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***Erysipelothrix rhusiopathiae*: genetic characterization of midwest US isolates and live commercial vaccines using pulsed-field gel electrophoresis**

T. Opriessnig, L. J. Hoffman, D. L. Harris, S. B. Gaul, P. G. Halbur¹

Abstract. This is the first report of molecular characterization of US erysipelas field isolates and vaccine strains of *Erysipelothrix rhusiopathiae* by pulsed-field gel electrophoresis (PFGE). Erysipelas in pigs is mainly caused by *E. rhusiopathiae* serotypes 1a, 1b, and 2. In 2001, erysipelas reemerged as a clinical problem in pigs in the midwestern United States. In this work 90 erysipelas isolates (58 recent and 28 archived field isolates as well as 4 live-vaccine strains) were genetically characterized. Because of the limited availability of antiserum, 74/90 isolates (44/58 recent isolates) were serotyped. The serotype of the majority (79.6%) of the 44 recent isolates tested was determined to be 1a, 13.6% were serotype 1b, and 6.8% of recent isolates were serologically untypeable. Among all 90 isolates, 23 different PFGE patterns were identified. There were 43 isolates identified as serotype 1a with 4 genetic patterns: 38/43, 1A(I); 3/43, 1A(III); 1/43, 1B(V); and 1/43, 3B. Sixteen serotype 1b isolates had 11 unique genetic patterns: 4/16 were genotype 1B(III), 2/16 were genotype 3A(I), and 1/16 was in genotype groups 1A(V), 1A(VI), 1A(VII), 1B(I), 1B(IV), 1B(VII), 2, 4, and 5. Six genetic patterns were distinguished among the 10 serotype 2 isolates: 1A(IV) (1/10), 1A(V) (1/10), 1B(VI) (1/10), 2 (4/10), 7 (1/10), and 8 (2/8). Erysipelas vaccine strains (modified live) were similar to each other but different from current field strains, sharing 78.6% identity with the most prevalent genotype 1A(I) based on the PFGE-*Sma*I pattern. Compared with serotyping, PFGE genotyping is a more distinguishing technique, easy to perform and not dependent on the limited availability of antiserum.

In the United States, during the summer of 2001, erysipelas began occurring with increasing frequency in both vaccinated and nonvaccinated pigs. The clinical picture was typically acute at onset, with mortality attributed primarily to sudden deaths among pigs in the late stages of the grow–finish period. A 9-year analysis from cases submitted to the Iowa State University Veterinary Diagnostic Laboratory, Ames, IA (ISU-VDL) demonstrated a 4-fold increase of erysipelas cases in 2001 compared with the years 1993–2000. There has also been a marked increase in carcass condemnations associated with erysipelas-like skin lesions for several months after the outbreaks, with a peak of 4,000 condemnations per million carcasses in August 2001 (M. Engle, personal communication). Practitioners and producers have raised questions on the emergence of new *Erysipelothrix rhusiopathiae* serotypes or strains, vaccine efficacy, and safety of live erysipelas vaccines.

The gram-positive, facultative anaerobic bacterium *E. rhusiopathiae* is widely distributed and causes erysipelas in swine, sheep, fish, reptiles, and birds, as well

as erysipeloid, a human skin disease.¹³ The genus *Erysipelothrix* is divided into the species *E. rhusiopathiae* (serovars 1a, 1b, 2, 4, 5, 6, 8, 9, 11, 12, 15, 16, 17, 19, 21, and N), *E. tonsillarum* (serovars 3, 7, 10, 14, 20, 22, and 23), *Erysipelothrix* sp. strain 1 (serovar 13), and *E. sp.* strain 2 (serovar 18). This distinction was made based on DNA–DNA hybridization and with polymerase chain reaction.^{8,9,11} The pig is known to be susceptible to at least 15 of the 28 described serotypes (Enoi C, Norrung V: 1992, Proc Int Pig Vet Soc: 345). Serotype 1a and 1b are associated with acute erysipelas, whereas serotype 2 is often associated with the chronic form of the disease (Yong-Jian S, Wei H: 1990, Proc Int Pig Vet Soc: 193).

