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Characterization of *Erysipelothrix* Species Isolates from Clinically Affected Pigs, Environmental Samples, and Vaccine Strains from Six Recent Swine Erysipelas Outbreaks in the United States[▽]

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The aim of this study was to characterize *Erysipelothrix* sp. isolates from clinically affected pigs and their environment and compare them to the *Erysipelothrix* sp. vaccines used at the sites. Samples were collected during swine erysipelas outbreaks in vaccinated pigs in six Midwest United States swine operations from 2007 to 2009. Pig tissue samples were collected from 1 to 3 pigs from each site. Environmental samples (manure, feed, central-line water, oral fluids, and swabs collected from walls, feed lines, air inlets, exhaust fans, and nipple drinkers) and live vaccine samples were collected following the isolation of *Erysipelothrix* spp. from clinically affected pigs. All *Erysipelothrix* sp. isolates obtained were further characterized by serotyping. Selected isolates were further characterized by PCR assays for genotype (*E. rhusiopathiae*, *E. tonsillarum*, *Erysipelothrix* sp. strain 1, and *Erysipelothrix* sp. strain 2) and surface protective antigen (*spa*) type (A, B1, B2, and C). All 26 isolates obtained from affected pigs were *E. rhusiopathiae*, specifically, serotypes 1a, 1b, 2, and 21. From environmental samples, 56 isolates were obtained and 52/56 were *E. rhusiopathiae* (serotypes 1a, 1b, 2, 6, 9, 12, and 21), 3/56 were *Erysipelothrix* sp. strain 1 (serotypes 13 and untypeable), and one was a novel species designated *Erysipelothrix* sp. strain 3 (serotype untypeable). Four of six vaccines used at the sites were commercially available products and contained live *E. rhusiopathiae* serotype 1a. Of the remaining two vaccines, one was an autogenous live vaccine and contained live *E. rhusiopathiae* serotype 2 and one was a commercially produced inactivated vaccine and was described by the manufacturer to contain serotype 2 antigen. All *E. rhusiopathiae* isolates were positive for *spaA*. All *Erysipelothrix* sp. strain 1 isolates and the novel *Erysipelothrix* sp. strain 3 isolate were negative for all currently known *spa* types (A, B1, B2, and C). These results indicate that *Erysipelothrix* spp. can be isolated from the environment of clinically affected pigs; however, the identified serotypes in pigs differ from those in the environment at the selected sites. At one of the six affected sites, the vaccine strain and the isolates from clinically affected pigs were of homologous serotype; however, vaccinal and clinical isolates were of heterologous serotype at the remaining five sites, suggesting that reevaluation of vaccine efficacy using recent field strains may be warranted.

Organisms of the genus *Erysipelothrix* are facultative anaerobic small, slender, Gram-positive rods and are distributed worldwide. *Erysipelothrix* spp. have been isolated from domestic and wild species of both birds and mammals and have been identified as the causative agent of the clinical disease known as "erysipelas" in animals and "erysipeloid" in humans (2). The genus *Erysipelothrix* consists of four species and 25 associated serotypes: *E. rhusiopathiae* (serotypes 1a, 1b, 2, 4, 5, 6, 8, 9, 11, 12, 15, 16, 17, 19, 21, N), *E. tonsillarum* (serotypes 3, 7, 10, 14, 20, 22, 23), *Erysipelothrix* sp. strain 1 (serotype 13), and *Erysipelothrix* sp. strain 2 (serotype 18) (27, 28). Among the four species, *E. rhusiopathiae* causes the greatest economic loss, primarily to the swine and turkey industries (34, 36).

Three clinical presentations of swine erysipelas are recognized, i.e., acute, subacute, and chronic, and serotypes 1a, 1b, and 2 are frequently isolated from all disease stages (36). The additional serotypes (3 to 23 and N) have little clinical significance in swine. It is estimated that 30 to 50% of healthy pigs harbor *E. rhusiopathiae* in tonsils and lymphatic tissue. Subclinically affected pigs are thought to be the source for acute erysipelas outbreaks due to shedding of the organism in urine, feces, saliva, and nasal secretions (36).

