

# Bacterial Isolation from Slaughtered Pigs Associated with Endocarditis, Especially the Isolation of *Streptococcus suis*

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**ABSTRACT.** Bacterial isolation from slaughtered pigs with endocarditis was carried out from 1985 to 1994. A total of 495 (0.025%) out of 2,006,127 pigs were diagnosed as having endocarditis. Though bacteria were significantly isolated from 399 of the 495 pigs, bacteria could not be isolated in 96 pigs (19.4%). In 11 pigs, 2 bacterial species were isolated from heart lesion. *Streptococcus suis* was isolated from 127 cases (25.7%), *Streptococcus dysgalactiae* from 75 (15.2%), *Erysipelothrix rhusiopathiae* from 63 (12.7%), *Actinomyces pyogenes* from 39 (7.9%), *Pasteurella multocida* from 11 (2.2%), *Staphylococcus aureus* from 10 (2.0%), and *Streptococcus porcinus* from 8 (1.6%). Among the 99 isolates biochemically identified as *S. suis*, the major serotype was *S. suis* type 2 (35.4%). The remainder of the typable isolates were identified as serotypes 1/2 (2.0%) and 9 (1.0%). A total of 61 isolates (61.6%) were untypable. — **KEY WORDS:** bacterial endocarditis, swine (slaughtered), *Streptococcus suis*.

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*Erysipelothrix rhusiopathiae*, *Actinomyces pyogenes* and streptococci are well known as causative agents of valvular-endocarditis in pigs. In the field, valvular-endocarditis is sometimes found in pigs which die suddenly after mixing or transporting [3]. During routine meat inspections of slaughter houses, however, valvular-endocarditis is frequently detected in pigs. In such cases, abattoirs suspect erysipelas and carry out bacterial examinations. In Japan, the isolation rate of *E. rhusiopathiae* from endocarditis in slaughtered pigs has been decreasing yearly, while that of the *Streptococcus* species has been gradually increasing since the 1970s [19].

Among the *Streptococcus* species, *Streptococcus suis* has lately attracted considerable attention in most countries with a developed swine industry [4, 6, 17, 20]. *S. suis* causes meningitis, septicemia, endocarditis, arthritis and pneumonia in mainly piglets and growing pigs [1, 2, 7, 10]. Endocarditis in particular, is frequently found in slaughtered pigs [11].

There are no detailed studies of streptococcal endocarditis in pigs and there is very little information available about its occurrence in Japan. In this paper, we carried out bacteriological examination of isolates from endocarditis in slaughtered pigs sampled for 10 years, and further examination of *S. suis* isolates.

From 1985 to 1994, 495 out of 2,006,127 slaughtered pigs were diagnosed with bacterial endocarditis during routine meat inspections at the Sendai Meat Inspection Center. A total of 410 isolates from 399 pigs were used in this study, 22 of which were isolated from eleven cases of mixed infection.

Bacterial isolation was carried out on Trypticase soy agar (Eiken Chemical Co., Ltd., Japan) containing 5% defibrinated horse blood. The plates were incubated in an atmosphere containing 5% CO<sub>2</sub> at 37°C for 48 hr, and in an anaerobic system using Gas Pak (BBL, U.S.A.) at 37°C for 72 hr. Bacterial isolates were identified by biochemical tests using commercial kits. First, Gram staining of bacteria

isolated from heart lesions was carried out, followed by catalase and oxidase reduction tests. Gram-negative rods, such as enterobacteriaceae to the API 20 E system (bioMérieux-Vitek Japan, Ltd., Tokyo), non-enterobacteriaceae to the API 20 NE system (bioMérieux-Vitek Japan, Ltd.); Gram-positive cocci, staphylococci to the API Staph system (bioMérieux-Vitek Japan, Ltd.), streptococci to the API 20 STREP system (bioMérieux-Vitek Japan, Ltd.); and anaerobic bacteria to the AN-IDENT system (bioMérieux-Vitek Japan, Ltd.) were all applied according to the instructions of the manufacturer. Gram-positive rods, especially *E. rhusiopathiae* and *A. pyogenes*, were examined for motility and H<sub>2</sub>S production in SIM agar (Eiken Chemical Co., Ltd., Japan), bottle-brush type growth and gelatin liquefaction in nutrient gelatin agar, and characteristic reactions in TSI semi-slant agar (Eiken Chemical Co., Ltd., Japan).

