Serovars of *Erysipelothrix* Strains Isolated from Pigs Affected with Erysipelas in Japan

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ABSTRACT. Serovars of 1,046 *Erysipelothrix* strains isolated from diseased pigs during a period from 1983 to 1993 in Japan were determined by an agar gel double-diffusion precipitation system using typing sera representing all the known serovars, 1 through 23 and type N, of 2 species of *Erysipelothrix*. A total of 943 strains could be serotyped within the serovars. The remaining 103 strains could not be determined for their serovars and were classified as untypable. Of the 938 (99.5%) strains serologically identified as *Erysipelothrix rhusiopathiae*, 423 (40.4%) belonged to serovar 1a, 359 (34.3%) to serovar 2, 65 (6.2%) to serovar 1b, 35 (3.3%) to serovar 6, 19 (1.8%) to serovar 5, 11 (1.1%) to serovar 21, and the remaining 26 to serovars 4, 8, 11, 12, 15, 17, or 19. Only 5 (0.5%) strains isolated from the cases of erysipelas belonged to *Erysipelothrix tonsillarum* and represented serovars 7 or 23. — **KEY WORDS:** *Erysipelothrix*, Japan, serovar.

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The genus *Erysipelothrix* was originally considered to consist of a single species biochemically, morphologically identified as *Erysipelothrix rhusiopathiae* despite its wide range of hosts and large variety of serological characteristics [3]. The organism is a causative pathogen for swine erysipelas, which causes enormous economic losses in pig production. The clinical signs of swine erysipelas can be divided into three types: acute (septicemia), subacute (urticaria), and chronic (arthritis, lymphadenitis, and endocarditis). *E. rhusiopathiae* has also been found in the tonsils of healthy pigs, slime on fish, and other environments [15].

Recently, on the basis of the levels of deoxyribonucleic acid relatedness among *E. rhusiopathiae* strains, represented by all 23 serovars presently recognized, we [10, 11] reported that the genus *Erysipelothrix* contains two main genomic species, *E. rhusiopathiae* including serovars 1a, 1b, 2, 4, 5, 6, 8, 9, 11, 12, 15, 16, 17, 19, 21, and type N, (type N strains do not produce any precipitating antibody against homologous and heterologous heat-stable extracts in rabbits), and *Erysipelothrix tonsillarum* including serovars 3, 7, 10, 14, 20, 22, and 23. *E. tonsillarum* can be distinguished from *E. rhusiopathiae* by the fermentation of saccharose and its lack of pathogenicity for the pig, as well as by its serology.

Some investigators [2, 4, 7, 12] in Japan have reported the results of serological classification of the *Erysipelothrix* strains isolated from diseased pigs. These data, dealing with 50–270 strains isolated from a limited district, did not necessarily reflect the present situation of the serovar distribution in Japan. Little has therefore been published on the epidemiological significance of *E. rhusiopathiae* and *E. tonsillarum* strains from the perspective of field surveys, although our previous investigations [10, 11] demonstrated differences in pathogenicity among *Erysipelothrix* strains after experimental inoculation of pigs. The aim of this work was to investigate the distribution of serovars in relation to various pathological conditions in pigs and to elucidate the epidemiological significance of the two serologically distinguished *Erysipelothrix* species, *E.*

rhusiopathiae and *E. tonsillarum*, by examining serovars of a large number of clinical strains obtained from a wide area of Japan over the past 11 years.

A total of 1,046 *Erysipelothrix* strains were isolated from pigs suspected of erysipelas in 18 meat inspection offices and 9 livestock hygiene service centers in Aomori, Yamagata, Fukushima, Niigata, Tokyo, Kanagawa, Shizuoka, Nagano, Toyama, Aichi, Kyoto, Osaka, Kochi, Ehime, Kumamoto, Nagasaki, and Kagoshima prefectures from 1983 to 1993. The erysipelas included arthritis (731 cases), lymphadenitis (174 cases), endocarditis (62 cases), urticaria (57 cases), and septicemia (22 cases). The strains were maintained on Stock Culture Agar (Difco Laboratories, Detroit, Michigan) at 4°C.

The serovars of the strains were determined by using an agar gel double-diffusion precipitation system previously described [5, 12]. Colonies from a 48-hr agar plate of each strain were inoculated into Tryptose Phosphate Broth (Difco; pH 7.6) containing 0.1% Tween 80. After incubation for 24 hr at 37°C, the broth culture was centrifuged at 12,000 × g for 20 min. The bacterial cells were washed three times with physiological saline and suspended in distilled water at 1/30 of the original volume. The bacterial suspension was autoclaved for 1 hr at 121°C, cooled, and clarified by centrifugation. The supernatant fluid was tested against typing sera, self-prepared in rabbit, representing all the known serovars, 1 through 23 and type N, of the *Erysipelothrix* species.

For pathogenicity tests of the *E. tonsillarum* strains, we used 3-months-old female and castrated male Yorkshire pigs which were purchased from the Minano Agricultural Cooperative Association for Laboratory Animals, Saitama, Japan. The sera of the pigs had growth agglutination titers [8, 9, 14] of less than 1:8. The levels of strain pathogenicity for the pigs were determined as described previously [13].

