NOTE *Bacteriology*

Characterization of *Erysipelothrix rhusiopathiae* Strains Isolated from Recent Swine Erysipelas Outbreaks in Japan

Ho TO,[1)]* Hiroko SATO[1)], Akihiro TAZUMI[1)], Nobuyuki TSUTSUMI[1)], Shinya NAGAI[1)], Akira IWATA[1)] and Tetsuji NAGANO[1)]

[1)]*Nippon Institute for Biological Science, 9–2221–1 Shinmachi, Ome, Tokyo 198–0024, Japan*

(Received 28 November 2011/Accepted 25 February 2012/Published online in J-STAGE 9 March 2012)

ABSTRACT. The objective of the present study was to characterize *Erysipelothrix* sp. strains from recent erysipelas outbreaks in Japan. Eighty-three (100%) strains were identified as *E. rhusiopathiae,* based on serotyping and *spaA* PCR. Fifty (60.3%), 5 (6.0%), and 28 (33.7%) strains were isolated from animals with acute, subacute and chronic outbreaks, respectively, of which 79 (95.2%), 1 (1.2%), and 3 (3.6%) belonged to serotypes 1a, 2a, and untypeable, respectively. Fifteen strains (including 3, 2, and 10 from acute, subacute, and chronic cases, respectively) were sensitive to acriflavine, and showed high levels of virulence in mice; of which strains from acute cases, and from subacute and chronic cases killed 100%, and 80 to 100% mice, respectively at challenge doses of 10^2 CFU per mouse. Based on sequence analysis of a 432-bp hypervariable region in *spaA* gene, 83 strains could be divided into 3 groups: (i) group 1 (3 strains of serotype 1a) had Ala-195 and Ile-203; (ii) group 2 (76 strains of serotype 1a and 3 of untypeable) had Asp-195 and Met-203; and (iii) group 3 (one strain of serotype 2a) had Asn-195 and Ile-203. The results of the present study suggest that the serotype 1a strains belonging to the group 2 might be widespread in pig populations in Japan.

KEY WORDS: acriflavine, *Erysipelothrix rhusiopathiae*, field strain, live vaccine, *spaA* sequence analysis.

doi: 10.1292/jvms.11-0533; *J. Vet. Med. Sci.* 74(7): 949–953, 2012

Erysipelothrix rhusiopathiae is a small Gram-positive rod bacterium that causes erysipelas in swine and a variety of diseases in other animals, as well as erysipeloid, a skin disease of humans [25]. Since swine erysipelas (SE) reappeared as a clinical problem in pig populations in Japan and in the Midwestern United States, it has been considered as a reemerging disease that contributes substantially to economic losses in the swine industry [1, 2, 5]. In Japan, to protect against SE, strain Koganei 65–0.15 (serotype 1a), which was attenuated by 65 passages on agar media containing 0.15% of an acriflavine dye, has been used as a seed strain for production of a live vaccine [16]. Later, an intensive vaccination program with the live vaccine decreased greatly the occurrence of SE [20]. However, after 1985 despite extensive vaccination, about 2000 pigs annually have been shown to have either acute or subacute infection, and also about 2,000 pigs have been condemned by meat inspection authorities each year due to the subacute or chronic erysipelas. Therefore, the economic losses caused by the acute, subacute, and chronic forms of erysipelas are not negligible. A recent investigation describes that some strains of *E. rhusiopathiae* isolated from chronic SE are closely related to the live vaccine strain, which has been arousing suspicion that the live vaccine might be the cause of the increase in chronic erysipelas cases in Japan [5]. Although several molecular biological methods such as randomly amplified polymorphic DNA (RAPD) typing [8, 11], ribotyping, restriction fragment length polymorphism (RFLP) [5], and pulse-field gel electrophoresis (PFGE) [12, 13] have been used to differentiate *Erysipelothrix* spp., only PFGE might differentiate the vaccine strain from field ones [13, 15]. Thus, the development of timely, accurate, and reliable diagnostic methods that can differentiate the vaccine strain from field ones is highly desired. Recent studies have focused on characterization of strains based on their *spa* type, whose gene encodes a surface protective antigen (Spa) protein [2, 4, 9, 17]. The Spa proteins of *E. rhusiopathiae* can be classified into 3 molecular species, named SpaA (produced by serovars 1a, 1b, 2, 5, 8, 9, 12, 15, 16, 17, and N), SpaB (produced by serovars 4, 6, 11, 19, and 21) and SpaC (produced by serovar 18) [6, 22, 23]. More recently, a method for discrimination between the vaccine strain and the field ones was developed based on the nucleotide sequencing of a 432-bp hypervariable region in the *spaA* gene, with the results being compared with those obtained by RAPD typing, ribotyping, and RFLP in combination with acriflavine resistance and mouse pathogenicity tests [10]. That study showed that *spaA* sequence analysis is in good agreement with those of genetic typing methods in combination with biological tests and is easy to perform and no ambiguity in the results obtained.