Economic losses due to swine erysipelas continue to occur worldwide. For this reason, accurate, reliable, and timely diagnostic strategies are important (4). Immunohistochemistry techniques have been shown to be highly sensitive and specific, especially when diagnostic specimens include lesions from antimicrobial-treated pigs or chronically affected pigs (16). Our previous studies confirmed that an *Erysipelothrix* species-selective broth technique is more sensitive than traditional direct plating of regular and contaminated specimens (1). Although the enrichment technique has been used by other countries for a number of years, it has only recently been adopted by diagnostic laboratories within the Midwestern United States (1). PCR technology is also being employed to complement traditional detection methods (10, 19, 28, 39). In addition to improved diagnostic assays, methods to further characterize and differentiate *Erysipelothrix* spp. through the use of randomly amplified DNA, pulsed-field gel electrophoresis, and ribotyping have been shown to be useful and credible (13–15, 17).

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TABLE 1. Characteristics of the six swine operations used in this study

Site	Type of facility	Pig capacity	% of pigs affected	Clinical signs
A	Finisher	1,200	3	Rhomboid skin lesions, swollen joints, lethargy
B	Finisher	2,200	2	Rhomboid skin lesions, swollen joints, lameness, pyrexia, lethargy
C	Breeding herd	1,600	2	Swollen joints, lameness, abortions
D	Boar stud	150	1	Rhomboid skin lesions, swollen joints, lameness
E	Finisher	2,400	2	Rhomboid skin lesions, lameness
F	Breeding herd	1,100	4	Acute death, rhomboid skin lesions, cyanosis of skin, abortions

Recent investigations have focused on antibodies against the cell surface components of *E. rhusiopathiae* and their protective role. Genes encoding surface protective antigens (Spa) have been cloned, and nucleotide sequences have been determined (11, 22). Spa-related genes of all *E. rhusiopathiae* serotypes and *Erysipelothrix* sp. strain 2 (serotype 18) were analyzed, and Spa proteins can be classified into three molecular species, SpaA, SpaB, and SpaC (29). The SpaA protein was identified in *E. rhusiopathiae* serotypes 1a, 1b, 2, 5, 8, 9, 12, 15, 16, 17, and N, the SpaB protein was identified in *E. rhusiopathiae* serotypes 4, 6, 11, 19, and 21, and the SpaC protein was identified only in serotype 18 (29). Additional work further differentiated SpaB into subtypes SpaB1 (serotypes 4, 6, 8, 19, 21) and SpaB2 (serotype 11) (21).

Previous characterization of *Erysipelothrix* sp. isolates from affected pigs or isolates from the environment of U.S. swine sites dates back to the 1970s. The objective of this study was to identify, characterize, and compare *Erysipelothrix* sp. isolates from affected pigs and the environment from erysipelas outbreaks in six swine operations and to compare those isolates to the vaccine strains routinely used for vaccination in those same six operations.

MATERIALS AND METHODS

Site selection. Submissions to the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) between December 2007 and February 2009 from pigs with a clinical history consistent with acute septicemia (fever, lethargy, skin lesions, decreased feed intake) or chronic changes (swollen joints, lameness) suggestive of swine erysipelas were cultured for *Erysipelothrix* spp. Following a positive isolation, the submitting veterinarian was contacted to determine the vaccination status of the herd. Six sites with a vaccination program against *E. rhusiopathiae* were identified for environmental sample collection. The six sites were located in Iowa (sites A to D), Indiana (site E), and Illinois (site F) and environmental sample collections were done on these sites from 2007 to 2009 within 7 to 14 days of a positive isolation. Samples were collected from the same barn in pens with additional clinically affected pigs or pens where clinically affected pigs had been housed recently. The site structures, types, and clinical signs present are summarized in Table 1. All sites housed pigs in confinement equipped with automatic feeders and waters.

Pig samples. Sections of spleen, liver, lung, tonsil, kidney, and skin were collected from clinically affected pigs. A total of 31 samples from 1 to 3 pigs from each of the six sites were cultured. All isolates obtained were frozen at −80°C for future evaluation.