Thirty-to-fifty percent of pigs are known to harbor *E. rhusiopathiae*.¹³ Usually, these pigs are healthy, yet the organism can be found in tonsils and lymphoid tissues. Subclinically infected pigs are thought to be the reservoir for acute erysipelas outbreaks. *Erysipelothrix rhusiopathiae* is shed in feces, urine, saliva, and nasal secretions. Generally, the most susceptible hosts are pigs between 3 months and 3 years of age. Pigs less than 3 months of age seem to be protected by maternal antibodies, and pigs over 3 years have acquired protective immunity by going through multiple subclinical infections during their life.¹³

Recently it has been demonstrated that pulsed-field gel electrophoresis (PFGE) can be used to differentiate

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Table 1. Differences in antibiotic susceptibility among erysipelas vaccine strains.

Vaccine strain	Florfenicol	Oxytetracycline	Trimethoprim
Suvaxyn® E-oral‡	intermediate	susceptible	resistant
ERY VAC 100†	intermediate	susceptible	susceptible
Erycell™*	susceptible	susceptible	resistant
Ingelvac® ERY-ALC§	intermediate	resistant	resistant

* Novartis Animal Vaccines, Inc.

† Arko Laboratories Limited.

‡ Fort Dodge Animal Health, Inc.

§ Boehringer Ingelheim Vetmedica, Inc.

strains of *Erysipelothrix* within a serotype.⁵ The objectives of this study were to adapt and shorten the PFGE technique for use in a diagnostic laboratory, to use the PFGE for differentiation of genotypes within a serotype, and to compare recent erysipelas field isolates with vaccine strains and field strains from previous outbreaks in the United States. Comparison of field isolates with vaccine strains should help determine whether the outbreaks are due to differences in genotype and a lack of heterologous protection and whether the live vaccines themselves may be responsible for some of the erysipelas outbreaks.

Materials and methods

Sample source. Fifty-eight recent erysipelas field isolates recovered from tissues and animals submitted to the ISU-VDL during the years 1999–2002 were evaluated in this study. In addition, 5 attenuated live-vaccine strains of *E. rhusiopathiae* (Ingelvac® ERY-ALC,^a Erycell™,^b Suvaxyn® E-oral,^c and ERY VAC 100^d) and several archived *E. rhusiopathiae* isolates of

known serotypes (10 serotype 2 isolates [1946–1988], 10 serotype 1b isolates [1948–1999], and 8 serotype 1a isolates [1975–1983]) were genetically compared with the recent field isolates. The recent field isolates had been collected and maintained at –80 C in PPLO medium (β -nicotinamide adenine dinucleotide,^e BBL® Mycoplasma broth base,^f horse serum^g) until further examination. All archived isolates had been stored lyophilized in sealed glass tubes.

Culture and antibiogram. Each isolate was cultured on 5% sheep blood agar and incubated at 37 C for 48 hr. After incubation the cultures were evaluated for purity. Antibiograms of all isolates were conducted according to a standardized protocol,⁴ using the National Committee for Clinical Laboratory Standards (NCCLS) microdilution technique. Susceptibility to the following antibiotics was tested: ampicillin, apramycin, ceftiofur, chlortetracycline, clindamycin, enrofloxacin, erythromycin, florfenicol, gentamicin, neomycin, oxytetracycline, penicillin, sulfachlorpyridazine, sulfadimethoxine, sulfathiazole, tiamulin, tilmicosin, trimethoprim, and tylosin. Quality control was performed with *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and

Table 2. Pulsed-field gel electrophoresis patterns (genotypes) of recent and archived serotype 1a field isolates.