Type specific antisera for *S. suis* types 1 to 28 were prepared using rabbits as described by Lancefield [12]. The organisms were inoculated in Todd-Hewitt broth (Difco, U.S.A.) and incubated at 37°C for 24 hr. After incubation, capsular polysaccharide antigen was extracted from the bacterial cells by autoclaving [15] and serological typing was determined by the capillary precipitation test [12].

Over the 10-year survey period, 495 (0.025%) out of 2,006,127 slaughtered pigs were found to have valvular endocarditis. Bacteria could only be isolated from 399 pigs. In 11 pigs, 2 bacterial species were isolated from heart lesions. The yearly incidence of endocarditis did not differ significantly (Table 1).

In the 495 endocarditis cases, *S. suis* was the most common bacteria detected and was isolated from 127 pigs (25.7%), followed by *Streptococcus dysgalactiae* from 75 pigs (15.2%), *E. rhusiopathiae* from 63 (12.7%), *A. pyogenes* from 39 (7.9%), *Pasteurella multocida* from 11 (2.2%), and *Staphylococcus aureus* from 10 (2.0%). Other bacterial species isolated included *Streptococcus porcinus*, *Actinobacillus pleuropneumoniae*, *Escherichia coli*,

Table 1. Incidence of endocarditis in slaughtered pigs and isolation of bacterial species

Year	pigs tested	Number of pigs with endocarditis	Isolation of bacterial species								None
			<i>S. suis</i>	<i>S. dysgalactiae</i>	<i>E. rhusiopathiae</i>	<i>A. pyogenes</i>	<i>P. multocida</i>	<i>S. aureus</i>	<i>S. porcinus</i>	Others	
1985	210,181	39 (0.019%)	2	6	2	6	2	2	1	10	9
1986	197,910	41 (0.021%)	9	5	1	3			1	17	5
1987	215,053	53 (0.025%)	6	9 (1)	6	3		1	2	11 (1)	16
1988	219,718	59 (0.027%)	12**	11 (1)	8 (1)	7		1		12	9
1989	221,996	53 (0.024%)	13**	9 (1)	8 (1)	5	3	2 (1)	1 (1)	3 (2)	12
1990	231,616	47 (0.020%)	13**	7	8	1	1	3	1	6	7
1991	206,231	53 (0.026%)	16** (1)*	10 (1)	6	6 (1)	1		1	3 (1)	12
1992	183,642	50 (0.027%)	22** (2)	4 (1)	10 (1)	1	2	1		5	7
1993	164,849	62 (0.038%)	24**	8	6	3 (1)	1		2 (1)	5	14
1994	154,931	38 (0.025%)	10**	6	8	4 (1)	1 (1)			5	5
Total	2,006,127	495 (0.025%) (11)*	127 (3) 25.7%	75 (5) 15.2%	63 (3) 12.7%	39 (3) 7.9%	11 (1) 2.2%	10 (1) 2.0%	8 (2) 1.6%	77 (4) 15.6%	96 19.4%

Others: *S. equinus*, *A. pleuropneumoniae*, *E. coli*, *S. hyicus*, *F. necrophorum*, *A. suis*, *E. faecalis*, etc.

None: No bacterial isolation from lesions of endocarditis.

\*: Number of pigs with mixed infection.

\*\*  $P < 0.05$  as compared with the incidence of *S. suis* endocarditis in 1985.