Environmental samples. Environmental samples were collected using swabs (Culturette, Becton Dickinson, Sparks, MD) from the water supply area and nipple drinkers, wall surfaces, feed lines, air inlets, and exhaust fans. Feed, manure, and central-line water samples were also collected in sterile 50-ml tubes (Thermo Fisher Scientific Remel, Lenexa, KS). For purposes of this study, oral fluid samples were also collected and classified as environmental samples, as they were from pigs on-site and not from the same pigs from which tissue specimens were collected. Oral fluids (mainly saliva) were collected only from sites C and D. In brief, a 3-strand, 1.27-cm-diameter cotton rope was placed in pens with 4 to 6 pigs. The rope was hung at approximately the height of the pigs' shoulders and left in place for 20 to 30 min. Oral fluids were then collected by mechanically compressing the rope and collecting the fluid in sterile 5-ml snap cap tubes (20). All environmental samples were immediately placed on ice following collection and stored at −20°C until microbiologic evaluation. Evaluation was done within 3 months of collection.

Vaccine strains. Four attenuated live vaccine strains (sites A, B, D, and E), one attenuated live autogenous vaccine strain (site F), and one inactivated vaccine strain (site C) were collected from the respective sites. Attenuated live vaccines included ERY VAC 100 (Arko Laboratories Limited, Jewell, IA), which was used at sites A and B; Suvaxyn E-oral (Fort Dodge Animal Health, Inc., Fort Dodge, IA), which was used at site D; and Ingelvac ERY-ALC (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO), which was used at site E. An attenuated live autogenous vaccine produced by using a site-specific *Erysipelothrix* sp. isolate (Newport Laboratories, Worthington, MN) was used at site F. An inactivated vaccine (Suvaxyn Parvo/E, Fort Dodge Animal Health, Inc., Fort Dodge, IA), which was serotype 2 based on the manufacturers' information, was used at site C. The five attenuated live vaccine strains were immediately placed on ice following collection and stored at −20°C until evaluation. Evaluation was done within 3 months of collection.

Bacterial isolation. An *Erysipelothrix* species-selective broth protocol as previously described was utilized for bacterial isolation (1). The selective broth was prepared as follows: 25 g heart infusion broth (Becton Dickinson) was dissolved in 1 liter of 0.1 phosphate buffer solution (12.02 g of Na_2HPO_4 and 2.09 g of KH_2PO_4 per liter of distilled water) and autoclaved. Sterile fetal bovine serum (5%; Sigma-Aldrich, St. Louis, MO), kanamycin (400 mg/ml; Sigma-Aldrich), and neomycin (50 mg/ml; Sigma-Aldrich) were added to the broth (31). Specimens were cultured on an *Erysipelothrix* species-selective agar as previously described (18).

Sample preparation. (i) Tissue specimens. Samples were cut into 2- by 3-cm sections, added to 2 ml of 0.85% physiologic saline solution, and homogenized using a stomacher (Seward, Bohemia, NY), and 300 μl of the resulting tissue homogenate was added to 3 ml of *Erysipelothrix* species-selective broth and incubated at 35°C for 24 to 48 h. At 24 h and again at 48 h, a 100-μl subculture from the *Erysipelothrix* species-selective broth was put onto a Trypticase soy agar plate containing 5% sheep blood, a colistin-nalidixic acid (Becton Dickinson) agar plate containing 5% sheep blood, and an *Erysipelothrix*-selective plate as described previously (1). Colonies were subcultured on sheep blood agar plates, incubated for 24 h, and then biochemically confirmed using standard laboratory methods (27, 31).

(ii) Water, oral fluids, and vaccines. Each specimen (300 μl) was added to 3 ml of *Erysipelothrix* species-selective broth and incubated.

(iii) Swabs. Culturette swabs were placed directly into the *Erysipelothrix* species-selective broth.

(iv) Manure and feed samples. A portion of the sample (75 to 100 g) was placed into sterile flasks, and 0.1 M phosphate buffer solution (pH 7.231) was added for a total volume of approximately 230 ml. The homogenate was mixed for 10 min with magnetic metal stir bars (37) and then transferred to centrifuge tubes and centrifuged for 10 min at 1,000 rpm. The supernatant (approximately 2 to 5 ml) was transferred to a flask to which 250 ml of the *Erysipelothrix* species-selective broth was added. Each flask was thoroughly mixed, incubated at 35°C for 24 h, and then subcultured to media in a manner similar to that used for tissue and liquid specimens (37).