Isolate No.	Date isolated	Genotype	Isolate No.	Date isolated	Genotype
HC-585	Feb 12, 1975*	1B(V)	48251/01	Aug 27, 2001	1A(I)
ME-7	Feb 10, 1976*	1A(III)	48554/01	Aug 28, 2001	1A(I)
2437	Apr 26, 1977*	1A(I)	49313/01	Aug 30, 2001	1A(I)
2449	Apr 26, 1977*	1A(I)	5024/01	Jan 23, 2001	1A(III)
2403	Apr 26, 1977*	1A(I)	51172/01	Sep 11, 2001	1A(I)
2329	Apr 26, 1977*	1A(I)	51675/01	Sep 14, 2001	1A(III)
2336	Apr 26, 1977*	1A(I)	53225/E/01	Sep 24, 2001	1A(I)
EI-6P	Apr 14, 1983*	3B	53815/01	Sep 26, 2001	1A(I)
34930/99	Oct 4, 1999	1A(I)	55600/01	Oct 8, 2001	1A(I)
35050/01	Jun 26, 2001	1A(I)	57146/01	Oct 16, 2001	1A(I)
37187/01	Jul 3, 2001	1A(I)	57592/01	Oct 19, 2001	1A(I)
37472/01	Jul 12, 2001	1A(I)	58689/01	Oct 25, 2001	1A(I)
40088/01	Jul 17, 2001	1A(I)	59086/01	Oct 30, 2001	1A(I)
40127/01	Jul 17, 2001	1A(I)	59172/01	Oct 30, 2001	1A(I)
41721/01	Jul 24, 2001	1A(I)	60716/01	Nov 8, 2001	1A(I)
41878/01	Jul 30, 2001	1A(I)	60785/01	Nov 8, 2001	1A(I)
44890/01	Aug 8, 2001	1A(I)	62490/01	Nov 20, 2001	1A(I)
45408/01	Aug 10, 2001	1A(I)	64765/01	Dec 4, 2001	1A(I)
45875/01	Aug 21, 2001	1A(I)	65403/01	Dec 7, 2001	1A(I)
45879/01	Aug 14, 2001	1A(I)	65408/01	Dec 7, 2001	1A(I)
47418/01	Aug 22, 2001	1A(I)	67002/01	Dec 15, 2001	1A(I)
48236/01	Aug 25, 2001	1A(I)			

* Date lyophilized.

Table 3. Pulsed-field gel electrophoresis patterns (genotype) of serotype 1b isolates and the 2 modified live-vaccine strains.

Isolate No.	Date isolated	Genotype
228 H	Oct 26, 1948*	1A(VII)
F-45	May 2, 1950*	1B(III)
HC2900	Jun 21, 1950*	1B(III)
422-1	Feb 16, 1961*	1B(IV)
De Castro	Jul 13, 1962*	2
EW2	Apr 24, 1974*	1B(I)
DG 1534	Jun 5, 1979*	1A(V)
OARDC 3	Jan 31, 1980*	1B(III)
OARDC 4	Nov 20, 1980*	1B(III)
DG1966 HT	Jun 5, 1999*	4
35078/00	Jul 28, 2000	3A(I)
18850/01	Apr 4, 2001	Undetected
22524/01	Apr 25, 2001	5
57586/01	Oct 19, 2001	1B(VII)
57742/01	Oct 19, 2001	3A(I)
58262/01	Oct 24, 2001	1A(VI)
ERY VAC 100†	...	1A(II)
Ingelvac® ERY-ALC‡	...	1A(II)

* Date lyophilized.

† Arko Laboratories Limited.

‡ Boehringer Ingelheim Vetmedica, Inc.