The serovars of the 1,046 *Erysipelothrix* strains examined are shown in Table 1. Of the strains, 943 (90.2%) could be serotyped within 15 serovars. The remaining 103 strains (9.8%) could not be serotyped and were classified as untypable. Of the serologically distinguished strains, 938

(99.5%) were of the *E. rhusiopathiae* serovars, and 5 (0.5%) were of the *E. tonsillarum* serovars. Of the *E. rhusiopathiae* strains, 423 (40.4%) belonged to serovar 1a, 359 (34.3%) to serovar 2, 65 (6.2%) to serovar 1b, 35 (3.3%) to serovar 6, 19 (1.8%) to serovar 5, 11 (1.1%) to serovar 21, and the remaining 26 (3.1%) to serovars 4, 8, 11, 12, 15, 17 or 19. The relative frequency of isolation of serovars in each calendar year is shown in Table 2. Patterns of the frequency of isolation of the serovars varied among years, however, the most common serovars in each year were serovars 1 and 2. All the *E. tonsillarum* strains were isolated from cases of arthritis or lymphadenitis and represented serovars 7 or 23. In swine, none of the 5 *E. tonsillarum* strains induced any clinical signs after intradermal inoculation (data not shown).

With many pathogenic bacteria, serovar determination is useful for understanding the epidemiology of an outbreak,

for preparing vaccines for the control of the disease, and for serological monitoring of infected herds. The present investigation demonstrated the presence of a wide variety of serovars of *Erysipelothrix* strains isolated from cases of swine erysipelas. The serovars 15, 17, 19 and 23 identified in this study have not been previously recognized in Japan. It has been generally known that most strains of *E. rhusiopathiae* from pigs affected with swine erysipelas fall into serovars 1a, 1b and 2 [15]. Kucsera [6], Cross and Claxton [1], Takahashi *et al.* [12] and Kubo *et al.* [4] reported that only 0.5% to 7.7% of strains, obtained mainly from the cases of chronic erysipelas, belonged to rare serovars other than serovars 1 and 2. In this study too, 81.0% of 1,046 strains examined were identified as serovars 1 (46.6%) and 2 (34.3%), indicating that during the last 11 years they have continued to remain dominant in Japan. It

Table 1. Serovars of the 1,046 *Erysipelothrix* strains isolated from pigs affected with erysipelas during a period from 1983 to 1993 in Japan

Origin	<i>E. rhusiopathiae</i> serovars														<i>E. tonsillarum</i> serovars		Untypable ^{a)}
	1a	1b	2	4	5	6	8	11	12	15	17	19	21	7	23		
Arthritis	302 ^{b)}	51	252	3	9	15	3	5		1	1	1	8	3		77	
Lymphadenitis	67	12	43		6	18	1	2	2		1	1	1	1	1	18	
Endocarditis	33	1	18	1					2	1			2			4	
Urticaria	5	1	40		4	2		1								4	
Septicemia	16		6														
Total	423	65	359	4	19	35	4	8	4	2	2	2	11	4	1	103	

a) The strains which could not serotyped with typing sera representing all the known serovars were designated as untypable.

b) Number of strains.

Table 2. Relative frequency of isolation of the various serovars of *E. rhusiopathiae* and *E. tonsillarum* strains from the diseased swine

Serovars	Relative frequency (%) of isolation of serovars in each year										
	1983 (n=63)	1984 (n=138)	1985 (n=342)	1986 (n=164)	1987 (n=29)	1988 (n=20)	1989 (n=118)	1990 (n=56)	1991 (n=61)	1992 (n=2)	1993 (n=53)
<i>E. rhusiopathiae</i>											
1a	3.2	29.7	56.7	36.0	13.8	15.0	11.9	71.4	34.4	50.0	83.0
1b		10.1	3.2		13.8		19.5		21.3		
2	92.1	58.7	33.6	32.3	20.7		15.3	5.4	31.1	50.0	9.4
4				0.6	6.9		0.8				
5						10.0	5.1	8.9	9.8		
6		0.7	0.9	4.9	10.3	5.8	16.1				
8			0.6				1.7				
11			0.6	0.6	6.9		1.7		1.6		
12					6.9	5.0	0.8				
15								3.6			
17	1.6						0.8				
19							1.7				
21	1.6			0.6	3.4	15.0	0.8		1.1		5.7
<i>E. tonsillarum</i>											
7			0.3	0.6	3.4		0.8				
23					3.4						
Untypable	1.6	0.7	4.1	24.4	10.3	50.0	22.9	10.7			1.9

should be noted, however, that 96 (9.2%) strains belonging to 12 serovars other than serovars 1 or 2, and 103 (9.8%) untypable strains, which could not be serotyped with known typing sera, represented by serovars 1 through 23 and type N, were isolated from the cases of swine erysipelas. Such a rate of occurrence, higher than before, would suggest a possible role of these strains in the cause of clinical erysipelas in swine. According to a previous report [6], it would be supposed that type N of the *E. rhusiopathiae* group would comprise the majority of the untypable strains in this study. Further studies are needed on the serotyping of the untypable isolates by testing the heat-extracted antigen of the serovar reference strains with antisera prepared in rabbits against the untypable isolates.

In this study, we clarified that 99.5% of the typable strains from pigs with erysipelas can be assigned to the *E. rhusiopathiae* group. We also demonstrated that all the *E. tonsillarum* strains isolated from chronic cases could not experimentally induce any clinical sign of erysipelas in pigs. This observation suggests that other bacterial infections such as *Streptococcus* species, *Actinomyces pyogenes*, *Mycoplasma hyosynoviae*, viral infection or uninfected wasting disease [16] may have taken part in the few clinical cases where the *E. tonsillarum* strains were isolated. Actually, the *Erysipelothrix*-selective medium containing sodium azide and several antibiotics has been widely used for isolation from cases suspected of arthritis or lymphadenitis in meat inspection offices. It is possible that *E. tonsillarum* is also ubiquitous in the environment of slaughter-houses as a soil contaminant, and might constitute a contamination source in the process of the bacterial isolation. In any case, the present data, in conjunction with some differences in genetic and pathogenic properties previously published [11], indicate the possibility that *E. tonsillarum* holds little of etiological significance.

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