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Characterization and Pathogenicity of *Yersinia pseudotuberculosis* Isolated from Swine and Other Animals

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Yersinia pseudotuberculosis serogroup III is most frequently detected in healthy pigs. *Y. pseudotuberculosis* strains were divided into two biogroups on the basis of their fermentation of melibiose. Melibiose-fermenting strains were distributed in all the hosts and serogroups examined. Melibiose-nonfermenting strains were limited to serogroup III isolated from healthy pigs. Autoagglutination and calcium dependency were not related to fermentation of melibiose. Melibiose-fermenting strains seem to be virulent for mice, but melibiose-nonfermenting strains showed no virulence for mice.

Systematic bacteriological examinations of pigs have confirmed that the pig is a symptomless carrier not only of *Yersinia enterocolitica* (1, 4, 7, 14–16, 19, 20, 21, 23) but also of *Yersinia pseudotuberculosis* (16, 18, 21–23).

Serogroup III strains of *Y. pseudotuberculosis* have rarely been isolated from either humans or animals (11, 13, 17; E. Thal, Ph.D thesis, Lund, Sweden), but recent investigators (16, 18, 23) showed that the serogroup isolated from healthy pigs was usually serogroup III.

We (18) reported that about half the number of the strains of serogroup III originating in healthy pigs did not ferment adonitol and melibiose. Mair et al. (12) also found that all serogroup III strains isolated from pigs and other sources did not ferment melibiose, and serogroup III porcine strains were avirulent and atoxic compared with strains of serogroup III isolated from other sources or serogroup I from healthy pigs.

In the present paper, we describe the sources and serogroups of *Y. pseudotuberculosis* isolated in Japan. The biochemical and serological characteristics, susceptibility to ampicillin, and pathogenicity of the strains originating in healthy pigs are given attention.

MATERIALS AND METHODS

Strains used. Two hundred and twenty-five strains were used. Of these strains, 197 were isolated in Japan, and the remaining 28 were supplied by European investigators (Table 1).

Biochemical reactions. All 225 strains were characterized by the conventional methods and media used in enterobacteriology (2). Incubation was carried out at 37 and 25°C. *Y. pseudotuberculosis* was grouped by fermentation of melibiose.

Serological test. Serological typing was performed with O antisera. Single-factor sera of IA, IB, IIA, IIB, IVA, IVB, VA, and VB serogroups were prepared by cross adsorption methods with the antiserum and antigen of each serogroup (17). Antisera of serogroups III and VI were used as the unabsorbed sera.

Susceptibility to ampicillin. A loopful of a 24-h tryptosoya broth (Nissui Pharmaceutical Co., Tokyo, Japan) culture of a test strain was streaked onto heart infusion agar (Nissui) plates containing serial twofold dilutions of sodium ampicillin (Toyama Chemical Industrial Co., Tokyo, Japan). After incubation at 25°C for 40 h, the MIC was determined.

Autoagglutination test. Autoagglutination tests for the strains were performed according to the method of Laird and Cavanaugh (10). The organisms (10^6 cells) were suspended in 2 ml of tissue culture medium (medium RPMI 1640 with 10% calf serum and 25 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid]) in each of two tubes. One tube was incubated for 18 h at 37°C, and the other was incubated for the same time at 25°C. A positive reaction was scored when bacterial agglutination occurred at 37°C but not at 25°C.

Calcium dependency. Magnesium oxalate agar, as described by Higuchi and Smith (5), was used. Strains were spread onto duplicate magnesium oxalate agar plates and incubated at 37 and 25°C, respectively. Calcium dependency was determined when an evident difference was found in the size and number of colonies after incubation at 37°C.

Mouse inoculation. Seventeen representative strains of serogroup III were used. They consisted of 7 strains which fermented melibiose and 10 strains which did not ferment melibiose. Three ICR mice weighing 20 to 22 g were inoculated intraperitoneally with 10^7 organisms of each strain. Survivors were killed after 21 days. Mice were examined for macroscopic lesions, and samples were taken for bacteriological examination.