Staphylococcus aureus. For inoculation the technical details of the veterinary NCCLS documents⁴ were followed. All results were within the limits established by NCCLS.⁴

Serotyping. A single colony was picked and inoculated into 60 ml of heart infusion broth^f with horse serum^g and incubated overnight at 37 C. Cells were then killed by addition of 1% formalin,^e held at room temperature overnight, harvested 1 day later by centrifugation, and washed twice in saline. After this step the cells were resuspended in 1.5 ml formalized saline^e and autoclaved for 1 hr at 100 C.¹⁵ The supernatant obtained from this step was collected and used as antigen in a gel precipitation test.² Homologous positive controls were used with each test. Reactions were recorded after 24 hr.¹⁵ *Erysipelothrix rhusiopathiae* isolates were serotyped at the National Veterinary Services Laboratories (NVSL), Ames, Iowa.

DNA preparation. The bacteria were picked with a swab and suspended in a cell suspension buffer (100 mM Tris-HCl, 100 mM ethylenediaminetetraacetic acid [EDTA] [pH 8.0]) to an absorbance of 1.0–1.1 in a spectrophotometer set at a wavelength of 612 nm. Ten microliters of lysozyme^e in a final concentration of 58.1 U/gml were added to 400 µl of the bacterial suspension and incubated for 30 min at 37 C. After incubation the plugs were prepared by adding 90 µl mutanolysin (5 U/gml),^e 20 µl proteinase K (20 mg/ml),^g and 400 µl melt plug agar.^h The solidified plugs were placed in cell lysis buffer (50 mM Tris-HCl, 50 mM EDTA [pH 8.0], 1% sarcosyl^e) and 40 µl proteinase K (20 mg/ml)^g in a shaking water bath at 54 C for 1.5 hr. After washing with autoclaved water and TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]), the plugs were stored in TE buffer at 4 C until use.

Pulsed-field gel electrophoresis. Digestion was done with *Sma*Iⁱ at a working concentration of 2.5%, with 1% bovine serum albuminⁱ and 10% buffer,ⁱ at 37 C for 2 hr. The di-

Table 4. Pulsed-field gel electrophoresis patterns (genotype) of serotype 2 isolates.

Isolate No.	Date isolated	Genotype
US 35	Nov 8, 1949*	1B(VI)
8943	Nov 10, 1971*	1A(IV)
R-32	Jan 24, 1973*	2
CN3461	Apr 12, 1979*	2
M-3	Dec 18, 1946*	8
830	Aug 16, 1949*	7
NF-4	Aug 22, 1972*	8
CN3342	Apr 19, 1979*	2
SE-9	Nov 8, 1983*	2
Jena 36	Feb 24, 1988*	1A(V)

* Date lyophilized.

gested plugs were loaded in their appropriate wells in the gel (pulsed-field certified agarose^h). Electrophoresis was carried out in a contour-clamped homogeneous electric field (CHEF DRIII^h) for 21 hr at 12 C and 6 V with pulse times from an initial 2.2 sec to a final 64 sec. Pulsed-field gel electrophoresis patterns were detected by UV transillumination after ethidium bromide staining. Lambda ladder^h was used as DNA size standard.

Data analysis. Pulsed-field gel electrophoresis patterns were analyzed visually and also compared with BioNumerics software. Dendograms used the unweighted pair group method using arithmetic averages (UPGMA), dice coefficient, and 0.9% optimization with 2.0% band position tolerance.³

Results

Culture and antibiogram. Colonies from all the erysipelas field isolates and the vaccine strains were similar. They appeared tiny and were transparent, rough-to-smooth colonies with a narrow zone of partial hemolysis in some of the isolates. Antibiograms showed that all isolates were susceptible to ampicillin, ceftiofur, clindamycin, enrofloxacin, erythromycin, penicillin, tiamulin, tilmicosin, and tylosin, whereas all isolates were resistant to apramycin, neomycin, sulfadimethoxine, sulfachlorpyridazine, and sulfathiazole. Differences in susceptibility among isolates were found with the following antibiotics: chlortetracycline (32/90 resistant, 20/90 intermediate, 38/60 susceptible), florfenicol (1/90 resistant, 62/90 intermediate, 27/60 susceptible), gentamicin (86/90 resistant, 2/90 intermediate, 2/60 susceptible), oxytetracycline (33/90 resistant, 18/90 intermediate, 39/60 susceptible), and trimethoprim (46/90 resistant, 44/60 susceptible). The susceptibility profiles seemed to follow no specific pattern and were independent of serotype or genotype. The vaccine strains showed differences among them with 3/19 antibiotics tested (Table 1).

