Notes for 10-herd NAS manuscript:

**Methods**

Would I be able to do a BLCM-ish analysis incorporating Se and Sp values for culture, to help correct for some misclassification bias?

**Discussion**

Include a section addressing possible biases; simon’s thesis papers provide a good model, good content, and can likely use some of the language from these

Discussion around agnetis and hyicus- Pamela’s 2017 paper (Species Identification and Strain Typing of Staphylococcus agnetis and Staphylococcus hyicus Isolates from Bovine Milk by Use of a Novel Multiplex PCR Assay and Pulsed-Field Gel Electrophoresis); how much did agnetis and hyicus elevate SCC at quarter level, did they act more like contagious or sporadic pathogens; persistent infections vs. transient? Repeat coag- which were positive, which were negative?

*tuf Gene Sequence Analysis Has Greater Discriminatory Power than 16S rRNA Sequence Analysis in Identification of Clinical Isolates of Coagulase-Negative Staphylococci*

16S rRNA and tuf gene sequencing. PCR amplifications were conducted in a total volume of 25 uL containing 2.5 mM deoxynucleoside triphosphates (dNTPs), 10 pmol of each PCR primer, 0.6 U Taq polymerase, 2.5 uL of 10x PCR buffer with 15 mM MgCl2 (Takara Bio, Inc., Shiga, Japan), and 2.5 uL of the template.

The tuf gene was amplified with primers TUF-F (5-GCCAGTTGAGGACGTATTCT-3) and TUF-R (5-CCATTTCAGTACCTTCTGGTAA-3). The PCR conditions for tuf were as follows: 15 min of initial denaturation at 95°C, followed by 35 cycles of 30 s at 95°C, 30 s at 56°C, and 45 s at 72°C, with a final 10-minextension at 72°C.

Gel electrophoresis was used to detect positive PCR signalsand to confirm amplicon lengths of 527 bp for 16S rRNA and 412 bp for thetufgene. Prior to sequencing, the PCR products were purified using the ExoSAP-ITreagent (USB Corporation, Cleveland, OH) according to the manufacturer’sinstructions. Forward and reverse sequencing reactions were conducted for eachof the amplified products. The sequencing reaction mixture for 16S rRNA con-sisted of 10l of MicroSeq 500 sequencing mix (containing 1.6 pmol of MSQ-For MSQ-R) primers, 2.9l of molecular-grade water, and 1l of the purifiedPCR product. For thetufgene, sequencing reactions were performed usingBigDye Terminator, version 3.1, reagents (Applied Biosystems Inc., Foster City,CA). Briefly, the sequencing reaction mixture consisted of 1l of BigDye ReadyReaction mix, 3.5l of BigDye sequencing buffer (5) (Applied BiosystemsInc.), 1.6l of a 1-pmol primer, 2.9l of molecular-grade water, and 1lofthepurified PCR product; the final reaction volume was 10l. The thermal cyclingconditions were as follows: 25 cycles of 10 s at 96°C,5sat50°C, and 4 min at60°C. The sequencing products were purified using ethanol–sodium acetate.Sequencing reactions were performed on an ABI Prism 3130xl genetic analyzer(Applied Biosystems Inc.) according to the standard automated sequencer pro-tocols