Title: Prevalence of slaughter house condemnation due to *Erysipelothrix* sp. and further characterization of isolates associated with these cases – NPB #07-072

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Industry Summary:

The objective of this study was to confirm the presence and investigate the identity of *Erysipelothrix* spp. in condemned tissues obtained from a regional abattoir. Tissue specimens from 70 carcasses with macroscopic lesions suspect of swine erysipelas were collected at a regional abattoir in Iowa from October 2007 to February 2009. *Erysipelothrix* spp. culture isolation procedures were performed and recovered suspect isolates were confirmed to be *Erysipelothrix* spp. by standard laboratory methods. The genotype and the surface protective antigen (Spa) type of selected isolates (one from each positive case) were determined by multiplex real-time PCR assays. *Erysipelothrix* spp. was isolated from 84.3% (59/70) of the carcasses. All of the isolates recovered from the same carcass were the same serovar. In the culture positive carcasses the following serovars were identified: Serovar 1 (40.7%; 24/59), serovar 2 (49.2%; 29/59) and untypeable (5.1%; 3/59). Fifty-seven of the 59 isolates from positive carcasses were determined to belong to *E. rhusiopathiae* and 2/59 of the isolates were determined to be *E. tonsillarum*. Spa A was detected in 57/59 isolates and 2/59 isolates were negative for all Spa types. *E. rhusiopathiae* serovars 1a and 2 continue to be the most commonly isolated serovars in pigs condemned due to erysipelas. *E. tonsillarum* on the other hand, previously reported to be of low pathogenicity for swine was identified in a few cases and may be more important than currently assumed.

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Scientific Abstract:

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Introduction

*Erysipelothrix* spp., facultative anaerobic slender gram positive rods and the cause of swine erysipelas, were first isolated from swine by Louis Pasteur in 1882. The clinical disease associated with *Erysipelothrix* is called “erysipelas” in birds and mammals, or “erysipeloid” in humans. The genus *Erysipelothrix* contains four species and associated serovars: *E. rhusiopathiae* (serovars 1a, 1b, 2, 4, 5, 6, 8, 9, 11, 12, 15, 16, 17, 19, 21, N), *E. tonsillarum* (serovars 3, 7, 10, 14, 20, 22, 23), *E. sp. strain 1* (serovar 13), and *E. sp. strain 2* (serovar 18).

Swine erysipelas of economic importance can occur in three clinical presentations: (1) acute infection characterized by sudden illness, rhomboid skin lesions, and sudden death, (2) subacute infection characterized by mild or subclinical infection, and (3) chronic infection characterized by arthritis and endocarditis. Acute septicemia cases in the United States are typically associated with serovar 1a. Subacute and chronic cases are typically associated with serovar 2; however, all clinical forms of erysipelas can be induced experimentally in susceptible swine with serovars 1a or 2. The additional serovars (3-26, N) have minimal clinical significance in swine. It has been estimated that 30-50% of healthy pigs harbor *E. rhusiopathiae* in tonsils and lymphoid tissues, and are the source for acute erysipelas outbreaks due to shedding of the organism in urine, feces, saliva, and nasal secretions.

Recent investigations have focused on the surface protective antigen (Spa) of *Erysipelothrix* spp. as a highly immunogenic and protective antigen. Four different Spa types have thus far been described, Spa A, SpaB1, SpaB2, and Spa C, with amino acid sequences found to be similar between members of the same Spa type, however different across Spa types. A cross protection study reported complete protection against homologous strains, however only partial protection against heterologous strains.

Economic losses due to *Erysipelothrix* spp. infection are caused by increased numbers of acute deaths, treatment costs, vaccination costs, and slow growth of diseased pigs. In addition, economic loss from abattoir condemnations or lesion trimming is often unexpected and economically significant. The United States Department of Agriculture (USDA) and USDA’s Food Safety Inspection Service (FSIS) collects data related to swine abattoir condemnations on an annual basis. Swine erysipelas continues to be associated with condemned swine carcasses, ranking in the top ten causes for swine carcass condemnations from 2003-2008 (Courtesy of Jackie Lenzy, FOIA-2008-000440).