Further characterization of the *Erysipelothrix* sp. isolates. (i) Serotyping. A pure culture was grown at 37°C for 36 h in 30 ml of heart infusion broth (Becton Dickinson) supplemented with 10% equine serum (Sigma-Aldrich) (35). The culture was then killed by adding 1% formalin (Sigma-Aldrich), held at room temperature for 12 h, harvested by centrifugation, and washed twice in 0.85% NaCl solution containing 0.5% formalin. Washed cells were suspended in 1.5 ml of distilled water and autoclaved at 121°C for 1 h (35). The supernatant was

TABLE 2. Isolation success of *Erysipelothrix* spp. in different environmental samples and associated genotypes and serotypes

Source of sample	No. of positive samples	No. of negative samples	% positive samples	Genotype(s) identified	Serotypes identified
Manure	13	15	46.4	*E. rhusiopathiae*, *Erysipelothrix* sp. strain 1, *Erysipelothrix* sp. strain 3[a]	1a, 2, untypeable
Oral fluid	5	6	45.5	*E. rhusiopathiae*, *Erysipelothrix* sp. strain 1	9, 12, 21, untypeable
Feed	9	8	52.9	*E. rhusiopathiae*	1a, 2
Feed line	0	7	0		
Wall	11	12	47.8	*E. rhusiopathiae*	1a, 2
Central-line water	6	22	21.4	*E. rhusiopathiae*, *Erysipelothrix* sp. strain 1	1a, 1b, 6, 13
Nipple drinker	9	8	52.9	*E. rhusiopathiae*	1a, 1b, 2, 6
Fan	3	8	27.3	*E. rhusiopathiae*	1a, 2

[a] Identified as and designated *Erysipelothrix* sp. strain 3 in the present study.

collected and used for the agar gel precipitation test (12). Homologous positive controls were used with each test run. Reactions were recorded after 24 h (35).

(ii) DNA extraction for PCR assays. For DNA extraction, bacterial colonies were suspended in 200 μl of sterile water by vigorous stirring. The suspension was then used for DNA extraction using the QIAmp DNA blood minikit (Qiagen, Valencia, CA) following the manufacturer's instruction, and the extracted DNA was kept at −20°C until use.

(iii) Genotype multiplex PCR assay. Further characterization was done on randomly selected representative isolates of each serotype collected at each site. A multiplex real-time PCR assay previously described was used to confirm the presence of *E. rhusiopathiae*, *E. tonsillarum*, or *Erysipelothrix* sp. strain 2 (19). A modified *Erysipelothrix* sp. strain 2 primer was utilized to increase the sensitivity of the assay described by Shen et al. (21).

(iv) Identification of *Erysipelothrix* sp. strain 1 by conventional PCR assay. A pair of specific primers, Sp11508F (5′-AGACGAAAGCGGCGATTACT-3′) and Sp12362R (5′-CCCCTACCACTTGCATTTAATGC-3′), were designed from the 16S rRNA gene of *Erysipelothrix* sp. strain 1 (GenBank accession no. AB019249). The PCR was performed in a model 9700 GeneAmp PCR system (Applied Biosystems, Foster City, CA) in 25-μl mixtures containing 1.25 U (0.25 μl) *Taq* DNA polymerase (Invitrogen, Carlsbad, CA), 0.2 mM deoxynucleoside triphosphate (dNTP), 0.4 μM each of the primers, 1.5 mM $MgCl_2$, and 4 μl DNA extract. The cycling conditions were 5 min at 95°C followed by 35 cycles of 40 s at 95°C, 40 s at 55°C, and 1 min at 72°C and a final extension at 72°C for 7 min. The amplified PCR products were separated by gel electrophoresis on a 1% agarose (Amresco, Solon, OH) gel and visualized by UV irradiation after ethidium bromide staining of the gel. The specificity of the *Erysipelothrix* sp. strain 1 conventional PCR assay was investigated by testing *E. rhusiopathiae* reference strain Tuzok (serotype 6), *E. tonsillarum* reference strain Lengyel-P (serotype 10), *Erysipelothrix* sp. strain 2 reference strain 715 (serotype 18), and *Erysipelothrix* sp. strain 1 reference strain Pécs 18 (serotype 13). A specific product of 855 bp was amplified from the *Erysipelothrix* sp. strain 1 reference strain, whereas no PCR products were amplified from the other isolates used. The sensitivity of this conventional PCR was determined to be 1×10^4 CFU per reaction (data not shown).