Serotyping. Because of a lack of antiserum, only 44/58 recent isolates from 1999 to 2001 were serotyped. Thirty-five of the isolates from the years 1999 to 2001 were serotype 1a (Table 2). One isolate from

Table 5. Pulsed-field gel electrophoresis patterns (genotype) of field isolates not tested because of unavailability of antiserum.

Isolate No.	Date isolated	Genotype	Isolate No.	Date isolated	Genotype
37289/00	Aug 9, 2000	1A(I)	37198/02	Jul 23, 2002	1A(I)
44997/00	Sep 22, 2000	1B(II)	37200/02	Jul 23, 2002	1A(I)
60682/01	Nov 7, 2001	6	38051/02	Jul 26, 2002	1A(I)
23581/02	May 10, 2002	1A(I)	38915/02	Jul 31, 2002	1A(I)
26914/02	Jun 3, 2002	1A(I)	40281/02	Aug 7, 2002	1A(I)
33644/02	Jul 8, 2002	1A(I)	26070/02	May 20, 2002	3A(I)
33882/02	Jul 9, 2002	1A(I)	30498/02	Jun 21, 2002	3A(II)
35030/02	Jul 12, 2002	1A(I)	Suvaxyn® E-oral*	...	1A(II)
35091/02	Jul 12, 2002	1A(I)	Erycell®†	...	1A(II)
36232/02	Jul 18, 2002	1A(I)			

* Fort Dodge Animal Health, Inc.

† Novartis Animal Vaccines, Inc.

2000 and 5 isolates from 2001 were serotype 1b (Table 3). The serotype could not be determined for two 2000 isolates and one 2001 isolate (Table 5).

Pulsed-field gel electrophoresis and data analysis. Pulsed-field gel electrophoresis patterns after restriction with *Sma*I were characterized by 8–14 bands in a 24.3–388-kb size range (Fig. 1). With PFGE it was possible to distinguish 23 different patterns from the 90 *E. rhusiopathiae* isolates (Tables 2–5; Fig. 2). Genetic labeling was done after comparison of the genetic relationships among the isolates. Dendrogram analysis revealed that at 30% divergence, 8 PFGE groups (A–H) were present (Fig. 2). At 27% divergence, groups A and C were divided into subgroups A1/A2 and C1/C2, respectively. Within the dendrogram the serotypes followed no specific pattern and were distributed throughout. The majority of the isolates (63/90) were within subgroup A1. Fifty-one of these isolates (38 serotype 1a, 12 not serotyped, 1 serotype undetected) belonged to genotype 1A(I). Erysipelas live vaccines

were slightly different from genotype 1A(I) but were identical to each other. They were designated as genotype 1A(II). Data analysis of the homogeneity of the PFGE patterns among the 1A genotypes revealed that 1A(I) and 1A(II) were 78.6% identical, 1A(I) and 1A(III) shared 91.7% identity, and the PFGE-patterns of 1A(II) and 1A(III) were 85.7% identical. Archived isolate 1A(IV) had the highest homology (92.9%) with the vaccine strains.