As a zoonotic disease, *E. rhusiopathiae* is becoming increasingly important to the pork industry. The risk of human infection is related to the opportunity for exposure. Individuals associated with occupations contacting animals, animal products, or animal wastes are at the greatest risk. Clinical manifestations in humans can resemble three forms similar to the disease in swine. The three human forms include: (1) erysipeloid which...
is the most common form of human infection, seen as localized cutaneous lesions, (2) diffuse cutaneous form where lesions can progress from the initial site with systemic signs that include fever, joint and muscle pain, and severe headaches, and (3) the form that is less common, septicemia and endocarditis.\(^\text{10}\)

The objective of this study was to confirm the presence and further characterize *Erysipelothrix* spp. in condemned tissues obtained from a regional abattoir in the Midwestern U.S.

**Objectives**

1. To determine the prevalence of *Erysipelothrix* sp. in lesions from slaughter house condemnations
2. To further characterize the *Erysipelothrix* sp. isolates found in slaughter house samples

**Materials and Methods**

*Abattoir condemnation specimens*-Tissue specimens (tonsil, skin, kidney, liver, spleen) from a total of 70 individual cases representing 70 different farm sites were collected from October 2007 to February 2009 by the veterinary inspector-in-charge at a single regional abattoir in Iowa. Utilizing the criteria shown in Table 1 (presence of lesions in the skin, lymph nodes, kidney, joints, and signs of septicemia), cases suggestive of swine erysipelas were visually identified.\(^\text{11}\) After condemnation, each case was classified as acute, subacute, or chronic based on the visual lesions (Table 1). Tissue specimens were collected, labeled, and frozen at -20°C in individual specimen bags. Frozen samples were transported to the Iowa State University Veterinary Diagnostic Laboratory and tested.

*Erysipelothrix* selective broth preparation*-Bacterial isolation was accomplished utilizing a selective broth and media technique.\(^\text{12}\) Briefly, an *Erysipelothrix* spp. selective broth base consisting of phosphate buffer solution and tryptose (Thermo Fisher Scientific Remel, Lenexa, KS) was dissolved and autoclaved. Fetal bovine serum (Sigma-Aldrich, St. Louis, MO), kanamycin (Sigma-Aldrich), and neomycin (Sigma-Aldrich) were added to the broth. The selective broth was then aseptically dispensed and stored at 5°C for a maximum of two weeks prior to use.

*Erysipelothrix* selective agar preparation*-Erysipelothrix* spp. selective agar was utilized as described.\(^\text{13}\) In brief, 33 g tryptose agar base, 5 g Bacto\(^\text{TM}\) tryptose, and 3 g granulated agar were dissolved in 920 ml distilled water. The agar was cooled and crystal violet stock solution (Thermo Fisher Scientific Remel, Lenexa, KS), sodium azide stock solution (Thermo Fisher Scientific Remel), and sterile bovine blood were added before aseptically dispensing into sterile petri plates.\(^\text{13}\)

Broth enrichment technique*-The broth enrichment technique utilized in this investigation has been previously described.\(^\text{14}\) Tissue specimens and physiologic saline were placed in a stomacher (Seward, Bohemia, NY) and processed for three-minutes. The tissue/fluid homogenate was removed and added to 3 ml of *Erysipelothrix* selective broth and incubated at 35°C. Subcultures were made from the *Erysipelothrix* selective broth onto a 5% sheep blood agar plate (Thermo Fisher Scientific Remel), a colistin-nalidixic acid agar plate (Thermo Fisher Scientific Remel), and an *Erysipelothrix* selective medium plate for both 24 and 48 hrs. Suspect colonies were subcultured on a 5% sheep blood agar plate and incubated at 35°C for 24 hrs. Standard laboratory methods including Gram stain, cell morphology, motility, oxidase, and catalase activity, and H\(_2\)S production on triple sugar iron agar (TSI) medium were used to confirm *Erysipelothrix* spp.\(^\text{2}\)