(v) Spa multiplex real-time PCR. A multiplex real-time PCR assay was utilized for identification of the Spa type present (*spaA*, *spaB1*, *spaB2*, and *spaC*) on the same isolates used for genotyping (21).

(vi) DNA sequencing. From selected samples (of undetermined serotype), the 16S rRNA gene was amplified as described previously (5). Amplified products were sequenced at the DNA facility of Iowa State University, Ames, IA. The consensus 16S rRNA sequence was evaluated by comparison with those available in GenBank using the Basic Local Alignment Search Tool (BLAST).

Nucleotide sequence accession number. *Erysipelothrix* sp. strain 3 was deposited in GenBank under accession no. HM216182.

RESULTS

Isolation and further characterization of isolates (genotype, serotype, and spa type). (i) Clinically affected pigs. Among the pig tissue samples examined, 83.9% (26/31) were found to be culture positive for *Erysipelothrix* spp. (see Table 3). Skin samples were received from all six sites, and 100% (11/11) were culture positive. *Erysipelothrix* spp. were also isolated from all tonsil (5/5) and kidney (3/3) samples obtained; however, these sample types were not submitted from every site. Table 3 describes the distribution of *Erysipelothrix* sp. isolates in tissues submitted from each site. The isolation success was 50% for heart (1/2) and liver (2/4) tissues and 66.7% for spleen tissues (4/6). Based on serotyping and multiplex PCR, all 26 isolates were found to be *E. rhusiopathiae*. The *E. rhusiopathiae* isolates recovered from each site were found to belong to the same serotype. The more common serotypes in affected pigs were serotypes 1a (sites D and F) and 2 (sites A and B). In addition, serotype 21 was present in pigs from site C, and serotype 1b was identified in pigs from site E. All 26 *E. rhusiopathiae* isolates recovered from pig tissues were positive for *spaA*.

(ii) Environmental samples. Of 142 environmental samples examined, 39.4% (56/142) were found to be culture positive for *Erysipelothrix* spp. (Table 2). Genotyping and serotyping revealed that 92.9% (52/56) of the environmental isolates belonged to *E. rhusiopathiae* and 5.4% (3/56) belonged to *Erysipelothrix* sp. strain 1, while in one isolate (1.8%) the genotype was novel and not previously described. For the purpose of this study, this genotype was designated *Erysipelothrix* sp. strain 3 (GenBank accession no. HM216182). The *Erysipelothrix* sp. strain 3 isolate was PCR negative for all known genotypes; however, its 16S rRNA region sequence was identical to those of GenBank accession no. AB055910.1 and AB055909 (*E. rhusiopathiae* strain KG-BB2), confirming its *Erysipelothrix* sp. origin. The most commonly identified serotype was 1a, which was identified in 37.5% (21/56) of the isolates, followed by serotype 2 (33.9% [19/56 isolates]), serotype 1b (10.7% [6/56 isolates]), serotypes 6 and 21 (each 3.6% [2/56 isolates]), and serotypes 9, 12, and 13 (each 1.8% [1/56 isolates]). The serotype of 5.4% (3/56) of the isolates was not determinable. All environmental isolates identified as *E. rhusiopathiae* were found to be positive for *spaA*, and all isolates identified as *Erysipelothrix* sp. strain 1 or with an undeterminable genotype were negative for *spaA*, *-B1*, *-B2*, and *-C*. The frequency of detection of *Erysipelothrix* spp. in environmental samples is summarized in Table 2, with nipple drinkers and feed as the sources where samples had the highest positive isolation rate. Serotypes that were detected in clinically affected pigs were also found in manure, feed, wall swabs, central-line water, nipple drinkers, and fans for serotype 1a; central-line water and nipple drinkers for serotype 1b; manure, feed, wall swabs,

TABLE 3. *Erysipelothrix* species isolation and serotypes from clinically affected pigs and environmental samples from six different sites

