Development and Validation of an Immunohistochemical Method for Rapid Diagnosis of Swine Erysipelas in Formalin-Fixed, Paraffin-Embedded Tissue Samples
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What is This?
Development and validation of an immunohistochemical method for rapid diagnosis of swine erysipelas in formalin-fixed, paraffin-embedded tissue samples

Tanja Opriessnig,1 Joseph S. Bender, Patrick G. Halbur

Abstract. The objective of the study was to develop an immunohistochemical (IHC) assay for rapid detection of Erysipelothrix rhusiopathiae. Serotypes 1a, 1b, and 2 are most frequently associated with clinical disease in pigs. Antiserum against serotypes 1a, 1b, and 2 was produced in rabbits, pooled, and applied to formalin-fixed, paraffin-embedded tissue sections of pigs (lungs, heart, spleen, and skin). The results obtained with the IHC assay were compared with direct culture on tissue samples from experimentally inoculated pigs either treated (n = 6) with antibiotics or untreated (n = 8) as well as on samples from field cases (n = 170) submitted to the Veterinary Diagnostic Laboratory at Iowa State University. The agreement between direct culture and IHC staining was found to be substantial. The results of the present study indicate that the IHC assay is highly sensitive and specific in detecting E. rhusiopathiae antigen in formalin-fixed, paraffin-embedded tissues. Results indicated that the IHC is particularly useful in cases in which pigs had been treated with antibiotics prior to submission and in which direct cultures of organs were negative. In addition, the IHC was found to be useful for detection of E. rhusiopathiae antigen in skin lesions, which are often culture negative.

Key words: Erysipelothrix spp.; immunohistochemistry; swine.
Table 1. Comparison of direct culture and immunohistochemical (IHC) staining in pigs experimentally inoculated with different *Erysipelothrix rhusiopathiae* serotypes.*

<table>
<thead>
<tr>
<th>Pig ID</th>
<th>Serotype</th>
<th>Inoculum</th>
<th>Treatment status</th>
<th>Necropsy DPI</th>
<th>Spleen</th>
<th>Heart</th>
<th>Lung</th>
<th>Skin</th>
<th>Pig basis†</th>
</tr>
</thead>
<tbody>
<tr>
<td>106</td>
<td>1a</td>
<td>EI-6P</td>
<td>Nonreated</td>
<td>1</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>148</td>
<td>1a</td>
<td>EI-6P</td>
<td>Treated</td>
<td>2</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>102</td>
<td>1b</td>
<td>422-1</td>
<td>Nonreated</td>
<td>2</td>
<td>++</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>103</td>
<td>1b</td>
<td>422-1</td>
<td>Nonreated</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>107</td>
<td>1b</td>
<td>422-1</td>
<td>Nonreated</td>
<td>5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>152</td>
<td>1b</td>
<td>422-1</td>
<td>Nonreated</td>
<td>5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>108</td>
<td>2</td>
<td>NF-4</td>
<td>Nonreated</td>
<td>2</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>159</td>
<td>2</td>
<td>NF-4</td>
<td>Nonreated</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>


Total number positive for treated pigs 0/6 2/6 0/6 0/6 0/6 3/6 0/6 2/6 0/6 4/6

* DPI = days postinoculation; + = detection of *Erysipelothrix* spp.; – = *Erysipelothrix* spp. was not detected.
† Summary of the results on each pig.

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Table 2. Comparison of direct culture and immunohistochemical staining on field samples.