Serotyping*-Isolate serovar type was determined using a method previously described.\(^\text{15;16}\) Serotyping was done on all recovered isolates. A pure culture was grown at 37°C for 36 hrs in 30 ml of heart infusion broth (Becton Dickinson, Sparks, MD) supplemented with 10% equine serum (Sigma-Aldrich). The culture was then killed by adding 1% formalin (Sigma-Aldrich) and held at room temperature for 12 hrs, harvested by centrifugation, and washed twice in 0.85% NaCl solution containing 0.5% formalin. Washed cells were then suspended in 1.5 ml of distilled water and autoclaved at 121°C for 1 hr. Following removal from the autoclave,
the washed cells and distilled water were centrifuged. The supernatant was collected and used for the agar gel precipitation test. Homologous positive controls were used with each test. Reactions were recorded after 24 hrs.

**Real-time PCR assays to determine the genotype and the Spa type**—Further characterization was done on one isolate from each of the culture positive carcasses. A multiplex real-time PCR assay was utilized to determine the *Erysipelothrix spp.* genotype as previously described with the following modification: The addition of primer (5'-CCTTATATCTTTAGCAGGTGATCTAG-3') for *E.* sp. strain 2 was added to increase the sensitivity of the assay (Shen HG et al. *J Appl Microbiol* Submitted 2010). In addition, a multiplex real-time PCR assay was utilized for identification of the Spa type present (Spa A, Spa B1, Spa B2, and Spa C) (Shen HG et al. *J Appl Microbiol* Submitted 2010).

**Results**

**Erysipelothrix spp. isolation**—The isolation results on the 70 condemnation cases collected at the regional abattoir are summarized in Tables 2 and 3. Of 70 condemned carcasses examined, 84.3% (59/70) were found to be culture positive for *Erysipelothrix spp.* Moreover, of the 350 tissue specimens cultured, 58.9% (206/350) were positive. In 11.9% (7/59) carcasses, all five tissues collected from the same carcass were culture positive, in 39.0% (23/59), four of five tissues from the same carcass were culture positive, in 37.3% (22/59), three of five tissues from the same carcass were culture positive, and in 8.5% (5/59) and 5.1% (3/59) two or one of the five tissues collected from the same carcass were culture positive, respectively. Overall, the highest isolation success was observed with tonsils with 53/70 (75.7%) of the samples being positive for *Erysipelothrix* spp.

**Serotypes identified in condemnation cases**—All isolates recovered from the same carcass were found to belong to the same serovar. The most common serovar was serovar 2 identified in 49.2% (29/59) of the carcasses, followed by serovar 1 identified in 40.7% (24/59) of the carcasses. One isolate each was identified for serovars 7, 10, and 11. Three of 59 isolates were found to be untypeable.

**Genotypes identified in condemnation cases**—Fifty-seven of 59 isolates belonged to *E. rhusiopathiae* and 2/59 isolates were found to be *E. tonsillarum*. All isolates that were untypeable by serotyping were found to belong to *E. rhusiopathiae*.

**Spa-types identified in condemnation cases**—96.6% (57/59) of the isolates were determined to be positive for Spa A. The three untypeable isolates were all found to be positive for Spa A. Two isolates (serovars 7 and 10; both *E. tonsillarum*) were negative for Spa A, B1, B2 and C.

**Association of Erysipelothrix spp. and visual erysipelas presentation**—Isolation was successful in 100% (8/8) of the cases with acute erysipelas presentation, 96.9% (31/32) of the cases with subacute presentation of swine erysipelas, and 66.6% (20/30) of the cases with chronic swine erysipelas presentation (Table 3). In general, serovar 1 and 2 were identified in all three presentations whereas serovars (7, 10, and 11) were only identified in chronic presentations of swine erysipelas (Table 3).