In the present study, the *spaA* sequence analysis in combination with serotyping, acriflavine resistance and mouse pathogenicity tests were used to identify and characterize recent field strains of *E. rhusiopathiae* from swine erysipelas outbreaks isolated from 2008 to 2011.

Three *E. rhusiopathiae* strains (Fujisawa, serotype 1a;

*CORRESPONDENCE TO: TO, H., Nippon Institute for Biological Science, 9–2221–1 Shinmachi, Ome, Tokyo 198–0024, Japan. e-mail: toho@nibs.or.jp

ATCC 19414^T, 2b; and Koganei 65–0.15, serotype 1a) were used as reference strains for acriflavine resistance test, and analysis of the 432-bp hypervariable region in the *spaA* gene. The Fujisawa strain is a virulent official challenge strain sensitive to acriflavine. The Koganei 65–0.15 strain is an attenuated live vaccine strain resistant to acriflavine.

Seventy-eight *Erysipelothrix* sp. strains isolated from pigs with erysipelas, and five strains isolated from sections of spleen, kidney, and heart from 5 pigs with a clinical history consistent with acute erysipelas submitted from pig farms, where acute erysipelas was occurring were used in the present study. Eighty-three strains were isolated from animals with acute (septicemia), subacute (urticaria), and chronic (arthritis, endocarditis, and lymphadenitis) erysipelas in Akita, Ibaraki, Gunma, Nagano, Yamaguchi, Kumamoto, Miyazaki, and Kagoshima prefectures during the years 2008–2011.

The procedure for isolating the bacteria was carried out as described previously [20], with some minor modifications. Brain heart infusion agar and broth supplemented with kanamycin (400 μg/m*l*) and gentamycin (25 μg/m*l*) instead of crystal violet (5 μg/m*l*) and sodium azide (0.03%) were used for isolation of *Erysipelothrix* spp. from tissue samples.

The procedure used to determine the serotypes of the isolates was similar to that described previously [7, 10, 19]. Briefly, colonies of each strain grown for 24 hr on agar plates were harvested and suspended in the sterilized distilled water. Bacterial cells were washed 1 time with saline and suspended in distilled water. The cell suspension was autoclaved for 1 hr at 121°C, and centrifuged at 15,000 rpm for 15 min. Rabbit antisera specific to serotypes 1 to 23 and type N were made in the authors' institute. The supernatant was collected and probed with rabbit antisera against serotypes 1a, 1b, 2a, and 2b of *E. rhusiopathiae* in 1% agarose agar (Sigma-Aldrich Co. Inc., St. Louis, MO, U.S.A.) with distilled water. After reacting for 16 hr at room temperature, formation of the precipitation line was observed. Isolates showing no precipitation line with these four sera were then probed with the remaining antisera. Isolates showing no precipitation line with any antisera were considered as untypeable.