<table>
<thead>
<tr>
<th>Pig ID</th>
<th>Serotype</th>
<th>Inoculum</th>
<th>Treatment status</th>
<th>Necropsy DPI</th>
<th>Isolation</th>
<th>IHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>103</td>
<td>1a</td>
<td>EI-6P</td>
<td>Nonreated</td>
<td>2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>42</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* E. rhusiopathiae, u = suspension, and the cell suspension was stored at 4°C until used. E. rhusiopathiae specific antigen was commonly identified in bacteria-like structures in the lung interstitium (Fig. 2), in lumina of small vessels in lung tissues (Fig. 3), between hepatocytes (Fig. 4), in the dermal- connective tissue sections including mesenteries, spleen, and kidney, we found and collected in acidified saline solution. For IHC detection, a 4°C 1 h incubation 0.05% protease type XIV (bacterial) from *Streptococcus pyogenes* for 2 min. After washing the slides 3 times with distilled water followed by quenching with 3% hydrogen peroxide for 5 min, the slides were washed with 3% hydrogen peroxide for 5 min. The slides were incubated with 0.05% protease type XIV for 1 h at room temperature. Cells were harvested by centrifugation, washed twice with chilled physiological saline at 14°C and 4°C until use. Hyperimmune sera samples were confirmed to be specific by testing sera against all of the 26 strains (serotypes 1–26) as previously described.11

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Summary of the results on each pig:

- Positive
- Negative

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In brief, the external surface of the tissue specimens was seared with a heated spatula to remove contaminants, the specimen was dewaxed and rehydrated, and the specificity was determined to be 100%. The sensitivity was evaluated by comparing the IHC results to those obtained with *E. rhusiopathiae* direct culture. In brief, the specificity was determined to be 100% and the sensitivity was evaluated by comparing the IHC results to those obtained with *E. rhusiopathiae* direct culture. In brief, the specificity was determined to be 100% and the sensitivity was evaluated by comparing the IHC results to those obtained with *E. rhusiopathiae* direct culture. In brief, the specificity was determined to be 100% and the sensitivity was evaluated by comparing the IHC results to those obtained with *E. rhusiopathiae* direct culture.
Figure 1. Skin, porcine. Immunohistochemical staining with (A) and without (B) a polyclonal antiserum against *Erysipelothrix rhusiopathiae* revealing dark staining in the dermis only with the appropriate antiserum. Streptavidin–biotin–peroxidase complex method counterstained with hematoxylin. Bar = 200 μm.

Figure 2. Lung, porcine. Immunohistochemical staining using a polyclonal antiserum against *Erysipelothrix rhusiopathiae* revealing dark brown staining of bacteria-like organisms in alveolar septa and capillaries. Streptavidin–biotin–peroxidase complex method counterstained with hematoxylin. Bar = 20 μm.

Figure 3. Lung, porcine. Immunohistochemical staining using a polyclonal antiserum against *Erysipelothrix rhusiopathiae* revealing dark brown staining in bacteria-like organisms in the lumen of small vessels. Streptavidin–biotin–peroxidase complex method counterstained with hematoxylin. Bar = 20 μm.
incised using a sterile scalpel blade, and a sterile swab was inserted for bacterial collection for culture. The swabs were inoculated on trypticase soy agar plates containing 5% sheep blood. Plates were aerobically incubated at 35°C and examined at 24 and 48 hr postinoculation. Suspect colonies with a characteristic appearance similar to *Erysipelothrix* spp. were subcultured to trypticase soy agar plates containing 5% sheep blood, incubated for 24 hr, and then biochemically confirmed using standard laboratory methods.11

For further evaluation of the assay, tissue samples were used from 14 pigs experimentally inoculated with *E. rhusiopathiae* serotypes 1a, 1b, or 2 and collected between 1 and 5 days postinoculation. The details of the experiment have been previously described. A portion of the pigs (6/14) were treated with antibiotics 24 hr postinoculation. The results obtained by direct culture and IHC assay are summarized in Table 1 individually by tissue (lungs, spleen, heart, and skin) and on a per pig basis. The tissues for the comparison were selected based on availability for isolation and IHC assay. Erysipelas was confirmed in 6 of 8 untreated, experimentally inoculated animals with both direct culture and IHC. Using direct culture, *E. rhusiopathiae* most often was isolated from the spleen and heart tissues, whereas *E. rhusiopathiae* antigen was demonstrated by IHC staining more commonly in lung and skin sections. In contrast, *E. rhusiopathiae* was not isolated using direct culture from any of the tissues (lungs, spleen, heart, and skin) in animals treated with antibiotics. However, *E. rhusiopathiae* antigen was demonstrated in 4 of 6 treated animals (Table 1), indicating that treatment did not interfere with IHC detection.

