Pathogenicity of *Erysipelothrix rhusiopathiae* Strains of Serovars 1a, 3, 5, 6, 8, 11, 21, and Type N Isolated from Slaughter Pigs Affected with Chronic Erysipelas

Toshio TAKAHASHI, Takuo SAWADA, Kenji SETO¹⁾, Masatake MURAMATSU, Tsutomu MARUYAMA²⁾, and Masako KANZAKI²⁾

National Veterinary Assay Laboratory, Kokubunji, Tokyo 185, ¹⁾Nippon Institute for Biological Science, Ohme, Tokyo 198, and ²⁾Tokyo Metropolitan Research Laboratory of Public Health, Tokyo 160, Japan

(Received 1 June 1984/Accepted 25 September 1984)

ABSTRACT. *Erysipelothrix rhusiopathiae* strains of serovars 1a (17 strains), 3 (1), 5 (2), 6 (7), 8 (1), 11 (5), 21 (1), or type N (3) isolated from slaughter pigs affected with chronic erysipelas, mainly arthritis or lymphadenitis, were tested for pathogenicity in mice and swine. One or more strains of serovars 5, 6, 8, 11, and type N induced local urticarial lesions in swine. Strains of serovar 1a showed a wide variety of pathogenic characteristics in swine, namely, induced generalized urticarial lesions with systemic symptom, local urticarial lesions, or no clinical sign. Likewise, most strains of serovar 1a were weakly virulent or non-virulent for mice, whereas most strains of the other serovars were highly virulent for mice. The existence of serovar 1a strains being more pathogenic for swine than mice was found. On the other hand, all strains of serovar 1a obtained from the cases of acute septicemic erysipelas killed mice, and mostly caused death in the acute phase in swine.—**KEY WORDS:** pathogenicity, slaughter pigs, swine erysipelas.

—Jpn. J. Vet. Sci. 47(1): 1–8, 1985

Erysipelothrix rhusiopathiae, the causative organism of swine erysipelas are classified into 22 serovars and N type. It has been generally known that most isolates of *E. rhusiopathiae* from swine erysipelas fall into serovars 1a, 1b, and 2, namely, most isolates from the cases of septicemia belonged to serovar 1a, and from arthritis or urticaria belonged to serovar 2 [1, 3, 5, 6, 7, 9]. We previously [10] reported that of 258 *E. rhusiopathiae* strains from slaughter pigs affected with arthritis or lymphadenitis, 26 (10.1%) belonged to serovar 1a, and 20 (7.8%) belonged to serovars 3, 5, 6, 8, 11, 21, and type N. Strains of such serovars isolated from chronic swine erysipelas have not been tested for pathogenicity yet. The purpose of present investigation was to clarify the epidemiological significance of these strains of *E. rhusiopathiae* by determining their pathogenic characteristics for mice and swine.

MATERIALS AND METHODS

Bacterial strains: A total of 258 *E. rhusiopathiae* isolates used in our previous experiments [10] were submitted. They were isolated mainly from arthritic stifle joints or inflamed inside iliac lymph nodes of slaughter pigs from 1980 to 1982 in Japan. Thirty seven strains were used for the determination of pathogenicity for mice and swine. The serovars of isolates included serovars 1a (17 strains), 3 (1), 5 (2), 6 (7), 8 (1), 11 (5), 21 (1), and type N (3). The remaining 221 strains of serovar 1a, 1b, or 2 were used for the determination of pathogenicity only for mice. Nine strains of *E. rhusiopathiae* of serovar 1a obtained from the cases of acute septicemic erysipelas from 1981 to 1982 in Japan were also used for comparison with the pathogenicity of serovar 1a isolates from the cases of chronic erysipelas. Fujisawa strain of *E. rhusiopathiae* (serovar 1a),

which had been isolated from septicemic swine, was used as a virulent strain for challenge exposure.

Animals: Four-week-old female mice of the outbred ddY strain and 3-5 months old conventional Yorkshire swine were used. Sera of the swine showed growth agglutination titer [8] of 8 or below.

Determination of pathogenicity of strains: Five mice were inoculated subcutaneously in the left flank and 2 pigs were inoculated intradermally in the back region with 0.1 ml of 24-hour beef infusion broth culture of each isolate. Each inoculum contained about 10^6 or 10^7 viable organisms. The mice were observed every day for 14 days after exposure and responses were determined by the quantal (live-dead) method. In survived mice after inoculation, the occurrence of arthritis were examined, and these mice were challenge-exposed subcutaneously in the right flank with 0.1 ml of the broth culture (about 10^6 viable organisms) of strain Fujisawa in order to surmise the propagation of inoculated organisms in the mice.