Discussion

This study represents the first genetic analysis of United States field isolates of *E. rhusiopathiae*. Diagnosis of erysipelas is typically confirmed by culture of the causative agent, and on rare occasions the isolates are serotyped. This service is offered only at the NVSL and has been temporarily terminated because of limited amounts of antiserum. Although most of the isolates are serotype 1a or 1b, it has not been possible in the past to genetically characterize strains within a

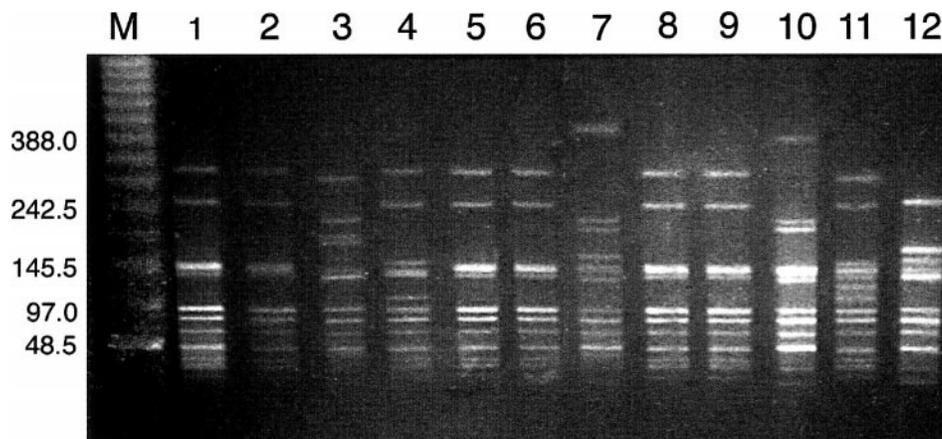


Figure 1. Pulsed-field gel electrophoresis gel showing 12 erysipelas field isolates (lanes 1–12) digested with *Sma*I. A 48.5-kb lambda DNA size standard (lane M), genotype 1A(I) isolates (lanes 1, 2, 5, 6, 8, and 9), genotype 1B(VII) isolate (lane 3), genotype 1A(III) isolate (lane 4), genotype 1B(II) isolate (lane 7), genotype 1B(I) isolate (lane 10), genotype 1A(II) isolate (lane 11), and genotype 1B(VI) isolate (lane 12).