In addition to tissues from experimentally inoculated pigs, diagnostic submissions were also included in the current investigation. The IHC procedure was tested on 170 field cases with clinical history and lesions suggestive of bacterial septicemia. Forty-four of these cases (25.9%) were confirmed to be positive by direct culture, and 126 of 170 cases (74.1%) were negative by direct culture. A comparison of results between direct culture and IHC are summarized in Table 2. The 2 tests agreed on 145 of the cases. Kappa statistics were calculated to measure statistical agreement between the 2 tests using a statistical software package. Values for kappa range from –1 to 1, where –1 indicates agreement worse than expected by chance, 0 equals agreement no better than expected by chance, and 1 equals perfect agreement. The following arbitrary standards for the strength of agreement as described by Landis and Koch were used: ≤0 = poor, 0.01–0.2 = slight, 0.21–0.4 = fair, 0.41–0.60 = moderate, 0.61–0.80 = substantial, and 0.81–1 = almost perfect.5 Under the experimental conditions described in the present report, the kappa statistic was 0.67 ± 0.06, indicating substantial agreement between direct culture and IHC assay.

Disagreement between direct culture isolation and the IHC assay was found in 25 of 170 (14.7%) cases. Two of the 25 cases were direct culture positive but IHC negative. In both cases, tissues were microscopically unremarkable, and low numbers of *E. rhusiopathiae* were isolated from lung tissues. Twenty-three of the 25 cases with conflicting results were direct culture negative but IHC positive. Interestingly, all 23 IHC-positive and direct culture–negative cases had microscopic lesions consistent with bacterial septicemia (fibrin thrombi in small vessels, suppurrative and necrotizing vasculitis, and necrosuppurative dermatitis). *Erysipelothrix rhusiopathiae* antigen was demonstrated in sections of skin in all cases that showed dermal lesions and where skin was submitted (21/23; Fig. 4), implying that affected sections of skin are ideal samples for IHC detection.

Immunohistochemistry approaches for detection of *E. rhusiopathiae* antigen in arthritic joints of pigs experimentally inoculated with *E. rhusiopathiae* serotype 2 have been described earlier.3 However, to the authors’ knowledge, the IHC assay described in the current study for detection of *E. rhusiopathiae* antigen is the first used for routine diagnostics. In summary, the IHC test was found to be highly sensitive and specific in detecting *E. rhusiopathiae* antigen in formalin-fixed, paraffin-embedded tissues. The authors concluded that IHC is particularly useful in cases in which the animals had been treated with antibiotics prior to submission and in which the cultures of organs were negative. In addition, the *E. rhusiopathiae* IHC was found to be useful for detection of erysipelas antigen in skin lesions, which are often culture negative.

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Sources and manufacturers

a. BD Diagnostic Systems, Sparks, MD.

b. Sigma-Aldrich, St. Louis, MO.

c. Dako North America Inc., Carpinteria, CA.

d. Thermo Fischer Scientific Inc., Waltham, MA.

e. JMP® Version 7, SAS Institute Inc., Cary, NC.

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**Figure 4.** Liver, porcine. Immunohistochemical staining using a polyclonal antiserum against *Erysipelothrix rhusiopathiae* revealing dark brown staining of bacteria-like organisms accumulating between hepatocytes. Streptavidin–biotin–peroxidase complex method counterstained with hematoxylin. Bar = 20 μm.

**Figure 5.** Skin, porcine. Immunohistochemical staining using a polyclonal antiserum against *Erysipelothrix rhusiopathiae* revealing abundant bacteria-like organisms (dark staining) in the dermis. Streptavidin–biotin–peroxidase complex method counterstained with hematoxylin. Bar = 50 μm.
References