Clinical signs in swine were observed every day for 14 days after exposure. Maximum size of local urticarial lesions in skin was measured. Survived animals were euthanatized and necropsied 21 days after exposure. In addition, stifle joints and inside iliac lymph nodes were opened for macroscopic examination, and bacterial isolation was done for the presence of *E. rhusiopathiae*.

RESULTS

Pathogenicity of *E. rhusiopathiae* strains of serovars 3, 5, 6, 8, 11, 21, and type N. Pathogenicity for mice and swine of 20 strains of serovars 3, 5, 6, 8, 11, 21, and type N isolated from pigs affected with chronic erysipelas is shown in Table 1. Eighteen strains of serovars 3, 5, 6, 8, 11, 21, and type N killed all mice within 10 days, usually within 3 to 5 days. The remaining 2 strains of serovar 5 and type N did not kill any mice.

However, none of these survived mice died after challenge exposure with the virulent strain Fujisawa.

In swine, 11 strains of serovars 5, 6, 8, 11, and type N induced local urticarial lesions at the site of inoculation 4 to 20 cm wide after intradermal exposure. Nine strains of serovars 3, 5, 6, 11, 21, and type N did not induce any clinical signs. At necropsy, stifle joints, inside iliac lymph nodes, and main systemic organs were macroscopically normal, and *E. rhusiopathiae* was not recovered from the joints and the lymph nodes.

Pathogenicity of *E. rhusiopathiae* strains of serovar 1a. Pathogenicity for mice and swine of 17 strains of serovar 1a isolated from pigs affected with arthritis or lymphadenitis of chronic erysipelas is shown in Table 2. Sixteen strains did not kill any mice, including 10 strains caused arthritis in one or more mice, and 6 strains did not cause any clinical signs in mice. The remaining one killed mice. All of the survived mice after inoculation with the 3 strains (491, 514, and 593), which had shown no clinical sign, died after challenge exposure with virulent strain Fujisawa. On the other hand, none of the survived mice inoculated with the other 13 strains died after challenge exposure.

In swine, however, 4 strains (547, 551, 552, and 530) induced generalized urticarial lesions with significant depression and anorexia after intradermal inoculation, in spite of their low virulence for mice. Eight strains (505, 528, 538, 914, S-13, 514, 592, and 593) induced local urticarial lesions 2 to 16 cm wide. The remaining 5 did not induce any clinical signs. At necropsy, stifle joints, inside iliac lymph nodes, and main systemic organs were macroscopically normal, and *E. rhusiopathiae* was not recovered from the joints and the lymph nodes.

Pathogenicity for mice and swine of 9 strains of serovar 1a isolated from pigs with septicemia of acute erysipelas is shown in Table 3. All strains killed mice within 10

Table 1. Pathogenicity of *E. rhusiopathiae* strains of rare serovars isolated from pigs with chronic erysipelas

Strain tested	Serovar	Origin	Pathogenicity for mice ^{a)}	Pathogenicity for swine	
				Erythema ^{b)}	Systemic
S-5	3	Arthritis	5/5	— ^{c)}	—
568	5	Arthritis	0/5	—	—
882	5	Urticaria	5/5	4×5 4×4	—
S-6	6	Arthritis	5/5	—	—
S-8	6	Arthritis	5/5	—	—
S-14	6	Arthritis	5/5	—	—
S-17	6	Arthritis	5/5	15×20	—
S-18	6	Lymphadenitis	5/5	10×13	—
566	6	Lymphadenitis	5/5	8×9	—
913	6	Lymphadenitis	5/5	—	—
911	8	Lymphadenitis	5/5	10×10 10×10	—
S-15	11	Arthritis	5/5	15×20 16×17	—
S-16	11	Arthritis	5/5	9×10 10×14	—
571	11	Arthritis	5/5	15×15 15×16	—
580	11	Arthritis	5/5	—	—
848	11	Arthritis	5/5	10×12 4×4	—
533	21	Arthritis	5/5	—	—
S-12	N	Lymphadenitis	5/5	15×15 10×10	—
S-44	N	Arthritis	5/5	6×7	—
506	N	Arthritis	0/5	—	—

a) No. of dead mice/No. tested.

b) Maximum size (cm) of erythema in skin observed for 2 weeks after intradermal inoculation with each strain.

c) No or none.