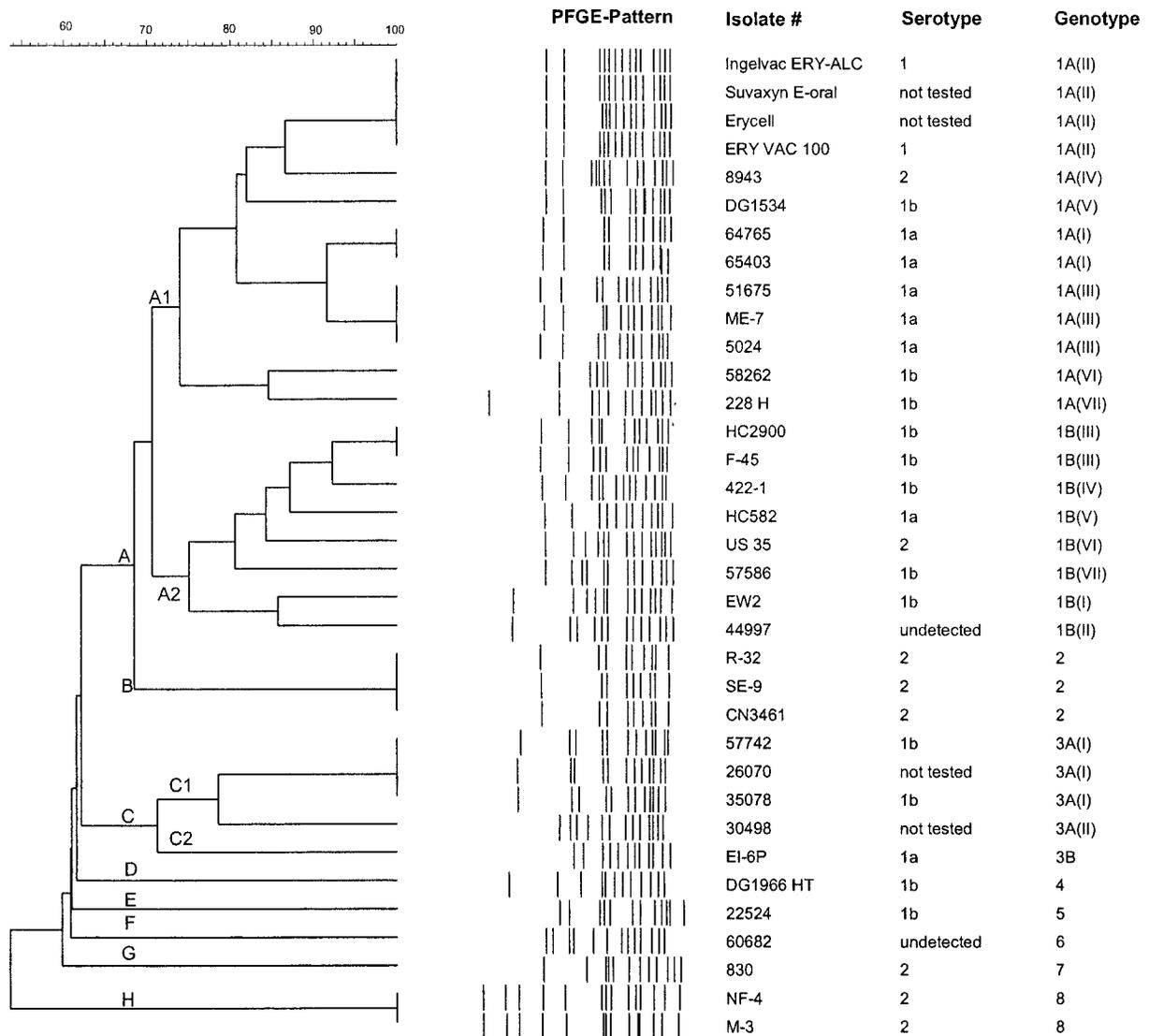


Figure 2. Genetic relationship between 32 erysipelas field isolates and 4 vaccine strains and schematic representation of 23 different PFGE patterns obtained after restriction with *Sma*I. The classification and divergence of isolates were calculated by the unweighted pair group method with averages from the PFGE results. At 30% divergence, 8 PFGE groups (A–H) were present; 2 of these groups, A and C, were subgrouped into A1/A2 and C1/C2 at 27% divergence, respectively.

given serotype. A recently published PFGE technology⁵ was modified for completion in 2 days rather than 5 days. Pulsed-field gel electrophoresis on recent serotype 1a isolates resulted in 2 patterns among 35 isolates. Thirty-three isolates were determined to be genotype 1A(I), and 2 isolates were designated as 1A(III). Among 6 recent serotype 1b isolates, 4 different patterns were identified. Two isolates were determined to be genotype 3A(I), and 1 isolate each was designated to be genotypes 1A(VI), 1B(VII), and 5. The pattern of 1 serotype 1b isolate remained undetected. It was found that the PFGE technique allowed for a reproducible determination of genotype, with the possibility of inferring serotype from such data, especially when identifying genotypes 1A(I), 1A(III),

and 3A(I), which currently seem to be the most common genotypes in the field.

By using PFGE, it was possible to compare recent isolates with archived ones. Because of availability of only a few older isolates in the archives, limited conclusions can be drawn in terms of whether genotype changes have occurred over time. Among 28 archived isolates, 5 archived 1a isolates were identified as genotype 1A(I), which was the major genotype among the 1999–2002 isolates. Also, genotype 1A(III) was found in an isolate from 1976, and it reappeared in 2 field isolates recovered in 2001. These findings disprove speculations over the appearance of new strains accounting for current outbreaks. The remaining 3 archived 1a isolates had unique patterns not observed in any of the recent field isolates.

This was also the case for archived serotype 1b isolates, which seemed not to share genetic profiles with recent isolates based on PFGE.