The acriflavine resistance test of 15 field strains (including 14 of serotype 1a and 1 of 2a), and 3 reference strains (Fujisawa, 1a; ATCC 19414^T, 2b; and Koganei 65–0.15, 1a) of *E. rhusiopathiae* was carried out as described elsewhere [5, 10].

The procedure used to determine pathogenicity of the 15 field strains (including 3, 2, and 10 from acute, subacute and chronic cases, respectively), and 2 reference strains (Fujisawa and Koganei 65–0.15) in mice was carried out according to the procedure described elsewhere [21, 24]. Briefly, 5 or 10 4-week-old female ddY mice (Nippon SLC, Shizuoka, Japan) were injected subcutaneously (s.c.) in the right inguinal region with 0.1 m*l* of approximately 10^2 colony-forming units (CFU) of the recent field strains, and Fujisawa strain. A group of 5 mice served as a nontreated control. Another group of ten 4-week-old female ddY mice was inoculated s.c. with 0.1 m*l* of approximately 10^7 CFU of live vaccine strain (Koganei 65–0.15). Mice were observed daily to detect clinical signs of the disease for the subsequent 10 days. The Japanese standards of Veterinary Biological Products indicate that the vaccine strain will not kill mice but will induce arthritis in more than 80% of animals at challenge dose of 10^7 CFU per mouse. For classification of virulence levels of *E. rhusiopathiae* strains, the strain that killed 100% mice within 3 days was considered to have very high virulence, and the strain that killed 80 to 100% mice within 4 to 5 days was considered to have high virulence. The animals used in the present study were cared for in accordance with the guidelines for animal treatment of Nippon Institute for Biological Science, which conform to the standard principles of laboratory animal care.

A 432-bp fragment of the N-terminal half of the *spaA* gene was PCR amplified and directly sequenced as described previously [10]. Briefly, genomic DNA of *E. rhusiopathiae* was prepared as previously described [22]. Primers Erko-1F (5′-GTGAAACACCGTATTTTAGTA-3′) and Erko-2R (5′- TTCAAGAAGTTCCTGTAGTTT-3′) are located at nucleotide positions 502 to 522 and 933 to 913, respectively, of the *spaA* gene were used to amplify the 432-bp fragment. Polymerase chain reaction was performed as described elsewhere [22] under the following conditions: a denaturation step at 94°C for 3 min and then 30 cycles of denaturation at 94°C for 45 sec; annealing at 55°C for 1 min and extension at 72°C for 2 min; and extension of 5 min for the final cycle. Sequencing reactions of the PCR products were performed with primers Erko-1F and Erko-2R. The DNA sequences were analyzed as described previously [10, 22].

The isolated bacteria were subcultured in the BHI agar and broth, and colonies with characteristics resembling *Erysipelothrix* species were Gram stained and examined microscopically. Suspected *Erysipelothrix* colonies were then identified as *E. rhusiopathiae* by PCR with the use of *spaA*-specific primers Erko-1F and Erko-2R.

Erysipelothrix sp. strains were isolated from the tissue samples of 5 pigs with clinical symptoms of acute septicemia erysipelas. Based on serotyping and *spaA* PCR, all 5 strains were identified as *E. rhusiopathiae* strains of serotypes 1a. The strains submitted during the years 2008–2011 belonged to serotypes 1a, 2a, and untypeable. Relationship between the strains and disease forms is shown in Table 1. Among 83 strains, 79 strains (95.2%), 1 (1.2%), and 3 (3.6%) belonged to serotypes 1a, 2a and untypeable, respectively; and 100% of strains were *E. rhusiopathiae*.

Two reference strains (Fujisawa, 1a; and ATCC 19414^T, 2b) and the 15 recent field strains were sensitive to acriflavine at concentration of 0.01%, whereas the live vaccine strain, Koganei 65–0.15, was resistant to acriflavine at the same concentration (Table 2).