Site (vaccine serotype)	Tissue samples			Environmental samples		
	Source	No. of positive samples/total no. of samples	Serotype	Source	No. of positive samples/total no. of samples	Serotype
A (1a)	Skin	2/2	2	Central-line water	0/6	2
	Tonsil	1/1	2	Nipple drinker	2/4	2
	Spleen	1/2	2	Manure	7/12	2
	Liver	1/2	2	Wall	6/8	2
				Feed line	0/4	
				Feed	3/5	1a (2/3), 2
				Fan/inlet	2/4	2
B (1a)	Skin	2/2	2	Central-line water	4/9	1a (2/4), 6, 13[a]
	Kidney	1/1	2	Nipple drinker	2/5	2, 6
	Spleen	1/1	2	Manure	1/5	1a
	Tonsil	1/1	2	Wall	2/8	1a
				Feed line	0/3	
				Feed	3/6	1a
				Fan/inlet	0/3	
C (2)	Skin	1/1	21	Oral fluid	2/4	21
	Tonsil	1/1	21	Central-line water	0/9	
	Spleen	1/1	21	Nipple drinker	0/3	
				Manure	0/6	
				Wall	0/3	
				Feed	0/3	
				Fan/inlet	0/3	
D (1a)	Skin	2/2	1a	Oral fluid	3/7	9, 12, untypeable[a]
				Central-line water	0/2	
				Manure	2/2	Untypeable[a]
				Wall	0/1	
E (1a)	Skin	2/2	1b	Central-line water	2/2	1b
	Tonsil	2/2	1b	Nipple drinker	2/2	1b
	Spleen	1/2	1b	Feed	2/2	1b
F (2)	Skin	2/2	1a	Nipple drinker	3/3	1a
	Liver	1/2	1a	Manure	3/3	1a
	Heart	1/2	1a	Wall swab	3/3	1a
	Kidney	2/2	1a	Feed	1/1	1a
				Fan/inlet	1/1	1a

[a] Characterized as a non-*E. rhusiopathiae* strain.

central-line water, and fans for serotype 2; and oral fluid for serotype 21.

(iii) Vaccine strains. All four commercially available attenuated live vaccine strains (sites A, B, D, E) were identified as *E. rhusiopathiae* serotype 1a, and the autogenous vaccine strain used on site F was identified as *E. rhusiopathiae* serotype 2. All five vaccine strains were positive for *spaA*.

Isolation success and distribution of isolates from the different sites. (i) Site A. A total of 7 tissue samples from affected pigs were obtained and 43 environmental samples were collected (Table 3). *E. rhusiopathiae* serotype 2 was isolated from 5/7 tissues and from 18/43 environmental samples (nipple drinker swabs, manure, wall swabs, feed, and fan swabs). In addition, *E. rhusiopathiae* serotype 1a was identified in 2/5 feed samples.

(ii) Site B. A total of five tissue samples from affected pigs were obtained and 39 environmental samples were collected (Table 3). *E. rhusiopathiae* serotype 2 was isolated from all tissue samples and from 1 nipple drinker swab. *E. rhusiopathiae* serotype 1a was isolated from 8 environmental samples (central-line water, manure, wall swab, and feed). In addition, *E. rhusiopathiae* serotype 6 was isolated from a central-line water sample and a nipple drinker swab, and serotype 13 (*Erysipelothrix* sp. strain 1) was isolated from a central-line water sample.

(iii) Site C. A total of three tissue samples from affected pigs were obtained and 31 environmental samples were collected (Table 3). *E. rhusiopathiae* serotype 21 was isolated from both tissue samples and from two oral fluid samples.

(iv) Site D. A total of two tissue samples from affected pigs were obtained and 12 environmental samples were collected (Table 3). *E. rhusiopathiae* serotype 1a was isolated from both tissue samples but was not identified in any of the environmental samples collected. Instead, *E. rhusiopathiae* serotypes 9 and 12 were isolated from oral fluids and *Erysipelothrix* sp. strain 1 was isolated from oral fluid and manure (serotype undeterminable). One additional isolate (novel *Erysipelothrix* sp. strain 3; serotype undeterminable) was isolated from manure.

(v) Site E. A total of six tissue samples from affected pigs were obtained and 6 environmental samples were collected (Table 3). *E. rhusiopathiae* serotype 1b was isolated from all tissue and environmental samples.

(vi) Site F. A total of eight tissue samples from affected pigs were obtained and 11 environmental samples were collected (Table 3). *E. rhusiopathiae* serotype 1a was isolated from all tissue and environmental samples.