Table 2. Pathogenicity of *E. rhusiopathiae* strains of serovar 1a isolated from pigs with arthritis or lymphadenitis of chronic erysipelas

Strain tested (origin ^a)	Pathogenicity for mice		Immunogenicity for mice ^d	Pathogenicity for swine	
	Mortality ^b	Arthritis ^c		Erythema ^e	Systemic ^f
487 (A)	0/5	0/5	5/5	—	—
491 (A)	0/5	0/5	0/5	—	—
505 (A)	0/5	1/5	5/5	13×15	—
528 (A)	0/5	3/5	5/5	10×15	—
538 (A)	0/5	0/5	5/5	10×13	—
547 (A)	0/5	5/5	5/5	7×10	—
548 (A)	0/5	5/5	5/5	7×8	—
549 (A)	0/5	1/5	5/5	9×10	—
550 (A)	0/5	1/5	5/5	Generalized	+
551 (A)	0/5	5/5	5/5	Generalized	+
552 (A)	0/5	0/5	5/5	—	—
914 (A)	5/5			—	—
S-13 (A)	0/5	5/5	5/5	15×16	—
514 (L)	0/5	0/5	0/5	—	—
530 (L)	0/5	5/5	5/5	12×13	—
592 (L)	0/5	1/5	5/5	10×13	—
593 (L)	0/5	0/5	0/5	Generalized	+
				5×6	—
				7×10	—
				8×12	—
				6×8	—
				2×2	—

a) A: arthritis, L: lymphadenitis.

b) No. of dead mice/No. tested.

c) No. of mice showing arthritis/No. tested.

d) No. of survived mice/No. challenge-exposed with about 10⁶ viable organisms of the virulent strain Fujisawa (serovar 1a) 14 days after subcutaneous inoculation with each strain.

e) Maximum size (cm) of erythema in skin observed for 2 weeks after intradermal inoculation with each strain.

f) +: with temporary depression and anorexia.

Table 3. Pathogenicity of *E. rhusiopathiae* strains of serovar 1a isolated from pigs with septicemia of acute erysipelas

Strain tested	Pathogenicity for mice ^{a)}	Pathogenicity for swine	
		Erythema	Systemic ^{b)}
F-1	5/5	Generalized	+
F-4	5/5	Generalized	Died
I-1	5/5	Generalized	Died
I-3	5/5	Generalized	+
N-1	5/5	Generalized	Died
N-4	5/5	Generalized	Died
N-7	5/5	Generalized	Died
N-10	5/5	Generalized	Died
N-16	5/5	Generalized	Died

a) No. of dead mice/No. tested.

b) Died: died in the acute phase within 4 days after intradermal inoculation with each strain.

+: with depression, anorexia, and difficulty in standing.

Table 4. The propagation of *E. rhusiopathiae* strain 514 (serovar 1a), which induced no clinical sign in mice in spite of pathogenic for swine, in systemic organs of mice

Time in days after inoculation ^{a)}	Recovery of organisms from ^{b)}							
	Lo	Ly	Ma	Li	Sp	Ki	He	Lu
3	+	+	—	—	—	—	—	—
	+	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
	+	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—

a) Mice were inoculated subcutaneously with 0.1 ml of broth culture (about 10^7 viable organisms) of strain 514.

b) Lo: local subcutaneous tissue, Ly: inguinal lymph node, Ma: marrow of thighbone, Li: liver, Sp: spleen, Ki: kidney, He: heart, Lu: lung.

Table 5. Relationship between serovars of *E. rhusiopathiae* isolates from pigs with chronic erysipelas and their pathogenicity for mice

Origin	Serovars										Total
	1a	1b	2	3	5	6	8	11	21	N	
Arthritis	21	6	107	1	1	4		5	1	2	148
Lymphadenitis	5	10	45			3	1			1	65
Endocarditis	3	2	25								30
Urticaria			14		1						15
Total	29	18	191	1	2	7	1	5	1	3	258
Highly virulent ^{a)}	2	18	183	1	1	7	1	5	1	2	221
Weakly virulent ^{b)}	19	0	4	0	0	0	0	0	0	1	24
Non-virulent ^{c)}	8	0	4	0	1	0	0	0	0	0	13

a) Killing all mice after subcutaneous inoculation with each strain.

b) Causing only arthritis in one or more mice.

c) Inducing no clinical signs.

days, usually within 3 to 5 days. In swine, 7 strains caused death in the acute phase within 4 days after inoculation, and the remaining 2 induced generalized urticarial lesions with significant depression, anorexia, and difficulty in standing.

