Development of a Real-Time PCR Assay for the Detection and Diagnosis of *Streptococcus suis* in Clinical Samples

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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.

Table 1. Primer and probe sequences

Oligonucleotide	Sequence
SsuisRecNFor:	5- CTTTTGGACAGTTTCGGAGAAGA -3
SsuisRecNRev:	5- TTT TCG TTT TCA AGA ACT CGT TTG -3
SsuisRecNPr:	5'- FAM- AAGACCGTTATCAGACAAC- NFQ -3'

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 PCR.
- *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

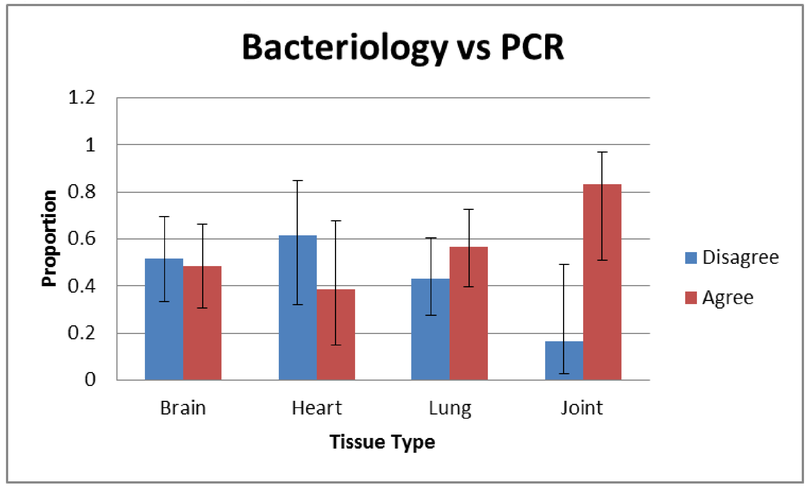
Anatomical Location

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

Bacteriology vs PCR

Anatomical Location	Count / Total % / Col % / Row %	Disagree	Agree	
Brain		**16** 17.2 38.1 51.61	**15** 16.13 29.41 48.39	31 33.33
Heart		**8** 8.6 19.05 61.54	**5** 5.38 9.8 38.46	13 13.98
Lung		**16** 17.2 38.1 43.24	**21** 22.58 41.18 56.76	37 39.78
Joint		**2** 2.15 4.76 16.67	**10** 10.75 19.61 83.33	12 12.9
		42 45.16	51 54.84	93

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



- Samples were determined positive when a sample showed a Ct value (cycle threshold) less than 40.
- Cultures weren't included in the sample analysis. 100% of cultures collected tested positive (average Ct = 22.3, standard deviation = 3.2).
- A Pearson χ^2 value of 5.918 and a P-value of 0.1157 across anatomical location are not considered significant when α=0.05. However, it is suspect and would suggest that there might be an effect across anatomical locations. More testing is needed to confirm.

Table 4. Limit of detection between the RGQ and the ABI thermal cyclers for Type 1 and Type 2 *S. suis.*

	Boil Prep		Regular Extraction	
	RGQ	ABI	RGQ	ABI
Type 1	3.90E+03	3.90E+04	3.90E+05	3.90E+05
Type 2	3.20E+03	3.20E+04	3.20E+04	3.20E+05

*Measured in CFU/mL

- A two log difference in sensitivity was found between the boil prep extraction and the regular magnetic extraction.
- Suggests that lost sensitivity might be due to the nucleic acid not being adequately extracted using the current ISU VDL protocol.
- Similar results were found to anatomical location when BacT and PCR were compared across sample type. A Pearson χ^2 value of 4.024 and a P-value of 0.1337 are not significant when α=0.05.
- Data were next aggregated based on case level. A case was considered to be positive by PCR, diagnostic code, or BacT if a sample per case tested positive
- Using this method, the PCR aggregate diagnosis agreed with diagnostic code 59.7±10.3% of the time, while BacT aggregate diagnosis agreed 75±8.2%.
- Possible future work could investigate the effects of extraction method on sensitivity and determine which anatomical locations serve as a better matrix for testing.