**Introduction**

- *Streptococcus suis* is a gram positive bacterium that most commonly infects swine.
- Transmission is thought to occur by nose to nose contact and by aerosol over short distances.
- S. suis can be zoonotic. It is also known to infect dogs, goats, sheep, cattle, and horses.
- Infection usually starts in the crypts of the palatine tonsils and mandibular lymph nodes.
- From there it can become systemic and invade joints, cerebrospinal fluid, heart, lungs, and brain.
- Diagnosis is usually determined based on the presence of lesions and isolation by bacterial culture.
- The Real-Time Polymerase Chain Reaction (qPCR) is a good alternative to traditional bacteriology since PCR assays are quicker and can more efficiently test a large volume of samples.

**Objectives**

- Develop a qPCR assay that is of similar or better sensitivity to bacterial culture and can be used in routine diagnostic testing for a variety of sample types.

**Materials and Methods**

**Sample Collection:**
- 174 clinical samples were obtained from the Iowa State University Veterinary Diagnostic Lab (ISU VDL), isolated by bacterial culture (BacT), and tested by qPCR.
- Samples were chosen prospectively and retrospectively based on presence of lesions, accession, and sample type.
- Homogenates, formalin blocks, and swabs collected from brain, lung, joint, and heart tissue were evaluated as potential matrices for testing.

**Primer and Probe Design:**
- Primers and probe sequences (Table 1) were developed using Primer Express® design software.
- The recombination repair protein gene (RecN) was chosen for amplification based on its high degree of conservation and target specificity.
- The probe contains a minor groove binder with a 5′-FAM fluorophore and a 3′-non fluorescent quencher.

**Sample Preparation**

- **Homogenates:** A 10% weight by volume tissue suspension in an Earle’s Balanced Salt Solution was prepared, centrifuged, and the resulting supernatant was tested by qPCR.
- **Swabs:** Placed in a 1% Phosphate Buffered Saline Solution prior to testing.
- **Formalin-fixed Paraffin Embedded (FFPE) Blocks:** No additional sample preparation. However, they required a separate extraction procedure.

**Nucleic Acid Extraction and PCR Setup**

- Samples were extracted using a KingFisher™ Flex Magnetic Particle Processor consistent with ISU VDL standard operating procedure.
- Joint fluid and FFPE samples were extracted with alternate methods.
- qPCR was performed with TaqMan® Virus 1-Step Master Mix, primers and probe (Table 1) and an internal control.
- A QIAGEN Rotor-Gene Q Thermal Cycler (RGQ) and 7500 Fast Real Time PCR System (ABI) were compared for performance.

**Results**

**Table 1. Primer and probe sequences**

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>SsuisRecNFor:</td>
<td>5′-CCITGGACAGTTGCGGAGAAGA-3′</td>
</tr>
<tr>
<td>SsuisRecNRev:</td>
<td>5′-TTTCGTTTCTACAAGTCTGTTG-3′</td>
</tr>
<tr>
<td>SsuisRecNP:</td>
<td>5′-FAM-AAGACGTTATCGAACAC-3′</td>
</tr>
</tbody>
</table>

**Sample Preparation**

- **Homogenates:** A 10% weight by volume tissue suspension in an Earle’s Balanced Salt Solution was prepared, centrifuged, and the resulting supernatant was tested by qPCR.
- **Swabs:** Placed in a 1% Phosphate Buffered Saline Solution prior to testing.
- **Formalin-fixed Paraffin Embedded (FFPE) Blocks:** No additional sample preparation. However, they required a separate extraction procedure.

**Nucleic Acid Extraction and PCR Setup**

- Samples were extracted using a KingFisher™ Flex Magnetic Particle Processor consistent with ISU VDL standard operating procedure.
- Joint fluid and FFPE samples were extracted with alternate methods.
- qPCR was performed with TaqMan® Virus 1-Step Master Mix, primers and probe (Table 1) and an internal control.
- A QIAGEN Rotor-Gene Q Thermal Cycler (RGQ) and 7500 Fast Real Time PCR System (ABI) were compared for performance.

**Table 2. Contingency table comparing BacT and PCR agreement across tissue type when bacteriology results were positive**

<table>
<thead>
<tr>
<th>Count</th>
<th>Total %</th>
<th>Col %</th>
<th>Row %</th>
<th>Disagree</th>
<th>Agree</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>16</td>
<td>17.2</td>
<td>18.1</td>
<td>29.41</td>
<td>41.61</td>
</tr>
<tr>
<td></td>
<td>38.1</td>
<td>52.61</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>5.3</td>
<td>8.6</td>
<td>19.05</td>
<td>38.46</td>
<td>51.54</td>
</tr>
<tr>
<td></td>
<td>13.98</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>22.7</td>
<td>17.2</td>
<td>38.1</td>
<td>49.34</td>
<td>50.66</td>
</tr>
<tr>
<td></td>
<td>39.78</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Joint</td>
<td>10.8</td>
<td>12</td>
<td>21.2</td>
<td>43.64</td>
<td>56.36</td>
</tr>
<tr>
<td></td>
<td>12.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Materials and Methods**

- **Sample Collection:**
  - 174 clinical samples were obtained from the Iowa State University Veterinary Diagnostic Lab (ISU VDL), isolated by bacterial culture (BacT), and tested by qPCR.
- Samples were chosen prospectively and retrospectively based on presence of lesions, accession, and sample type.
- Homogenates, formalin blocks, and swabs collected from brain, lung, joint, and heart tissue were evaluated as potential matrices for testing.

**Primer and Probe Design:**

- Primers and probe sequences (Table 1) were developed using Primer Express® design software.
- The recombination repair protein gene (RecN) was chosen for amplification based on its high degree of conservation and target specificity.
- The probe contains a minor groove binder with a 5′-FAM fluorophore and a 3′-non fluorescent quencher.

**Results**

**Anatomical Location**

Table 3. Contingency table comparing BacT and PCR agreement across tissue type when bacteriology results were positive.

<table>
<thead>
<tr>
<th>Anatomical Location</th>
<th>Count</th>
<th>Total %</th>
<th>Col %</th>
<th>Row %</th>
<th>Disagree</th>
<th>Agree</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>16</td>
<td>17.2</td>
<td>18.1</td>
<td>29.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>5.3</td>
<td>8.6</td>
<td>19.05</td>
<td>38.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>22.7</td>
<td>17.2</td>
<td>38.1</td>
<td>49.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Joint</td>
<td>10.8</td>
<td>12</td>
<td>21.2</td>
<td>43.64</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Graph 1. Bar plot comparing PCR and BacT agreement across tissue type when bacteriology results were positive**

** Graph 1. Bar plot comparing PCR and BacT agreement across tissue type when bacteriology results were positive.

**More testing is needed to confirm.**