Strain Fujisawa and 3 strains from acute cases killed 100% animals within 3 days, whereas 2 and 10 strains from subacute and chronic cases, respectively, killed 80 to 100% within 4 to 5 days at challenge dose of approximately 10^2 CFU per mouse (Table 2). Live vaccine strain, Koganei 65–0.15, induced arthritis in 100% mice.

The different nucleotide sequences in the 432-bp hyper-

Table 1. Serotypes of the 83 *E. rhusiopathiae* field strains isolated from erysipelas outbreaks during the years 2008 to 2011

Disease	No. of strains of serotype			
	1a	2a	Untypeable[a)]	Total
Septicemia	50			50
Urticaria	3		2	5
Lymphadenitis	6			6
Endocarditis	5			5
Arthritis	15	1	1	17
Total	79	1	3	83

a) Strains untypeable with antisera against serotypes 1 to 23 and N.

Table 2. Mouse pathogenicity, acriflavine resistance of 15 *E. rhusiopathiae* strains

Origin[a)]	Group[b)]	No. of strains for which mouse pathogenicity was as follows[c)]		Acriflavine resistance[d)]
		Very high	High	
Septicemia	1	1		S
	2	2		
Urticaria	2		2	S
Lymphadenitis	2		1	S
Endocarditis	2		1	S
Arthritis	2		7	S
	3		1	S

a) Two and four strains associated with subacute (urticaria) and chronic (arthritis) infections, respectively, originated from slaughterhouses, whereas 1, 1, and 4 strains also from chronic cases (lymphadenitis, endocarditis, and arthritis, respectively) originated from pig farms.
b) Strains were grouped based the *spaA* sequence similarities of hypervariable region.
c) Five or ten mice each received a subcutaneously injection of 10^2 CFU of strain Fujisawa and the field strains. Vey high, all mice died within 3 days; high, 80 to 100% mice died within 4 to 5 days after challenge.
d) S, sensitive to 0.01% acriflavine. Vaccine strain is resistant to 0.01% acriflavine.

variable region of *spaA* gene of 83 field strains, and the high virulent strain Fujisawa compared with the corresponding sequence of the attenuated live vaccine strain, Koganei 65–0.15 are shown in Table 3. Based on the sequence analysis of *spaA* gene, 83 strains could be divided into 3 groups; group 3 consisted of 1 strain of serotype 2a, group 2 consisted of 76 and 3 strains of serotype 1a and untypeable, respectively; and group 1 consisted of 3 strains of serotype 1a. The strains of group 1 had 2 nucleotide differences with those of strains of group 2 (positions 584 and 609) and of strains of group 3 (positions 583 and 584). These nucleotide differences resulted in amino acid differences as follows: (i) groups 1 had Ala-195 and Ile-203; (ii) groups 2 had Asp-195 and Met-203; and (iii) group 3 had Asn-195 and Ile-203. Strains of groups 1, 2, and 3 also showed 2 nucleotide differences each with that of Fujisawa at positions 584 and 769; 583 and 769; and 609 and 769, respectively, which induced amino acid exchanges at positions 195, 203, and 257. Strains of groups 1, 2, and 3 had only one nucleotide difference with that of Koganei 65–0.15 (live vaccine strain) at postions 583, 584, and 609, respectively, which also induced amino acid exchanges at positions 195, and 203.

It was reported that serotyping 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 [18]. Many previous investigations show that most strains of *E. rhusiopathiae* from pigs with SE fall into serotypes 1a, 1b and 2 [1, 3, 5, 14, 18, 25]. Our observation that the presence of a high rate (95.2% of 83 strains) of serotype 1a among *E. rhusiopathiae* strains isolated from SE outbreaks from 2008 to 2011 is consistent with data from a recent report where 79.6% of 44 strains of serotype 1a were found in the isolates originating from swine erysipelas outbreaks in the Midwestern United State from the years 1999–2001 [13]. However, our results agree partially with those