The propagation of strain 514 (serovar 1a), being more pathogenic for swine than mice, in mice. Strain 514 of serovar 1a isolated from the pig affected with lymphadenitis of chronic erysipelas was non-pathogenic for mice. Then, propagation of the organisms in systemic organs of mice were examined. The results presented in Table 4 indicate that strain 514 scarcely propagated in any organs of inoculated mice.

Relationship between serovars of E. rhusiopathiae isolates and their pathogenicity for mice. Relationship between serovars of 258 isolates from pigs affected with chronic erysipelas and their pathogenicity for mice is shown in Table 5. Of 29 strains belonging to serovar 1a, 27 were weakly virulent or non-virulent. On the other hand, 183 of the 191 strains of serovar 2, all of the 18 strains of serovar 1b, and 18 of the 20 strains of serovars other than 1a, 1b, or 2 were highly virulent.

DISCUSSION

E. rhusiopathiae organisms of serovars 1a, 1b, and 2 has been generally believed to be predominant causative agent of swine erysipelas. However, in the present experiments, more than half of *E. rhusiopathiae* strains of serovars 3, 5, 6, 8, 11, 21, and type N from pigs with chronic erysipelas: mainly arthritis or lymphadenitis were found to be pathogenic for swine, capable of inducing local urticarial lesions. Kucsera [5], and Cross and Claxton [1] also isolated the organisms belonging to serovars 4, 5, 7, or 10 from lesions of chronic erysipelas, but they did not test for their pathogenicity with inoculation studies of swine. Wood and Harrington [11, 12] reported that approximately 20% of isolates from pigs under conditions not involving an epizootic of clinical erysipelas belonging to serovars other than 1a, 1b, or 2, and some isolates were pathogenic for mice and swine. It is possible, therefore, that *E. rhusiopathiae* belonging to serovars other than 1a, 1b, and 2 are also involved in the causes of clinical erysipelas, and should be considered as important factors in the epizootiologic features of the disease.

E. rhusiopathiae strains of serovar 1a reportedly have been most often isolated from pigs with acute septicemic erysipelas [3, 6, 9], and generally considered to be highly pathogenic for swine. However, in the present investigation, strains of serovar 1a isolated from pigs with arthritis or lymphadenitis of chronic erysipelas showed a wide variety of pathogenic characteristics in swine, namely, induced generalized urticarial lesions with significant depression and anorexia, local urticarial lesions, or no clinical sign. The variability in their pathogenic characteristics may suggest the possibility of alteration in their pathogenicity during long-term habitation in porcine joints or lymph nodes. Likewise, most strains of serovar 1a were weakly virulent or non-virulent for mice, whereas most strains of the other serovars were highly virulent for mice. On the other hand, it is confirmed that all strains of serovar 1a obtained from the cases of acute septicemic erysipelas used for comparison with their pathogenicity were highly virulent for mice, and caused either death in acute phase or generalized urticarial lesions with serious systemic symptom in swine. In any case, our results indicate the absence of a relationship between pathogenicity and serovars of *E. rhusiopathiae* strains. Further studies on the virulence factor of *E. rhusiopathiae* are necessary to account for the remarkable difference of pathogenicity among serovar 1a strains.

Some strains of serovar 1a from pigs with chronic erysipelas induced either generalized urticarial lesions with systemic symptom or local urticarial lesions, in spite of their low or non virulence for mice. It has been known that mice are highly susceptible to *E. rhusiopathiae* strains. The existence of non-virulent *E. rhusiopathiae* strains for swine, in spite of their high virulence in mice, has been reported [2, 4, 13], but the reverse cases were not reported yet. The occurrence of *E. rhusiopathiae* strains which are more pathogenic

for swine than mice is considered to have significant aspect in susceptibility of animal species to *E. rhusiopathiae*. Further investigation will be required to clarify how the virulence factor of *E. rhusiopathiae* is concerned in such difference of susceptibility between mice and swine.

ACKNOWLEDGEMENTS. The authors wish to express their gratitude to the veterinarians of Fukuoka Prefectural Chūō Live Stock Hygiene Service Center, Hokkaidō Ishikari Live Stock Hygiene Service Center, and Niigata Prefectural Chūō Live Stock Hygiene Service Center for supplying of *E. rhusiopathiae* strains obtained from the cases of acute septicemic erysipelas.