RESULTS

Frequency of detection of *Y. pseudotuberculosis* and serogroups from healthy pigs. A total of 197 strains isolated from individual cases in Japan are shown in Table 1. The strains isolated from humans, monkeys, goat, rabbits, and guinea pigs were from ill humans and animals, whereas the strains isolated from pigs, dogs, cats, and rats were from healthy animals, except for three strains from pigs. Many more strains were recovered from pigs, possibly because more surveillance had been performed on them. Many strains belonged to serogroup III, followed by serogroups IVB and IB; in particular, serogroup III was the predominant one isolated from healthy pigs.

Biogrouping of *Y. pseudotuberculosis*. Although the biochemical characteristics of the 225 strains used were mainly identical with those of *Y. pseudotuberculosis*, melibiose-nonfermenting strains were also present. Fermentation of melibiose was closely related to sources or serogroups of the strains. The melibiose-fermenting strains were distributed

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TABLE 1. Sources and serogroups of *Y. pseudotuberculosis* strains used

Source	No. of strains and serogroup									
	IA	IB	IIA	IIB	III	IVA	IVB	VA	VB	VI
Japanese strains										
Human		4	1	2	6		11	8	6	
Monkey		16					3			
Pig		8[a]		1	51		11		1	
Goat		1								
Dog		7					10			
Cat		1			4		11			
Rabbit		3		1	1		2		9	
Guinea pig		2			2			3		3
Rat		1				7				
European strains										
Human								1		
Rabbit	2	1	1							
Hare	1	1								
Guinea pig	1							4		
Turkey		1								
Pigeon	5	2			1					
Duck	2	1						4		

[a] Including three morbid cases.

most frequently in all hosts and serogroups. Melibiose-nonfermenting strains were limited to only serogroup III derived from healthy pigs, except one strain of human origin (Table 2).

It seems that the distribution of melibiose-fermenting strains varied geographically in Japan. For instance, a rate of detection of the melibiose-fermenting strains was 10.7% (3 of 28) in Tokyo, 46.2% (6 of 13) in Fukuoka, and 0% (0 of 6) in Tottori Prefecture.

Susceptibility to ampicillin. The majority of the organisms (80.9%; 182 of 225) were susceptible to ampicillin and had a peak MIC of ≤0.39 μg/ml. Four strains that were resistant to ≥100 μg/ml of the drug existed. They did not ferment melibiose.

Autoagglutination and calcium dependency. The majority of the strains of serogroup III autoagglutinated and were calcium dependent. These characteristics did not relate to fermentation of melibiose (Table 3). Autoagglutination and calcium dependency of the strains were not always in accord in the present study. Three strains autoagglutinated and were not calcium dependent, and five did not autoagglutinate and were calcium dependent.

Virulence for mice. Virulence for mice by intraperitoneal inoculation correlates with fermentation of melibiose. Six of seven melibiose-fermenting strains killed mice within 20 days. Necropsy showed enlarged spleens, and many various-sized grayish-white foci were present in the spleens and livers; numerous colonies of the inoculum were recovered from viscera and heart blood. Ten melibiose-nonfermenting strains did not kill mice. Most of the mice that were sacrificed 21 days after inoculation showed no macroscopic lesions and no recovery of inocula. One each of the mice inoculated with strains T312 and Tp1030 revealed grayish-white foci in the liver (T312) and an enlarged spleen (Tp1030), and inocula were detected from these organs.

The findings show that fermentation of melibiose was related to virulence for mice. However, virulence for mice does not always seem to be related to autoagglutination and calcium dependency.

DISCUSSION

In recent studies, it was shown that *Y. pseudotuberculosis* was retained by healthy pigs; moreover, a large number of strains isolated from these animals belonged to serogroup III (16, 18, 23). The above findings were confirmed by the present study. Out of 197 strains of *Y. pseudotuberculosis* detected in Japan, 69 strains were isolated from healthy pigs. They were classified into five serogroups: IB, IIB, III, IVB, and VB. Serogroup III was predominant.