Table 2. Monthly distribution of bacterial isolates from endocarditis

Bacterial species	Number of isolates	Month											
		1	2	3	4	5	6	7	8	9	10	11	12
<i>S. suis</i>	127 (3)*	12	11	9	16 (1)	8	8	7 (2)	5	8	11	14	18
<i>S. dysgalactiae</i>	75 (5)	4	5	12	10 (1)	8 (1)	5	5 (1)	8	5 (1)	6 (1)	1	6
<i>E. rhusiopathiae</i>	63 (3)	4	4	4	1	4 (1)	5	12 (1)	8	7	4 (1)	4	6
<i>A. pyogenes</i>	39 (3)	4	5 (1)	7 (1)	2	5	2	3	4	2	1		4 (1)
<i>P. multocida</i>	11 (1)		2	2 (1)		1	2	2	2				
<i>S. aureus</i>	10 (1)		4 (1)	1		2	2				1		
<i>S. porcinus</i>	8 (2)	1	2 (2)	1	1					1	2		
Others	77 (4)	9	9	7 (2)	9	6	5	6	7	8 (1)	3	2	6 (1)
None	96	7	7	6	6	9	16	4	8	13	6	10	4
Total	495 (11)	41	49 (2)	49 (2)	45 (1)	43 (1)	45	39 (2)	42	44 (1)	34 (1)	31	44 (1)

Others: *S. equinus*, *A. pleuropneumoniae*, *E. coli*, *S. hyicus*, *F. necrophorum*, *A. suis*, *E. faecalis*, etc.

None: No bacterial isolation from lesions of endocarditis.

\*: Number of pigs with mixed infection.

*Staphylococcus hyicus*, *Fusobacterium necrophorum*, *Actinobacillus suis*, and *Enterococcus faecalis*. Streptococci made up 51.5 percent of all endocarditis cases. The occurrence of endocarditis caused by *S. suis* markedly increased from 1988, and reached a peak from 1992 to 1993 ( $P < 0.05$ ). However, the isolation rates of the other bacteria did not fluctuate between 1985 and 1994. The number of isolates of the bacteria grouped as others decreased rapidly in 1989 (Table 1).

The monthly distribution of the bacterial isolation is shown in Table 2. The monthly incidence of endocarditis cases lacked diversity. The number of isolates of *S. suis* was lowest in August but comparatively high between October and April ( $P < 0.05$ ). That of *S. dysgalactiae* was high in March and April, while *E. rhusiopathiae* numbers increased in July and August. The isolation rates of *A.*

*pyogenes* fluctuated little between months (Table 2).

Of the 99 isolates biochemically identified as *S. suis*, only 38 (38.4%) could be serotyped with one of the antisera against serotypes 1 to 28. Of all the isolates, 35 (35.4%) were identified as serotype 2, with the remainder of the typable isolates identified as serotypes 1/2 (2.0%) and 9 (1.0%). A total of 61 isolates (61.6%) were untypable. The number of non-typable strains gradually increased year by year, reaching 70% (7 of 10 isolates) of all *S. suis* isolates in 1994 (Table 3).

The results of examinations using the API 20 STREP system are summarized in Table 4. Sixty eight of the 127 *S. suis* isolates (53.5%) were identified as either *S. suis* I and 59 strains (46.5%) were identified as *S. suis* II. However, 19 (54.3%) of 35 isolates serologically identified as *S. suis* type 2 were identified as *S. suis* I using this

Table 3. Serotyping of *Streptococcus suis* isolated from pigs associated with endocarditis

Year	Number of <i>S. suis</i> isolates	Number of strain tested	<i>S. suis</i> serotypes (%)			
			2	1/2	9	NT*
1985	2					
1986	9					
1987	6					
1988	12	6	3			3
1989	13	10	3		1	6
1990	13	11	5			6
1991	16	16	5			11
1992	22	22	9	1		12
1993	24	24	7	1		16
1994	10	10	3			7
Total	127	99	35 (35.4%)	2 (2.0%)	1 (1.0%)	61 (61.6%)

\* NT: Non typable strain.

Table 4. Identification of *Streptococcus suis* using API STREP 20 system

Identification	Number of <i>S. suis</i> isolates	Number of strain used serotyping	<i>S. suis</i> serotypes (%)			
			2	1/2	9	NT*
<i>S. suis</i> I	68 (53.5%)	51	19 (54.3%)			32
<i>S. suis</i> II	59 (46.5%)	48	16 (45.7%)	2	1	29
Total	127	99	35	2	1	61

\* NT: Non typable strain.

system.