Table 3. Substitutions in nucleotide and amino acid in a 432-bp hypervariable region on the *spaA* gene of 83 *E. rhusiopathiae* strains and Fujisawa strain compared with the corresponding sequence of the vaccine strain Koganei 65–0.15

Strain or group	Prefecture	No. of strains	Serotype	Substitutions in nucleotide (amino acid position)[a, b)]		
				Nucleotide (aa 195)	Nucleotide (aa 203)	Nucleotide (aa 257)
Koganei 65–0.15			1a	GAT (Asp)	ATT (Ile)	ATT (Ile)
Fujisawa			1a	GAT (Asp)	ATT (Ile)	CTT (Leu)
Group 1	Kumamoto	2	1a	GCT (Ala)	ATT (Ile)	ATT (Ile)
	Yamaguchi	1	1a			
Group 2	Miyazaki	2	1a			
	Kagoshima	3	1a			
	Nagano	35	1a			
	Gunma	31	1a	GAT (Asp)	ATG (Met)	ATT (Ile)
	Ibaraki	2	1a			
	Akita	3	1a			
	Nagano	3	Untypeable			
Group 3	Nagano	1	2a	AAT (Asn)	ATT (Ile)	ATT (Ile)

a) Asp, aspartic acid; Ala, alanine; Asn, asparagine; Ile, isoleucine; Leu, leucine; Met, methionine.
b) Underlined letter, nucleotide different from those at the same position; and underlined word, amino acid different from those at the same position.

obtained previously [1, 3, 5, 14, 18]. Those studies found high rates of serotypes 1a and 2 from pigs with erysipelas from 2007–2009 in Iowa (40.7% and 49.2% of 59, respectively), from pigs affected with erysipelas in Japan from the years 1983–1993 (40.4% and 34.3% of 1046, respectively) [18], from the years 1992–2002 (47.6% and 31.8% of 800, respectively) [5], and from 1994–2001 (28.8% and 47% of 66, respectively) [14]. The current results, taken together with previously described observations of other groups, strongly suggest that serotype 1a has continued to remain to be the serotype commonly isolated from pigs affected with erysipelas in the world. The great difference in occurrence rates between serotypes 1 and 2 (95% versus 1.2%) among strains isolated from SE outbreaks from 2008 to 2011 was unexplained.

Our observation that strains with nucleotide and amino acid sequences in the 432-bp hypervariable region of *spaA* gene different from those of the live vaccine strain, Koganei 65–0.15, were virulent for mice is consistent to data from a recent report by Nagai *et al.* [10]. Those investigators examined biological and genetic characteristics of 16 *E. rhusiopathiae* strains (including 10 strains of serotype 1a, and 6 of 1b) isolated from 2001 to 2002 from chronic (arthritis) cases in the Chubu area, and showed that (i) three strains of serotype 1a showing an identical nucleotide sequence to the live vaccine strain seem to be closely related to the live vaccine strain; and (ii) strains of serotype 1a or 1b showing the nucleotide sequence of *spaA* gene different from that of the live vaccine were virulent for mice and considered to be wild-type strains. In this study, except that 3 strains serotype 1a from South of Japan (Kumamoto and Yamaguchi prefectures) had 1 nucleotide substitution at position 584; 76 and 3 strains of serotype 1a and untypeable, respectively, from 7 prefectures of Japan (Miyazaki, Kagoshima, Nagano, Gunma, Ibaraki, Miyagi, and Akita) had 1 nucleotide substitution at position 609 compared with the nucleotide sequence of the vaccine strain Koganei 65–0.15. Nucleotide sequences of 79 strains of serotype 1a found in the present study were different from those reported previously [10], in which 3, 5, and 2 strains had 0, 1, and 2 nucleotide substitutions, respectively, at position 591, 711, 729, 796 or 911 (with 1-nucleotide substitution); 511 and 638; 555 and 773 compared with the corresponding sequence of the live vaccine strain Koganei 65–0.15. The present results suggest that 83 strains of *E. rhusiopathiae* could be divided into 3 groups, based on their *spaA* sequence similarities: (i) group 1 (including 3 strains serotype 1a) with Ala-195 and Ile-203 was shown to be causing acute septicemia erysipelas on pig farms in South of Japan, (ii) group 2 (including 76 and 3 strains of serotypes 1a and untypeable, respectively) with Asp-195 and Met-203 was shown to be causing acute, subacute, and chronic cases on pig farms in many prefectures of Japan, and (iii) group 3 (including 1 strain of serotype 2a) with Asn-195 and Ile-203 was shown to be causing chronic arthritis case in a farm in Nagano prefecture. Our results show that the serotype 1a strains of group 2-SpaA might be widespread in pig populations in Japan.