The strains used in this study were isolated in a wide variety of districts in Japan, and sources of the strains were not limited to specific areas or specific farms. In the United States (3) and Canada (16), all *Y. pseudotuberculosis* strains isolated from feces or alimentary tracts of healthy pigs belonged to serogroup III. In Germany, however, serogroup III strains were not always frequently detectable from feces of healthy pigs (21); moreover, strains isolated from tonsils of healthy slaughter pigs fell into serogroups I and II, and none of serogroup III was detected (22). These findings suggest that the frequency of isolation of serogroup III from healthy pigs is different among countries.

The epidemiology of *Y. pseudotuberculosis* has not been established. Kanazawa et al. (9) isolated a serogroup III strain from a housewife, and they suspected contaminated pork to be the vehicle since she often ate this meat. This is the only case of isolation of a melibinose-nonfermenting strain from a host (human) other than healthy pigs (Table 2). This finding supports their speculation that the infectious source might be pork.

Mair et al. (12) described 19 porcine strains of serogroup III from Great Britain, Australia, and Canada that did not ferment melibiose at 37 and 30°C. Thus, further study was needed on whether melibiose-fermenting strains were really limited to Japan.

TABLE 2. Relationship between sources, serogroups, and melibiose fermentation of *Y. pseudotuberculosis* strains

Source	Serogroup	Melibiose fermentation (no. of strains)	
		+	−
Pig	III	12	39
	Other than III	21[a]	
Other than pig	III	13[b]	1[c]
	Other than III	139	

[a] Serogroups IB (8 strains). IIB (1 strain), IVB (11 strains), and VB (1 strain).

[b] Human (five strains), cat (four strains), guinea pig (two strains), rabbit (one strain), and pigeon (one strain).

[c] Human.

TABLE 3. Relationship between autoagglutination, calcium dependency, and melibiose fermentation of serogroup III strains of *Y. pseudotuberculosis*

Source	No. of strains						
	Melibiose fermentation		Autoagglutination			Calcium dependency	
	+	−	+	−	F[a]	+	−
Healthy pig	12		9	0	3	10	2
Other than pig[b]	13		12	0	1	11	2
Healthy pig		39	26	8	5	32	7
Human		1	1	0	0	1	0

[a] False; agglutinated at both 37 and 25°C.

[b] Human (five strains), cat (four strains), guinea pig (two strains), rabbit (one strain), and pigeon (one strain).

Y. pseudotuberculosis was susceptible to benzylpenicillin, whereas *Y. enterocolitica* was resistant to the drug (8). Also, in our experiment, the majority of the organisms were susceptible to ampicillin. However, some melibiose-nonfermenting strains are resistant. Although the mechanism was not investigated, it is suggested that they possess penicillinase activity, as has been widely observed in *Y. enterocolitica* (8).

Mair et al. (12) reported that the serogroup III strains of porcine origin were avirulent and atoxic. These strains did not ferment melibiose. In the present study, the serogroup III strains isolated from healthy pigs were divided into two groups on the basis of pathogenicity for mice. It seems that the property of fermentation of melibiose was closely related to virulence for mice. Most melibiose-fermenting strains were virulent for mice, whereas melibiose-nonfermenting strains were avirulent for mice. On the other hand, however, one strain (Tateishi) isolated from a patient suffering from terminal ileitis (9) did not ferment melibiose and was avirulent for mice. Consequently, the mouse virulence test used in this study may be inadequate for evaluation of pathogenicity of *Y. pseudotuberculosis*.

Laird and Cavanaugh (10) reported that virulence of *Yersinia pestis*, *Y. pseudotuberculosis*, and *Y. enterocolitica* for mice was related to autoagglutination. In the present study, there was no relation between virulence for mice autoagglutination or calcium dependency. Although the reason for this difference is unknown, it may involve the route of inoculation and dose of the inocula.

Recently, a plasmid associated with the production of V and W antigens (calcium dependency) and with pathogenicity of *Y. pseudotuberculosis* was reported (6). As mentioned above, there was no relationship between fermentation of melibiose and autoagglutination or calcium dependency. Further study is necessary on the pathogenicity of serogroup III strains originating in healthy pigs, on plasmids, and on the property of melibiose fermentation.

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