DISCUSSION

Results from this investigation indicate that during a clinical outbreak of swine erysipelas, *Erysipelothrix* spp. can be isolated from a variety of environmental samples. Earlier investigations on swine erysipelas conducted in the United States have reported similar results in identifying *Erysipelothrix* spp. from swine and swine production premises; however, studies have not been conducted on sites with acute swine erysipelas outbreaks (35, 37). Interestingly, in this study we identified three *Erysipelothrix* sp. strain 1 isolates (1/3 was serotype 13 and 2/3 were untypeable), indicating the possibility of one or more new serotypes within *Erysipelothrix* sp. strain 1 in addition to serotype 13. Moreover, a new genotype of *Erysipelothrix* designated *Erysipelothrix* sp. strain 3, whose serotype and *spa* type were also unknown, was identified in an environmental sample, indicating the possibility of a new serotype within the species.

When tissues from affected pigs were investigated, it was found that skin specimens with visible rhomboid lesions were consistently culture positive, implicating skin as the tissue of choice for isolation of *Erysipelothrix* spp., which is in agreement with previous work (1). In addition, all tested tonsil samples (5/5) were found to be culture positive. The existence of *Erysipelothrix* spp. in the tonsils of healthy pigs is suspected (24); however, in the current study, all selected isolates obtained from tonsils had the same serotype as isolates recovered from other organs of affected pigs. *E. rhusiopathiae* serotypes 1a, 1b, and 2 are commonly associated with clinical disease in pigs and were associated with clinically affected pigs in five of the six sites. Interestingly, serotype 21 was found in affected pigs from one of the six sites. While uncommon, this has been reported previously in a larger study that characterized 1,046 isolates recovered from pigs with swine erysipelas in Japan and found that 1.1% of *Erysipelothrix* sp. isolates belonged to serotype 21 (26). In addition, isolates with reactivity to both serotypes 1b and 21 (termed 1b $\times$ 21) have been isolated on four occasions from affected pigs in three Australian herds with clinical erysipelas (3).

Among the environmental samples, *Erysipelothrix* spp. were most frequently isolated in feed and nipple drinkers (both 9/17 samples). This was followed by isolation from wall swabs (11/23 samples) and manure (13/28 samples). While isolation of *Erysipelothrix* spp. has been described from manure and soil (31), to our knowledge this is the first description of isolation of *Erysipelothrix* spp. from nipple drinkers, walls, and ventilation fans. Although clinical disease was present at all sites, relatively high percentages of environmental samples were culture negative (53.5% at site A, 69.2% at site B, 93.5% at site C, and 41.7% at site D). This may have to do with the total number of samples collected, the sample types, storage, or a delay between the original case submission and follow-up site visit or may be related to shedding mechanisms of *Erysipelothrix* spp. Previous studies demonstrated no evidence of growth or maintenance of *Erysipelothrix* spp. in soil or manure samples from swine pens (32), but the role of soil, manure, and pit slurry as reservoirs or sources of infection is not completely understood (34).

The attenuated live vaccine strains utilized on the different sites were also collected, cultured, and characterized. All sites in this investigation utilized a vaccine as part of an erysipelas control plan. Four of six sites (sites A, B, D, and E) utilized attenuated live vaccines, produced by three manufacturers. All three of these commercially available vaccines were found to be positive for *E. rhusiopathiae* serotype 1a. Interestingly, two of the four sites using these vaccines had clinical infections with serotype 2, one of the four sites had infections with serotype 1b, and one site had infections with serotype 1a based on the isolation of these serotypes from affected pigs. One site (F) used an autogenous attenuated live vaccine based on a site-specific isolate. The isolate recovered from the autogenous vaccine was identified as serotype 2; however, *E. rhusiopathiae* serotype 1a was isolated from affected pigs from this site and was also the only serotype present in the environment. Many factors affect the ability of a live vaccine to elicit protection, including but not limited to vaccine storage, route and dose of administration, age, maternal immunity, antimicrobial therapy, and the vaccine strains used. Based on cross-protection studies done in the 1980s, it was found that live serotype 1a protected against serotypes 1b and 2 (25). At site D, we found that the serotype of the live vaccine used was identical to that isolated from the pig tissues. In Japan in 1932, an acriflavine-resistant attenuated live vaccine was developed; this vaccine has been used intensively since the erysipelas outbreaks in 1966 and 1967. However, since the 1990s, approximately 2,000 pigs annually have been shown to have acute and subacute septicemia. It is believed that the live vaccine was the causal agent for this high number of pigs with swine erysipelas (6), and this could also be a possibility in herd D. Earlier studies have shown that serotype 2 bacterins can protect against serotype 1 challenge (33, 38). Cross-protection was apparently not sufficient to prevent clinical disease in this case, providing evidence of the need to further evaluate cross-protection in the swine model using recent field isolates.

