Molecular Characterization of Recent and Archived *Erysipelothrix rhusiopathiae* Isolates

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Summary and Implications

Cases of erysipelas have increased considerably in 2001–2002. Diagnosis of erysipelas is typically confirmed by culture and in a limited number of cases the isolates are serotyped. Reagents for serotyping are limited and are available only at National Veterinary Service Laboratory (NVSL). In this study, we utilize pulsed-field gel electrophoresis (PFGE) to differentiate genotypes and compare archived and recent isolates. Seventy-three erysipelas field isolates (58 recent, 15 historical) and four live vaccine strains were genetically characterized. Forty-six isolates were found to belong to genotype 1A(I), three were genotype 1A(III), each one was genotype 1A(IV), 1A(V), 1A(VI), and two isolates were designated as 1A(VII). Nine different genotypes were identified among the serotype 1b isolates [1B(I-IX)]. Within serotype 2, three genotypes were identified: 2A, 2B, and 2C. The four vaccine strains tested in this study belong to the genotype group 1A(II), closely related to genotype 1A. The vaccine strains and the most common field isolates genotype 1A(I) shared 78.6% identity based on PFGE pattern.

Introduction

The gram-positive, facultative anaerobic bacterium *Erysipelothrix rhusiopathiae* causes erysipelas in swine, sheep, fish, reptiles, and birds, as well as Erysipeloid, a human skin disease.[4] 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), *E. sp. strain 1* (serovar 13), and *E. sp. strain 2* (serovar 18). This distinction was made on the basis of DNA-DNA hybridization, and with PCR.[1-3]

In the United States, erysipelas in pigs reemerged in the summer of 2001 and outbreaks have persisted since that time. The outbreaks occurred in both vaccinated and non-vaccinated pigs, and the clinical picture typically was acute in onset with mortality attributed primarily to sudden deaths in pigs in the late stages of the grow-finish period. Practitioners and producers have raised questions over emergence of new *E. rhusiopathiae* serotypes or strains, vaccine efficacy, or safety of live erysipelas vaccines.

The objectives of this study were to establish a PFGE technique that allows differentiation within serotypes, to compare recent isolates with historical isolates, and to compare recent field isolates with current live commercial vaccines.

Materials and Methods

Fifty-eight erysipelas field isolates from the years 1999–2002 were the basis of this work. In addition, four attenuated live vaccines (Ingelvac® ERY-ALC, Boehringer Ingelheim Vetmedica, St. Joseph, MO; Erycell®, Grand Laboratories, Larchwood, IA.; Suvaxyn® E-oral, Fort Dodge Animal Health, Fort Dodge, IA; and ERY VAC 100, Arko Laboratories, Jewell, IA), and several archived erysipelas isolates with known serotype [7 isolates with serotype 2 (1946–1983); 5 isolates with serotype 1b (years 1950–1979); and 3 isolates with serotype 1a (1975–1983)] were genetically compared with recent isolates.

The bacteria were cultured on sheep-blood agar plates and the cells were suspended in a cell suspension buffer to an absorbance of 1.0–1.1 at a wavelength of 612 nm. To 400 µl of the bacteria suspension 10 µl lysozyme (Sigma-Aldrich, Inc.) was added and incubated for 30 minutes at 37ºC. After incubation the plugs were prepared by adding 90 µl of mutanolysin (Sigma-Aldrich, Inc., St. Louis, MO), 20 µl of Proteinase K (Invitrogen, Carlsbad, CA), and 400 µl of melt plug agar (Bio-Rad, Hercules, CA). The solidified plugs were placed in cell lysis buffer and 40 µl of Proteinase K in a shaking water bath at 54ºC for 1.5 hours. After washing with autoclaved water and TE-buffer the plugs were stored in TE buffer at 4ºC until use.

Digestion was done with *Sma*I (Promega, Madison, WI) with a working concentration from 2.5%, with 1% BSA (Promega) and 10% buffer (Promega) at 37ºC for 2 hours. The digested plugs were loaded in their appropriate wells in the gel (Pulsed Field certified agarose; Bio-Rad). Electrophoresis was carried out for 21 hours at 12ºC and 120 V with pulse times from 2.2 to 64 seconds.

For analysis of the data the PFGE BioNumerics Software (Applied Maths, Kortrijk, Belgium) was used.

Results and Discussion

This study represents the first genetic analysis of *E. rhusiopathiae* on United States field isolates. Genotype 1A(I) group consisted of 58 isolates. Forty-six isolates 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). A

different pattern was manifest by three genotype 1A(III) isolates, one of them from the year 1976, the other two from 2001. The remaining genotype 1A patterns were composed of archived isolates. Genotype 1A(I) was the most common among the 1999-2002 isolates; however, no genotype 1A(I) isolates were found in the archived isolate collection.

Genotype 1B was composed of a wider variety of genotypes. All in all, nine different patterns were identified, and the genotype 1B was separated into 1B(I) to 1B(IX). Within the genotype 1B, three isolates were found to belong to genotype 1B(I) and remaining genotype 1B isolates had only one representative each. The genotype of one serotype 1b isolate remained unconfirmed, it was not possible to get bands strong enough for evaluation. A comparison between archived and recent 1b isolates based on serotyping revealed that none of the five historical isolates tested were identical to recent 1B isolates. This may be due to the general high variability within genotype 1B. The most prominent 1B genotype within the recent isolates is 1B(I) with three representatives.

Seven archived serotype 2 isolates were distinguished genetically into three groups: two isolates were found to belong to genotype 2A, four isolates composed genotype 2B, and one isolate was found to belong to genotype 2C.

The PFGE will be a useful epidemiological tool to monitor future erysipelas outbreaks.

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