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Citation for published version:

Digital Object Identifier (DOI):
10.1177/104063871102300126

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published in:
Journal of Veterinary Diagnostic Investigation

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J VET Diagn Invest 2011 23: 139
DOI: 10.1177/104063871102300126

The online version of this article can be found at:
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What is This?
Erysipelothrix spp. genotypes, serotypes, and surface protective antigen types associated with abattoir condemnations

Joseph S. Bender, Christa K. Irwin, Hui-Gang Shen, Kent J. Schwartz, Tanja Opriessnig

Abstract. The objective of the current study was to investigate characteristics of Erysipelothrix spp. from slaughter condemnations. Specimens from 70 carcasses with lesions suspect for swine erysipelas were collected at an abattoir in Iowa from October 2007 to February 2009. Erysipelothrix spp. were isolated from 59 of 70 carcasses (84.3%). Abattoir inspectors classified lesions as acute, subacute, or chronic; 8 of 8 (100%) were acute cases, 31 of 32 (96.9%) were subacute cases, and 20 of 30 (66.6%) were chronic cases that were isolation positive. The following serotypes were identified: 1a (40.7%; 24/59), 2 (49.2%; 29/59), 7 (1/59), 10 (1/59), 11 (1/59), and untypeable (5.1%; 3/59). Serotypes 1a and 2 were identified in pigs with acute, subacute, or chronic clinical manifestations, whereas serotypes 7, 10, and 11 were only present in chronic cases. Fifty-seven of the 59 isolates were determined to belong to E. rhusiopathiae, and 2 of 59 of the isolates were determined to be E. tonsillarum by multiplex real-time polymerase chain reaction. Surface protective antigen (spa) A was detected in all E. rhusiopathiae isolates but not in E. tonsillarum serotypes 7 and 10. The results of the present study indicate that E. rhusiopathiae serotypes 1a and 2 continue to be commonly isolated from condemned pig carcasses and that spaA is the exclusive spa type in U.S. abattoir isolates. Interestingly, E. tonsillarum, thought to be avirulent for swine, was isolated from systemic sites from 3.4% of the carcasses that were negative for E. rhusiopathiae, indicating the potential importance of this genotype in erysipelas pathogenesis.

Key words: Abattoir; condemnation; Erysipelothrix; genotype; surface protective antigen; swine.

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Members of the genus Erysipelothrix are facultative anaerobic, slender, Gram-positive, rod-shaped bacteria that cause swine erysipelas. The clinical disease associated with Erysipelothrix spp. is called erysipelas in birds and mammals or erysipeloid in humans. Current taxonomy recognizes the genus Erysipelothrix with 2 species, each with differentiable serotypes: Erysipelothrix rhusiopathiae (serotypes 1a, 1b, 2, 4, 5, 6, 8, 9, 11, 12, 15, 16, 17, 19, 21, N) and Erysipelothrix tonsillarum (serotypes 3, 7, 10, 14, 20, 22, 23). Two proposed Erysipelothrix spp. consisting of serotypes 13 (E. sp. strain 1) and 18 (E. sp. strain 2) have been described. In addition, another proposed species, Erysipelothrix inopinata, has also recently been described. Acute septicaemia in U.S. swine is typically associated with serotype 1a. Recent subacute and chronic cases are typically associated with serotype 2; however, all clinical forms of erysipelas can be induced experimentally in susceptible pigs with serotypes 1a or 2. Other serotypes have less clinical significance in pigs. Economic losses associated with swine erysipelas are from increased numbers of deaths, treatment costs, vaccination costs, and slower growth of diseased pigs. In addition, financial loss associated with abattoir condemnations or lesion trimming is of economic significance. The U.S. Department of Agriculture (USDA) and USDA Food Safety Inspection Service (FSIS) collect data related to swine abattoir condemnations on an annual basis. Swine erysipelas continues to be ranked as one of the top 10 causes for swine carcass condemnations (Courtesy of Jackie Lenzy, USDS FSIS, FOIA-2008-000440). Few studies have investigated isolates obtained from condemned carcasses. The objective of the current study was to confirm the presence of Erysipelothrix spp. in condemned carcasses and to characterize the isolates obtained from a regional abattoir in the Midwestern United States.