In this study, *Erysipelothrix* spp. were isolated from the environments of clinically affected swine. In addition, the *Erysipelothrix* sp. isolates recovered from clinically affected pigs and the majority of the *Erysipelothrix* sp. isolates recovered from the pigs' environments were found to be identical in four of the six sites (site A, serotype 2; site C, serotype 21; site E, serotype 1b; and site F, serotype 1a; Table 3). However, dissimilarities between isolates in pigs and isolates from their environments were identified in two of the six sites investigated. While pigs in these two sites were infected with very common serotypes (serotypes 1a and 2), a variety of serotypes were identified in the environment (serotypes 1a, 2, 6, 9, 12, 13, and untypeable) (Table 3). It has been determined that *E. rhusiopathiae* serotypes remain stable through swine serial passage or under different storage conditions (23, 30). In this investigation, serotypes identified in the environment were both identical to (4/6 sites) and different from (2/6 sites) what was isolated from clinically affected pigs from these sites; however, affected sites

were visited only once. To fully understand the interactions and relations between isolates associated with disease in pigs and isolates present in the environment, repeated collection over time of samples would provide more complete information.

In this study, all recovered isolates were also tested for their dominant *spa* type. The Spa protein of *E. rhusiopathiae* has been shown to be attached at the cell surface level of the bacteria and also to be a major protective antigen against infection by *E. rhusiopathiae* (7, 8, 11, 29). All *E. rhusiopathiae* isolates obtained from affected pig tissues (serotypes 1a, 2, and 21), environmental samples (serotypes 1a, 1b, 2, 6, 9, 12, and 21), and vaccine strains (serotypes 1a and 2) were found to contain a single *spa* type, *spaA*. The identification of *spaA* in serotype 21 is in contrast to a previous report where *spaB* was identified (29) but in agreement with another study which reported serotype 21 as expressing SpaA (9). Moreover, the findings of this study support previous results that *spaA* is highly conserved in serotypes most often associated with clinical swine erysipelas (9). All five vaccine strains characterized were found to be positive for *spaA*, and based on the current state of knowledge, cross-protection should have occurred (9, 29). However, the role of vaccine handling, administration, and timing should not be overlooked and can be considered a potential explanation for the lack of protection. In addition, vaccine efficacy testing of vaccine batches may be unreliable, as testing is often done in mice, in which vaccine efficacy may or may not mimic what occurs in pigs. All three *Erysipelothrix* sp. strain 1 isolates and the *Erysipelothrix* sp. strain 3 isolate were found to be negative for all *spa* types investigated (A, B1, B2, C). These isolates were identified in two sites and were present in central-line water, oral fluid, and manure. No recent reports have implicated *Erysipelothrix* sp. strain 1 to be pathogenic. Moreover, as this strain was not isolated from pig tissue based on present evidence, it is unlikely that *Erysipelothrix* sp. strain 1 is an important pathogen.

Much interest has recently been generated regarding *spa* types. Results of this study agree with previous work and indicate that *spa* types are likely to be quite conserved among swine isolates of *Erysipelothrix* spp. associated with disease. However, all of the isolates obtained from clinically affected pigs were *E. rhusiopathiae* strains, which are known to contain only either *spaA* or *spaB*. In this study, *spaA* was the only type found, which is consistent with findings of others who have reported that serotypes 1a, 1b, and 2 (all *spaA*) predominate in clinically affected pigs. What is interesting is that the serotype 21 isolate from pig tissue from site C contained *spaA* and not *spaB* as previously described (29). In one investigation in Australia, four farms were found to have isolates that were reactive to both 1b and 21 (3), which could indicate a possible reason for why *spaA* was found in this case.

Data from acute swine erysipelas outbreaks investigated in this study indicate that during an acute outbreak situation, *Erysipelothrix* spp. can be isolated from both clinically affected pigs and their environments. Characterization of the *Erysipelothrix* sp. isolates using serotyping and genotyping assays indicated that isolates from affected pigs and the environment are not necessarily the same on individual sites.

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