**Discussion**

Results from this investigation indicate that, based on culture results alone, 84.3% (59/70) of the carcasses were appropriately condemned as “swine erysipelas” at a regional abattoir as the etiologic agent *Erysipelothrix* spp. was isolated. Based on USDA/FSIS data collected from 2003 to 2008, septicemia was identified as being the most frequent cause (15.5%) of postmortem swine condemnation in the United States. In
addition, 4.1% of postmortem swine condemnations were attributed to arthritis. However, the number of swine condemnations classified as septicemia or arthritis actually caused by *Erysipelothrix* spp. is unknown, as several conditions manifest similarly to erysipelas. Previous work by Miniats *et al.* (1989) demonstrated difficulties differentiating swine erysipelas in the acute stage from other causes of septicemia. Hariharan *et al.* (1992) investigated bacterial causes of arthritis in Canadian slaughter hogs and reported *E. rhusiopathiae* as the most common bacterial pathogen (45%) isolated from arthritic joints. For these reasons, the full economic and public health impact of swine erysipelas may be greatly underestimated in respect to the U.S. swine industry. Due to constraints at the abattoir, condemnations due to septicemia or arthritis not highly suspected of swine erysipelas were not included in this study. With the development and validation of improved diagnostics assays, further investigation into cases of septicemia or arthritis condemned without classic “diamond skin” lesions is warranted.

In this study isolate serovar distribution in different presentations of erysipelas was evaluated. Ninety-six point six percent (57/59) of the isolates collected belonged to *E. rhusiopathiae* which is similar to previous reports.\(^{19,20}\) Interestingly, the association between serovar 1 (acute) and serovar 2 (subacute or chronic) was not as clear as previously reported. Both serovars 1 and 2 were found to be present in all three presentations of erysipelas; however, serovars 1 and 2 were the most common serovars identified agreeing with previous literature.\(^{6,19,20}\) Also consistent with previous findings was the association between the most commonly isolated serovars (1a and 2) and the identification of Spa A.\(^{21}\)

All three isolates which were determined to be untypeable utilizing serotyping techniques were found to belong to the *E. rhusiopathiae* genotype and were positive for Spa A. Serovar N has been described as lacking a type-specific antigen thus failing to induce antibody production in rabbits which are utilized to produce typing antisera.\(^{15,22}\) The lack of an antigen antibody reaction results in a lack of visible precipitation lines when performing the agar diffusion test. Isolates that belong to serovar N also belong to the genotype *E. rhusiopathiae* and have been shown to be positive for Spa A which is very similar pattern to what was observed with the untypeable isolates in this study. Therefore, it can be concluded that they likely belong to serovar N.

An unexpected finding was the presence of *E. tonsillarum* (serovars 7 and 10) in two cases of condemned chronic erysipelas. *E. tonsillarum* has been reported to be of minimal etiologic significance.\(^1\) Recent work demonstrated that strains belonging to *E. tonsillarum* serovar 10 induced generalized urticarial skin lesions after intradermal inoculation; however, *E. tonsillarum* serovar 7 induced no clinical signs or macroscopic lesions.\(^1\) Additional investigations to determine the full impact of *E. tonsillarum* strains is warranted, as previous studies have reported minimal significance related to clinical swine erysipelas. The Spa PCR was negative for Spa A, B1, B2, and C on the four cases of cases of septicemia or arthritis condemned without classic “diamond skin” lesions is warranted.

It can be speculated that based on recent evidence of the immunogenic properties of the Spa protein, the actual serovar of the isolate may be less responsible and thus less consistent with lesions associated with swine erysipelas. Previous investigations have described the strains utilized in attenuated-live vaccines as serovar 1a.\(^{16}\) Due to constraints at the abattoir, the ability to trace back condemned cases to the farm of origin in a timely fashion was not feasible and it remains unknown if the condemned carcasses had been vaccinated against erysipelas. It can be speculated that a pig vaccinated with a product containing serovar 1a should be protected against serovars 1 and 2 based on previous studies using homologous Spa types.\(^8\) However, *E. tonsillarum* isolates were found to contain no Spa types, possibly implicating a lack of protection from current available vaccines. Future investigations of swine erysipelas should include Spa typing of vaccines if utilized on site. In addition even if vaccinated, other possibilities such as improper administration or duration of immunity must be evaluated.