Our findings that strains isolated from chronic SE had high levels of virulence in mice are consistent with data reported previously. Imada *et al*. [5] showed that 21 of 50 strains (42%) of serotype 1a isolated from pigs with chronic cases between 1992 and 2002 have high levels of virulence at challenge doses of 10^4 CFU per mouse [5]. In 2008, Nagai *et al*. [10] reported that 8 of 16 (50%) isolated from pigs with chronic arthritis cases have high levels of virulence at challenge dose of 10^7 CFU per mouse [10]. Recently, Ozawa *et al*. [14] found that 39 of 55 (70.9%) isolated from pigs with chronic cases from 1994 to 2001 have high levels of virulence at challenge dose of 10^8 CFU per mouse [14]. In the present study, 10 strains (including 9 of serotype 1a, and 1 of serotype 2a) from pigs with chronic cases, which had biological and genetic characteristics different from the live vaccine strain showed high levels of virulence at challenge doses of approximately 10^2 CFU per mouse. The findings, together with data reported by other investigators support the idea that the majority of chronic strains not related to the live vaccine strain have high levels of virulence in mice.

The most important advantage of nucleotide sequence analysis of a 432-bp hypervariable region in the *spaA* gene demonstrated here is its simplicity and applicability to screen more samples at the same time. The results show that sequence analysis also parallelled the mouse pathogenicity and the acriflavine resistance tests for discrimination of the live vaccine strain from *E. rhusiopathiae* field strains. It has been reported that the sequence analysis is easy to perform and no ambiguity in the results obtained, affords the same results irrespective of the laboratory involved, and can screen more samples at the same time [10]. Results of the present study demonstrate that (i) most strains of *E. rhusiopathiae* (95.2% of 83) isolated from recent erysipelas outbreaks belonged to serotype 1a; (ii) based on similarities of amino acid sequences of the 432-bp hypervariable region in the *spaA* gene, 83 strains could be divided into 3 groups, (iii) none of 83 field strains is vaccine strain. Currently, to fully understand the relations between strains associated with swine erysipelas outbreaks, studies on PFGE profile and on the protection by currently available vaccines in the swine model using recent isolates are under way.

ACKNOWLEDGMENTS. Authors thank Drs. Tetsuo Nunoya, Koji Uetsuka, and Tomohiro Koyama for helpful advices and critical suggestions; and technicians Rie Kitahara, Takashi Kamada, and Yuu Yamazaki for laboratory assistance during the study; Yukiko Abe (Gunma prefecture), Akira Kanda (Nagano prefecture) and the many veterinarians at Liver stock Hygiene Service Centers and pig farms of the prefectures for the kind gift of many field isolates together much invaluable information.