REFERENCES

1. Cross, G. M. R., and Claxton, P. D. 1979. Serological classification of Australian strains of *Erysipelothrix rhusiopathiae* isolated from pigs, sheep, turkey, and man. *Aust. Vet. J.* 55: 77-81.
2. Hashimoto, K., Sugawara, H., Ando, K., Muramatsu, U., and Kojima, K. 1972. Serotyping and pathogenicity of *Erysipelothrix insidiosa* isolated from tonsils of swine and fishes. *Jpn. J. Vet. Sci.* 34 (Suppl.): 11-12 (in Japanese).
3. Hashimoto, K., Yoshida, Y., and Sugawara, H. 1974. Serotypes of *Erysipelothrix insidiosa* isolated from swine, fish, and birds in Japan. *Natl. Inst. Anim. Health Q.* (Jpn). 14: 113-120.
4. Kucsera, G. 1971. Detection of new serotypes among *Erysipelothrix rhusiopathiae* strains of different origin. *Acta Vet. Acad. Sci. Hung.* 21: 211-219.
5. Kucsera, G. 1979. Serological typing of *Erysipelothrix rhusiopathiae* and the epizootiological significance of the typing. *Acta Vet. Acad. Sci. Hung.* 27: 19-28.
6. Murase, N., Suzuki, K., Nakahara, T., Araumi, W., and Hashimoto, K. 1959. Studies on the typing of *Erysipelothrix rhusiopathiae*. I. Serological behaviours of *Erysipelothrix rhusiopathiae* isolated from pigs. *Jpn. J. Vet. Sci.* 21: 113-121.
7. Sasaki, Y., Omura, K., Kawashima, Y., and Okaniwa, A. 1970. Clinical pathological and bacteriological observations on erysipelas arthritis in piglets. *Bull. Nat. Inst. Anim. Health* (Jpn). 60: 13-21 (in Japanese).
8. Sawada, T., Muramatsu, M., and Seto, K. 1979. Responses of growth agglutinating antibody and protection of pigs inoculated with swine erysipelas live vaccine. *Jpn. J. Vet. Sci.* 41: 593-600.
9. Seto, K., Muramatsu, M., and Yoshida, K. 1972. Serotypes of isolates from pigs affected with three

- types of erysipelas and attenuated strains of *Erysipelothrix insidiosa*. *Ann. Rep. Nat. Vet. Assay Lab.* 9: 31-36 (in Japanese with English summary).
10. Takahashi, T., Sawada, T., Takagi, M., Seto, K., Kanzaki, M., and Maruyama, T. 1984. Serotypes of *Erysipelothrix rhusiopathiae* strains isolated from slaughter pigs affected with chronic erysipelas. *Jpn. J. Vet. Sci.* 46: 149-153.
 11. Wood, R. L., and Harrington, R. 1978. Serotypes of *Erysipelothrix rhusiopathiae* isolated from swine and from soil and manure of swine pens in the United States. *Am. J. Vet. Res.* 39: 1833-1840.
 12. Wood, R. L., Haubrich, D. R., and Harrington, R. 1978. Isolation of previous unreported serotypes of *Erysipelothrix rhusiopathiae* from swine. *Am. J. Vet. Res.* 39: 1958-1961.
 13. Wood, R. L., and Packer, R. A. 1972. Isolation of *Erysipelothrix rhusiopathiae* from soil and manure of swine-raising premises. *Am. J. Vet. Res.* 33: 1611-1620.

要 約

屠場豚の慢性型豚丹毒例から分離された1a型, 3型, 5型, 6型, 8型, 11型, 21型およびN型の豚丹毒菌株の病原性: 高橋敏雄・沢田拓士・瀬戸健次¹⁾・村松昌武・丸山 務²⁾・神崎政子²⁾(農林水産省動物医薬品検査所, ¹⁾日本生物科学研究所, ²⁾東京都立衛生研究所)——屠場豚の慢性型豚丹毒例(主に, 関節炎およびリンパ節炎)から分離された1a型(17株), 3型(1株), 5型(2株), 6型(7株), 8型(1株), 11型(5株), 21型(1株), およびN型(3株)の豚丹毒菌の豚およびマウスに対する病原性を調べた。5型, 6型, 8型, 11型, およびN型には豚に局所発疹をひきおこす株が含まれていた。1a型株は豚に対し種々の病原性を示し, 全身発疹, または局所発疹をひきおこし, あるいは全く臨床症状をひきおこさなかった。また, 1a型以外のほとんどの株はマウスに強毒であったのに対し, 1a型株のほとんどはマウスに対して弱毒あるいは無毒であり, マウスよりも豚に対して強い病原性を示す豚丹毒菌の存在が初めて確認された。一方, 急性敗血症型豚丹毒例由来の1a型の9株すべてはマウスに対し強毒で, ほとんどの株が豚を急性経過でへい死させた。