Results of this study indicate cases condemned at an abattoir suspected of swine erysipelas were appropriately condemned. In addition, the majority of isolates recovered continue to belong to *E. rhusiopathiae* serovars 1a and 2. A difference however is the distribution of serovar and lesion staging. Furthermore, results of this study show that *E. tonsillarum* strains can be isolated from condemned tissue specimens and may play a more significant role than previously reported. Alternatively, the findings could be due to carcass contamination. Investigations at additional abattoirs in the United States are necessary as these results are based
on condemnations at a single abattoir utilizing a single inspector. Unknown is the number of condemnations in the United States that are classified as “septicaemia” or “arthritis” that may actually be due to swine erysipelas, possibly underestimating the full economic impact of swine erysipelas.
**Tables**

**Table 1** - Swine erysipelas suggestive lesions and criteria for determination of the lesion stage at the abattoir.

<table>
<thead>
<tr>
<th>Presentation</th>
<th>Location</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>Skin</td>
<td>Raised dark red to dark purple urticarial changes</td>
</tr>
<tr>
<td></td>
<td>Lymph node</td>
<td>Hyperemic, hyperplastic, or hemorrhagic changes</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>Hemorrhagic lesions</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>Additional systemic organ signs of septicemia</td>
</tr>
<tr>
<td>Subacute</td>
<td>Skin</td>
<td>Light pink to light purple discoloration</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>Minimal renal changes</td>
</tr>
<tr>
<td>Chronic</td>
<td>Lymph node</td>
<td>Edematous changes</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>Renal infarcts</td>
</tr>
<tr>
<td></td>
<td>Joints</td>
<td>Arthritic joint changes</td>
</tr>
</tbody>
</table>

**Table 2** - Association of lesion duration and successful *Erysipelothrix* spp. isolation in selected tissues. Data presented as number isolated/total number tested for each tissue.

<table>
<thead>
<tr>
<th>Presentation</th>
<th>Tonsil</th>
<th>Skin</th>
<th>Kidney</th>
<th>Liver</th>
<th>Spleen</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>8/8</td>
<td>8/8</td>
<td>5/8</td>
<td>4/8</td>
<td>5/8</td>
<td>30/40</td>
</tr>
<tr>
<td>Subacute</td>
<td>26/32</td>
<td>24/32</td>
<td>19/32</td>
<td>16/32</td>
<td>21/32</td>
<td>106/160</td>
</tr>
<tr>
<td>Chronic</td>
<td>19/30</td>
<td>10/30</td>
<td>14/30</td>
<td>11/30</td>
<td>16/30</td>
<td>70/150</td>
</tr>
<tr>
<td>Total</td>
<td>53/70</td>
<td>42/70</td>
<td>38/70</td>
<td>31/70</td>
<td>42/70</td>
<td>206/350</td>
</tr>
</tbody>
</table>

**Table 3** - Number of *Erysipelothrix* spp. isolation positive cases by lesion stage with associated serovars

<table>
<thead>
<tr>
<th>Presentation</th>
<th>Successful isolation</th>
<th>Serovar</th>
<th>Genotype</th>
<th>Spa Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>8/8</td>
<td>Serovar 1 (5/8)</td>
<td><em>E. rhusiopathiae</em></td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serovar 2 (3/8)</td>
<td><em>E. rhusiopathiae</em></td>
<td>A</td>
</tr>
<tr>
<td>Subacute</td>
<td>31/32</td>
<td>Serovar 1 (15/31)</td>
<td><em>E. rhusiopathiae</em></td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serovar 2 (14/31)</td>
<td><em>E. rhusiopathiae</em></td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Untypeable (2/31)</td>
<td><em>E. rhusiopathiae</em></td>
<td>A</td>
</tr>
<tr>
<td>Chronic</td>
<td>20/30</td>
<td>Serovar 1 (4/20)</td>
<td><em>E. rhusiopathiae</em></td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serovar 2 (12/20)</td>
<td><em>E. rhusiopathiae</em></td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serovar 7 (1/20)</td>
<td><em>E. tonsillarum</em></td>
<td>ND*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serovar 10 (1/20)</td>
<td><em>E. tonsillarum</em></td>
<td>ND*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serovar 11 (1/20)</td>
<td><em>E. rhusiopathiae</em></td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Untypeable (1/20)</td>
<td><em>E. rhusiopathiae</em></td>
<td>A</td>
</tr>
</tbody>
</table>

*ND: Isolates were negative for Spa A, B1, B2, and C*
References


