colony nos. 14 to 26, their antibody titers were 1:5, 1:10 or 1:20, and never exceeded 1:20. However, the results

shown in Table 1 were, obtained from surveys of rats older than 6 months of age or retired, and never from the young of 8 weeks or more ages. From these results, it was considered that antibody titers of 1:20 or lower did not always indicate *C. kutscheri* infection in rats. Therefore, in the case of rats, antibody titers of 1:40 or higher were regarded as a reliable indication of *C. kutscheri* infection.

Results obtained from serological surveys of mice and rats for infection with *C. kutscheri* are summarized in Table 2. According to the criterion mentioned above, 87 or 13%, of 669 conventional rats were found to be reliably positive and positive rats were obtained from 20 or 32.8%, of 61 rat colonies. Many seropositive rats were older than 6 months of age.

In order to compare the findings of gross lesions, organisms and antibodies, 192 rats derived from 15 colonies positive for *C. kutscheri* infection were examined by autopsy, cultivation of the organism and serological test. The results are shown in Table 3. Lesions caused by *C. kutscheri* were detected in 3 or 1.6%, of animals suffering from pneumonia or abscess from which the organism was isolated

Table 2. Summary of serological surveys of *Corynebacterium kutscheri* infection in mice and rats.

Animal	Bacteriological grade of animal	No. Examined		No. Positive*			
		Colony	Animal	Colony	%	Animal	%
Mouse	Germ-free	1	5	0	0	0	0
	SPF	21	399	0	0	0	0
	Conventional	73	756	3	4.1	4	0.5
Rat	Germ-free	2	7	0	0	0	0
	SPF	33	439	0	0	0	0
	Conventional	61	669	20	32.8	87	13.0

*Antibody titers of 1:5 or higher in mice, and 1:40 or higher in rats, were regarded as positive. Germ-free and SPF animals were all negative at 1:5 serum dilution.

Table 3. Comparison of findings on gross lesion, organism and antibody in rats derived from colonies positive for *Corynebacterium kutscheri* infection.

No. Examined		No. Positive			
Colony	Animal	Lesion (%)	Organism from		Antibody*(%)
			Lesion (%)	Caecum (%)	
15	192	3 (1.6)	3 (1.6)	8 (4.2)	74 (38.5)

*Antibody titers of 1:40 or higher were regarded as positive.

in pure culture. These animals developed antibody titers of 1:2,560 to 1:5,120. By cultivation of the caecal content, 8 or 4.2%, were positive for the organism, but did not show any significant development of the antibody, being limited to low levels such as 1:5 or 1:10. However, the total frequency of the antibody detection among these 192 rats was 74 or 38.5%. In addition, 206 conventional rats from 17 colonies as well as 7 germ-free and 254 SPF rats, were all negative for evidence of the infection on gross lesion, organism and antibody.

Compared with some results reported by previous investigators [4, 5, 6, 7], the present studies indicated that the frequency *C. kutscheri* infection might not have altered in mice but might have increased in rats in recent years.

In the case of rats, we regarded antibody titers of 1:40 or higher as being reliably positive judging from seroepidemiological data. Although low titers such as 1:5 or 1:10 might indicate positive candidates, they could not be regarded as evidence of infection in serodiagnosis. In spite of this rather severe criterion, 32.8% of conventional rat colonies and 38.5% of rats obtained from positive colonies were obviously seropositive for *C. kutscheri* infection. From these results, it seems that great attention must be paid to *C. kutscheri* infection in rat colonies.

In our previous study, we examined *C. kutscheri* infection by the cultivation of fecal content, and showed that the frequency of positive animals was only 0.3% in mice and 3.6% in rats, respectively (unpublished data).

In the present study, macroscopic lesions caused by *C. kutscheri* infection were rarely found in seropositive rats, although a good correlation was observed between serological results and postmortem findings in mice. Detection of *C. kutscheri* by cultivation in rats was also low, showing 1.6% from lesions and 4.2% from caecal content. Although *C. kutscheri* infection without significant development of the

antibody existed in some rats, only when the organism was present in the caecum, the frequency of the antibody detection in rats from positive colonies was as high as 38.5%. Therefore, serological examination was considered to have a great advantage as compared with bacteriological and pathological examinations for detection of *C. kutscheri* infection, especially in rats.

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マウスおよびラットにおけるネズミコリネ菌感染の 血清学的調査

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マウスおよびラットのネズミコリネ菌 (C, k) 感染の現状を知る目的で、1981年から1983年にかけて抗体調査を行った。凝集試験は市販 C, k 抗原を用いて、マイクロ法で実施した。コンベンショナルマウスは73コロニー、756匹中3コロニー (4.1%)、4匹 (0.5%) に抗体が検出され、40-2,510倍の抗体価を示した。それらのうち3匹の抗体陽性マウスは C, k による膿瘍がみられた。

コンベンショナルラットでは40倍以上の抗体価を陽性と判定すると、61コロニー、669匹中20コロニー (32.8%) の87匹 (13.0%) に感染が認められた。抗体は両動物の6ヵ月齢以上で検出された。一方、無菌およびSPFマウスでは、抗体はすべて5倍以下で陰性であった。ラットの C, k 感染において、病巣および菌検出率は非常に低かったが、抗体は有意に検出された。