Tissue specimens (tonsil, skin, kidney, liver, and 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 previously described criteria, cases suggestive of swine erysipelas were visually identified and classified as acute, subacute, or chronic. 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 (Ames, Iowa) and tested.

Bacterial isolation was accomplished by utilizing a previously described selective broth enrichment and media technique. Standard laboratory methods (Gram staining, hydrogen sulfide production) were used to confirm Erysipelothrix spp. All isolates were serotyped by using an agar gel precipitation test as previously described. One isolate from all culture-positive carcasses was additionally characterized by using a multiplex real-time polymerase chain reaction (PCR) assay to determine the Erysipelothrix spp. genotype as previously described with the following modification: the addition of primer (5'-CTTTATATTTAGCAGGTGATCTAG-3') for Erysipelothrix spp. strain 2 was incorporated to increase the sensitivity of the assay. All isolates were also evaluated by using a multiplex real-time PCR assay to identify the spa types (spaA, spaB1, spaB2, and spaC).

The isolation results of 70 condemned cases collected at the regional abattoir are summarized in Table 1. Of 70 cases examined, 84.3% (59/70) were found to be culture positive for Erysipelothrix spp. Moreover, of 350 tissue specimens cultured, which included tonsil, skin, kidney, liver, and spleen, 58.9% (206/350) were positive. In 11.9% (7/59) of the carcasses, all 5 tissues collected from the same carcass were culture positive; in 39.0% (23/59), 4 of 5 tissues from the same carcass were culture positive; in 37.3% (22/59), 3 of 5 tissues from the same carcass were culture positive; and in 8.5% (5/59) and 5.1% (3/59), 2 or 1 of the 5 tissues collected from the same carcass were culture positive, respectively. Overall, the highest isolation success was observed with tonsils for which 75.7% (53/70) of the samples were positive for Erysipelothrix spp.

All isolates recovered from different tissues of the same carcass were found to belong to the same serotype. The most common serotype was serotype 2 identified in 49.2% (29/59) of the carcasses, followed by serotype 1a identified in 40.7% (24/59) of the carcasses. Other serotypes detected were serotype 7 (1 isolate), serotype 10 (1 isolate), serotype 11 (1 isolate), and untypeable (3 isolates). Serotypes 1a and 2 were generally identified in tissues from pigs with acute, subacute, or chronic clinical manifestations, whereas serotypes 7, 10, and 11 were only identified in cases with a chronic presentation (Table 1). Previous investigations reported an association of serotype 1a with acute disease manifestation and an association of serotype 2 with subacute or chronic disease manifestation. In the current study, both serotypes 1a and 2 were found to be present in

### Table 1. Number of Erysipelothrix spp. isolation-positive cases by lesion stage with associated serotypes.

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

* ND = isolates were negative for spaA, B1, B2, and C.
all 3 clinical presentations of erysipelas. Consistent with previous reports is the finding that serotypes 1a and 2 are
the most common serotypes associated with disease.4,11,16

Fifty-seven of 59 isolates belonged to E. rhusiopathiae
(including the untypeable isolates), and 2 of 59 isolates were
found to be E. tonsillarum, which was isolated from the spleen (serotype 7) or from spleen, liver, and kidney (serotype
10). Spa typing revealed that 97% (57/59) of the isolates were
positive for the spaA type, which also includes all 3
untypeable isolates. Two isolates of E. tonsillarum (serotypes
7 and 10) were found to be negative for the spaA type as well
as for other spa types. To the authors’ knowledge, the present
study is the first to determine the spa type in recent
Erysipelothrix spp. isolates recovered from field cases of
swine erysipelas. On the basis of reference strain analysis, it is
speculated that spa types associated with swineherds are
likely highly conserved; however, additional field isolates
need to be screened to prove the speculation. Results of the
present study are consistent with previous observations
associating serotypes 1a and 2 with spaA.3