This study has demonstrated that *S. suis* is a major causative agent of bacterial endocarditis in slaughtered pigs in Japan. While bacterial valvular-endocarditis is generally known to be caused by *E. rhusiopathiae*, the isolation rate of this bacteria from endocarditis did not change greatly during the 10-year period of this study. On the other hand, the detection of *S. suis* from swine endocarditis has gradually increased since 1988. This result agrees with our previous study [9], which showed that *S. suis* infection in the field has been increasing since 1988. This increase may be responsible for the increase in detectable bacterial endocarditis associated with this bacteria in meat inspections at slaughter houses.

Pederson *et al.* [13] reported on the bacteriological examination of endocarditis in slaughtered pigs in Denmark. According to their results, in the majority of cases, the causative agent of bacterial endocarditis was *E. rhusiopathiae*. The isolation rate of *E. rhusiopathiae* from endocarditis was 63.6%, while that of *S. suis* was only 10.3%. In another report from England, the majority of endocarditis cases, 23 (76.7%) out of 30, were caused by group C and L streptococci, namely *S. dysgalactiae*, while the isolation rate of *E. rhusiopathiae* was 20.0% [8]. These results, however, included clinical acute cases of bacterial endocarditis in the field. In just slaughtered pigs, *E. rhusiopathiae* was isolated from 2 (40.0%) out of 5 cases

and *S. dysgalactiae* from the remaining 3 (60.0%). Thus, the results of the present study showed a very high isolation rate of *S. suis*. In Japan, there have been few epidemiological studies of bacterial endocarditis in pigs. Tomita *et al.* [19] reported that streptococci and *E. rhusiopathiae* were isolated from most of the bacterial endocarditis cases in slaughtered pigs. Generally, the distribution of bacterial species from swine endocarditis varies according to the area, the age and the state of breeding, and consequently a wide range of data are reported.

Monthly incident rates of *S. suis* infection have also been reported by Higgins *et al.* [4] and by Reams *et al.* [16]. According to their results, the incidence of *S. suis* infection in pigs increased due to sudden changes in climate. The results of the present study also showed that the isolation rate of *S. suis* from endocarditis in slaughtered pigs was comparatively high between October and April. The monthly incidence of *S. suis* endocarditis each year also tended to be higher between October and April except for 1987. Although our results did not directly indicate the incidence of *S. suis* infection in the field, it was thought that chronic *S. suis* infection detected in slaughtered pigs occurred 2 to 4 weeks after sudden changes in weather. In contrast to the incidence of endocarditis associated with *S. suis*, it is notable that the detection of *E. rhusiopathiae* from endocarditis greatly increased in summer.

*S. suis* type 2 was the major serotype detected throughout the 10-year study period. This result agrees with other reports as well as our previous study on the epidemiology of *S. suis* infection in pigs [4, 9, 16]. It was, however, very interesting that more than half of the isolates from endocarditis were untypable strains. A similar result was obtained in our previous study [9]. Although a high percentage of isolates from meningitis (86.9%) and pneumonia (91.3%) could be serotyped, only 46% of the isolates from endocarditis could be serotyped. Higgins *et al.* [4] and Pederson *et al.* [13] also reported that most of the isolates from swine endocarditis were untypable. This suggests that the organism exists in a special lesion, such as a granuloma, of valvular-endocarditis for a long period, and that the capsular antigen of the surface of the organism changes or disappears. Although there are some reports on the properties of the capsule of *S. suis* [14, 18], the epitope of the capsular antigen is not clear at present.

The result of the identification of *S. suis* by using API 20 STREP was in accordance with the results of Hommez *et al.* [5] and our previous study [9]. Discrimination of *S. suis* type 1 from 2 is mostly based on the result of acidification from raffinose. However, most cases of *S. suis* type 2 (19 strains) could not be correctly identified. Therefore, it must be emphasized that only serological tests are suitable for *S. suis* serotyping classification.

It is important to continue the epidemiological study of *S. suis* infection in pigs, and to further investigate the epitope of capsular antigen and the pathogenesis of *S. suis*.

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