REFERENCES

1. Bender, J. S., Irwin, C. K., Shen, H. G., Schwartz, K. J. and Opriessnig, T. 2011. *Erysipelothrix* spp. genotypes, serotypes, and surface protective antigen types associated with abattoir condemnations. *J. Vet. Diagn. Invest.* **23**: 139–142. [Medline] [CrossRef]

2. Bender, J. S., Shen, H. G., Irwin, C. K., Schwartz, K. J. and Opriessnig, T. 2010. Characterization of *Erysipelothrix* species isolated from clinically affected pigs, environmental samples, and vaccine strains from six recent swine erysipelas outbreaks in the United States. *Clin. Vaccine Immunol.* **17**: 1605–1611. [Medline] [CrossRef]
3. Eamens, G. J., Forbes, W. A. and Djordjevic, S. P. 2006. Characterization of *Erysipelothrix rhusiopathiae* isolates from pigs associated with vaccine breakdowns. *Vet. Microbiol.* **115**: 329–338. [Medline] [CrossRef]
4. Imada, Y., Goji, N., Ishikawa, H., Kishika, M. and Sekizaki, T. 1999. Truncated surface protective antigen (SpaA) of *Erysipelothrix rhusiopathiae* serotype 1a elicits protection against challenge with serotypes1a and 2b in pigs. *Infect. Immun.* **67**: 4376–4382. [Medline]
5. Imada, Y., Takase, A., Kikuma, R., Iwamaru, Y., Akachi, S. and Hayakawa, Y. 2004. Serotyping of 800 strains of *Erysipelothrix* isolated from pigs affected with erysipelas and discrimination of attenuated live vaccine strain by genotyping. *J. Clin. Microbiol.* **42**: 2121–2126. [Medline] [CrossRef]
6. Ingebritson, A. L., Roth, J. A. and Hauer, P. L. 2010. *Erysipelothrix rhusiopathiae:* association of Spa-type with serotype and role in protective immunity. *Vaccine* **28**: 2490–2496. [Medline] [CrossRef]
7. Kucsera, G. 1977-1979. Serological typing of *Erysipelothrix rhusiopathiae* strains and the epizootiological significance of the typing. *Acta Vet. Acad. Sci .Hung.* **27**: 19–28. [Medline]
8. Makino, S., Ishizaki, H., Shirahata, T., Fujiwara, S. and Sawada, T. 1998. Isolation of acriflavine resistant *Erysipelothrix rhusiopathiae* from slaughter pigs in Japan. *J. Vet. Med. Sci.* **60**: 1017–1019. [Medline] [CrossRef]
9. Makino, S., Yamamoto, K., Murakam, S., Shirahata, T., Uemura, T., Sawada, T., Wakamoto, H. and Morita, Y. 1998. Properties of repeat domain found in a novel protective antigen, SpaA, of *Erysipelothrix rhusiopathiae*. *Microb. Pathog.* **25**: 101–109. [Medline] [CrossRef]
10. Nagai, S., To, H. and Kanda, A. 2008. Differentiation of *Erysipelothrix rhusiopathiae* strains by nucleotide sequence analysis of a hypervariable region in the *spaA* gene: discrimination of a live vaccine strain from field isolates. *J. Vet. Diagn. Invest.* **20**: 336–342. [Medline] [CrossRef]
11. Okatani, A.T., Hayashidani, H., Takahashi, T., Taniguchi, T., Ogawa, M. and Kaneko, K. 2000. Randomly amplified polymorphic DNA analysis of *Erysipelothrix* spp. *J. Clin. Microbiol.* **38**. 4332–4336. [Medline]
12. Okatani, A. T., Uto, T., Taniguchi, T., Horisaka, T., Horikita, T., Kaneko, K. and Hayashidani, H. 2001. Pulsed-field gel electrophoresis in differentiation of *Erysipelothrix* species strains. *J. Clin. Microbiol.* **39**: 4032–4036. [Medline] [CrossRef]
13. Opriessnig, T., Hoffman, J., Harris, D. L., Gaul, S. B. and Halbur, P. G. 2004. *Erysipelothrix rhusiopathiae*: genetic characterization of Midwest US isolates and live commercial vaccines using pulsed-field gel electrophoresis. *J. Vet. Diagn. Invest.* **16**: 101–107. [Medline] [CrossRef]