The culture results from the current study confirm that
84.3% (59/70) of the carcasses were appropriately con-
demned as swine erysipelas at a regional abattoir. On the
basis of USDA and/or FSIS data collected from 2003 to
2008, the predominant cause for postmortem swine
condemnation in the United States was septicemia (15.5%) followed by arthritis (4.1%). However, the number of
swine condemnation classified as septicemia or arthritis
that may actually be caused by Erysipelothrix spp. is
unknown, because the criteria of gross lesions are not
etiologic specific and because a previous work demonstrat-
ded difficulties differentiating the acute stage of swine
erysipelas from other causes of septicemia.10 Bacterial
causes of arthritis in Canadian slaughter hogs were
investigated in 1992, and E. rhusiopathiae was identified
as the most common bacterial pathogen (45%) isolated
from arthritic joints.5 For these reasons, the full economic
and public health impact of swine erysipelas may be greatly
underestimated. Because of constraints at the abattoir,
condemnations as a result of septicemia or arthritis not
highly suspected of swine erysipelas were not included in
the present study. With the development and validation of
improved diagnostics assays, additional investigation into
cases of septicemia or arthritis condemned without classic
diamond-skin lesions is warranted.

The 3 E. rhusiopathiae isolates found positive for spaA
type were untypeable by utilizing serotyping techniques.
Earlier studies have indicated that serotype N strains lack
a type-specific antigen; as a result, they fail to induce antibo-
dy production in rabbits, which were used for producing typing antisera.7,23 This could be the probable
reason for lack of visible precipitation lines while perform-
ing the agar diffusion test in the current study. Therefore,
it can be concluded that the isolates that were untypeable in
the present study may likely belong to serotype N.

An unexpected finding was the presence of E. tonsillarum
(serotypes 7 and 10) in 2 cases condemned for chronic
erysipelas. Interpretation of the importance of E. tonsil-
larum is difficult as it can be frequently isolated from tonsils
of normal swine,24 and it is reported to be of little
pathologic significance.17 A 1987 study demonstrated that
strains belonging to E. tonsillarum serotype 10 induced
generalized urticarial skin lesions after intradermal inocu-
lation; however, E. tonsillarum serotype 7 induced no
clinical signs or macroscopic lesions.17 In the current study,
E. tonsillarum was the only pathogen (E. rhusiopathiae was
not detected) isolated from internal organs (spleen, liver,
and kidney) of the 2 condemned cases, suggesting that E.
tonsillarum may be more important in pigs than previously
speculated. The spa PCR was negative for spaA, spaB1,
spaB2, and spaC on the E. tonsillarum isolates recovered
from the carcasses, which is consistent with previous
studies.20 Additional investigations to determine the full
impact of E. tonsillarum strains is warranted. Recent
evidence of the immunogenic properties of the spa protein
suggests this virulence factor may better predict patho-
nicity than the serotype of the isolate.

Constraints at the abattoir prevented trace-back of
condemned cases to the farm of origin; therefore, it remains
unknown if the condemned carcasses had been vaccinated
against erysipelas. Commercial killed and attenuated-live
vaccines are derived from serotype 1a.11 It can be
speculated that a pig vaccinated with a product containing
serotype 1a should be protected against serotypes 1a and 2
on the basis of previous studies using homologous spa
types.20 The E. tonsillarum isolates were found to contain
no spa types, suggesting a mechanism for a lack of
protection from currently available vaccines. Future
investigations of swine erysipelas should include spa typing
of vaccines if utilized on site, recognizing that immuniza-
tion failures also occur for other reasons.

Results of the current study indicate that cases of
suspected swine erysipelas condemned at an abattoir were
appropriately classified. In addition, the majority of
isolates recovered indeed belong to E. rhusiopathiae
serotypes 1a and 2. In contrast to previous studies,
however, the presence of these serotypes was demonstrated
in carcasses with lesions at all stages (acute, subacute, and
chronic). Furthermore, an important novel finding in this
study is the association of E. tonsillarum strains with
condemned tissue specimens. On the basis of the findings,
E. tonsillarum may play a more significant role than previously
suspected. Alternatively, the findings could be
due to carcass contamination. Investigations at additional
abattoirs in the United States are necessary, as results of the
present study are based on condemnations at a single
abattoir by utilizing a single inspector.

Acknowledgements. The authors thank Dr. Howard
Lindaman for assistance procuring samples. This study was
supported by the Pork CheckOff Dollars from the National
Pork Board, the Iowa Livestock Health Advisory Council,
and Schering-Plough Animal Health.

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Comparison of conventional direct and enrichment culture
methods for Erysipelothrix spp. from experimentally and