In addition to PFGE, all isolates were tested for susceptibility to antibiotics for additional evidence on relationships of the isolates, as is commonly done with *Salmonella* sp.⁶ The current resistance analysis showed that all isolates were susceptible to a variety of antibiotics and also resistant to others. The resistance or susceptibility of erysipelas to 14 antibiotics appeared to be stable over time because the oldest isolate was lyophilized in 1948. Differences in susceptibility were found in only 5 antibiotics (chlortetracycline, florfenicol, gentamicin, oxytetracycline, and trimethoprim). Susceptibility to these antibiotics appeared arbitrary and did not seem to follow a specific pattern in terms of serotypes or genotypes, or time recovered.

After analysis of 90 *E. rhusiopathiae* isolates, the question remains why a significant increase of erysipelas cases occurred in Iowa during 2001–2002. Vaccines have been available for prevention of clinical erysipelas since 1947, and there are at least 4 modified live vaccines and 12 killed bacterins currently available in the United States. Prevention through vaccination is usually effective, and challenge studies performed on vaccinated pigs with vaccines containing serotype 2 demonstrated protection from acute erysipelas caused by serotypes 1 and 2.^{10,12,14} Thus far, the authors have not been able to obtain strains of *E. rhusiopathiae* that were used to prepare inactivated vaccines. Two of the live-vaccine strains were serotype 1, whereas the other 2 had to remain untested because of the unavailability of antisera. The genotyping of all 4 live-vaccine strains indicated the same 1A(II)-PFGE pattern, suggesting that they are the same strain. The majority of the recent isolates submitted to the ISU-VDL were genotype 1A(I), and all the genotype 1A(I) isolates serotyped were serotype 1a.

Among the current cases, 8 outbreaks were reported in herds using an erysipelas vaccination program. There were no reports regarding erysipelas vaccination in the other cases. The recovered isolates in vaccinated herds were all identified as genotype 1A(I). Based on the *Sma*I pattern, genotypes 1A(I) and 1(AII) appear to be closely related, which means that the data from this study are not definitely able to prove or disprove whether reversion occurred. Attempts using other enzymes like *Xba*I and *Not*I for erysipelas digestion failed to provide readable bands for differentiation of isolates (data not shown). In this regard, future work should focus on identifying other enzymes that will allow detection of differences or confirm the similarity between genotype 1A(I) isolates and vaccine strains.

The results of this study do not provide an explanation for the reemergence of erysipelas cases in 2001.

The recent erysipelas outbreaks, as in the past, were most likely due to failure to use vaccines. Two doses of the killed bacterins are recommended for protective immunity,¹ and sometimes producers give only 1 dose. A recent study showed that a single oral dose of attenuated live vaccine provides immunity against swine erysipelas for at least 18 weeks (Sick F, Hayes P: 2001, Proc Leman Conf: 45). Efficacy of delivery of vaccine to pigs via the water system may also be problematic. Use of multiple antigen products has also been a concern. One group recently compared the antibody titers of gilts vaccinated with single-antigen erysipelas vaccines with those vaccinated with products containing multiple antigens. They found that all vaccinated gilts were protected against the disease; however, gilts vaccinated with single-antigen vaccines had significantly higher titers than the gilts vaccinated with multiple-antigen vaccines (Ritzmann M, Heinritzi P: 2001, Proc Leman Conf: 43).

Another explanation for the outbreaks of erysipelas in well-vaccinated herds may be improper vaccine management (timing, proper storage, administration, etc.). The vaccine may be administered at an age when the pigs still have high maternal antibody titers that interfere with development of active immunity. It is also possible that concurrent infection with viruses such as porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), or other agents may suppress the immune response and compromise development of protective immunity. A trial with erysipelas vaccination and infection with PRRSV recently demonstrated that pigs developed erysipelas after immunization for *E. rhusiopathiae* and challenge with PRRSV.⁷ More work on the effect of viruses such as PRRSV and PCV2 on the safety and efficacy of live erysipelas vaccines is warranted.

The PFGE described in this work will provide a useful epidemiological tool to monitor future erysipelas outbreaks.

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