14. Ozawa, M., Yamamoto, K., Kojima, A., Takagi, M. and Takahashi, T. 2009. Etiological and biological characteristics of *Erysipelothrix rhusiopathiae* isolated between 1994 and 2001 from pigs with swine erysipelas in Japan. *J. Vet. Med. Sci.* **71**: 697–702. [Medline] [CrossRef]
15. Sawada, T., Hassanein, R., Takahashi, E., Ando, S., Entomack, B., Kataoka, Y., Takagi, M., Yamamoto, K. and Kojima, A. 2006. Pathogenic and genetic characterization of acriflavine resistant *Erysipelothrix* isolates from arthritic pigs. *J. Vet. Epidemiol* **10**: 21–28. [CrossRef]
16. Seto, K., Nishimura, M., Fujiki, M., Azechi, H. and Suzuki, K. 1971. Studies on acriflavine-fast attenuated *Erysipelothrix insidiosa*. Comparison on pathogenicity and immunogenicity between mice and pigs. *Jpn. J. Vet. Sci* **33**: 161–171. [CrossRef]
17. Shimoji, Y., Mori, Y. and Fischetti, V. A. 1999. Immunological characterization of a protective antigen of *Erysipelothrix rhusiopathiae*: identification of the region responsible for protective immunity. *Infect. Immun.* **67**: 1646–1651. [Medline]
18. Takahashi, T., Nagamine, N., Kijima, M., Suzuki, S., Takagi, M., Tamura, Y., Nakamura, M., Muramatsu, M. and Sawada, T. 1996. Serovars of *Erysipelothrix* strains isolated from pigs affected with erysipelas in Japan. *J. Vet. Med. Sci.* **58**: 587–589. [Medline] [CrossRef]
19. Takahashi, T., Fujisawa, T., Tamura, Y., Suzuki, S., Muramatsu, M., Sawada, T., Benno, Y. and Mitsuoka, T. 1992. DNA relatedness among *Erysipelothrix rhusiopathiae* strains representing all twenty-three serovars and *Erysipelothrix tonsillarum*. *Int. J. Syst. Bacteriol.* **42**: 469–473. [Medline] [CrossRef]
20. Takahashi, T., Nitta, H., Morioka, A. and Yamamoto, K. 2006. The history and current issues with the use of live vaccines against swine erysipelas. *Annu. Rep. Natl. Assay Lab* **43**: 1–7.
21. To, H., Koyama, T., Nagai, S., Tuchiya, K. and Nunoya, T. 2009. Development of quantitative real-time polymerase chain reaction for detection of and discrimination between *Erysipelothrix rhusiopathiae* and other *Erysipelothrix* species. *J. Vet. Diagn. Invest.* **21**: 701–706. [Medline] [CrossRef]
22. To, H. and Nagai, S. 2007. Genetic and antigenic diversity of the surface protective antigen proteins of *Erysipelothrix rhusiopathiae*. *Clin. Vaccine Immunol.* **14**: 813–820. [Medline] [CrossRef]
23. To, H., Someno, S., Nagai, S., Koyama, T. and Nagano, T. 2010. Immunization with a truncated recombinant protein SpaC of *Erysipelothrix rhusiopathiae* strain 715 (serovar 18) confers protective immunity against challenge with various serovars. *Clin. Vaccine Immunol.* **17**: 1991–1997. [Medline] [CrossRef]
24. Watarai, M., Sawada, T., Nakagomi, M., Amao, H., Yoshida, T. and Takahashi, I. 1993. Comparison of etiological and immunological characteristics of two attenuated *Erysipelothrix rhusiopathia*e strains of serotype 1a and 2. *J. Vet. Med. Sci.* **55**: 595–600. [Medline] [CrossRef]
25. Wood, R. L. 1999. Erysipelas. pp. 419–430. *In:* Diseases of Swine, 8th ed. (Straw, B.E., D'Allaire, S., Mengeling, M.L. and Taylor, D.J. eds.), Iowa State University Press, Ames, IA.