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Title: Investigating differences in the source of Serratia and other bacteria in boar semen Final Report

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Introduction

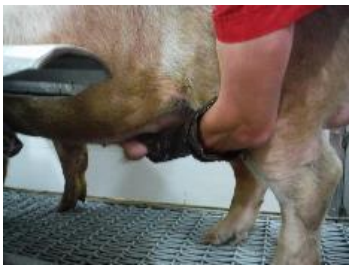
The presence of certain bacteria in the extended semen can cause loss of sperm viability and fertility. Serratia marcescens contamination is of concern due to its severe and unpredictable impact on downstream fertility. The antibiotics used and approved in semen extenders are not effective against Serratia. A series of experiments have been conducted by the author over the last few years to help determine the effectiveness of various collection strategies to the presence of bacteria in the semen, including an experiment using 16S sequencing techniques and presented at the 2021 AASV meeting. The purpose of this experiment was to expand on previous studies and conduct PCR testing to determine the source of Serratia.

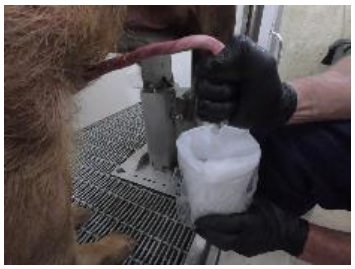
Materials and Methods

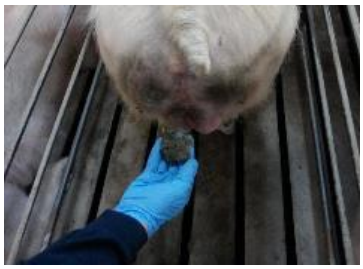
Three boar studs, all with at least 15 years of extended semen culture data from every collection day, were sampled. The studs ranged from 300-450 boars. Two have routinely found Serratia in extended semen and one has never identified Serratia.

10 boars from each stud were sampled by: prepuce fluid, raw semen, feces, and Swiffer of boar stall floor (see pictures below). The semen was collected using the three-glove method described previously (Reicks, 2018). For the Swiffer sample, 20 ml of saline was added to the pad and the slat wiped under boar.

Samples were frozen and ran all at once by traditional PCR for Serratia marcescens per published protocol (Bussaleu and Althouse, 2018). Three positive cultures from extended semen from two of the studs served as positive controls for the PCR.


Prepuce fluid


Raw semen


Feces


Environment

Results

Traditional PCR

All three known positive cultures from the two studs with history of Serratia reacted strongly on the PCR (figure 1). 12 of 120 samples reacted (one example - blue arrow below in figure 2). The reactions were not strong, which was expected from random samples.

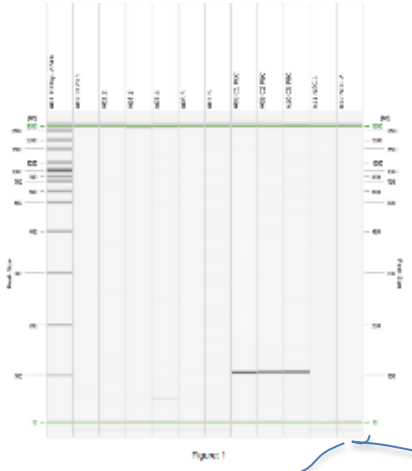


Figure 1: Three positive cultures from the two boar studs with a history of Serratia showing a strong reaction to the traditional PCR test.

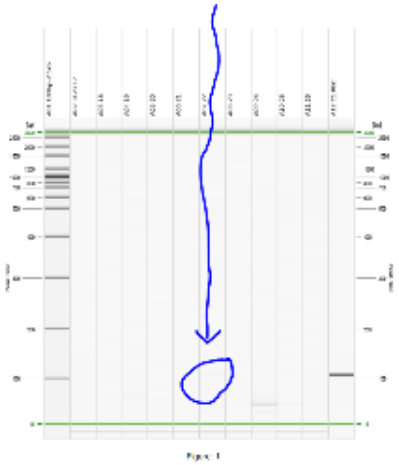


Figure 2: An example of a positive reaction (circled in blue) one of the fecal samples to Serratia marcescens on the traditional PCR test.

Samples of prepuce fluid and feces were positive from all three studs, while semen and environmental Swiffers were negative. One boar was positive on feces and prepuce fluid. 11 of 30 boars overall had at least one positive sample.

Stud	A	B	C
History of Serratia in semen?	yes	yes	no
Number of boars positive out of 10			
Prepuce fluid	1	0	3
Semen	0	0	0
Feces	2	5	1
Environmental Swiffer (20 ml saline)	0	0	0

Conclusions

This is a first of its kind study comparing studs with a history of Serratia, to one without, regarding hypothesized reservoirs of the bacteria in the barn. All three studs had positive samples, even though one of the studs has never cultured Serratia. Positives were found in prepuce fluid and feces, suggesting both are a significant reservoir. Using the three-glove method, all 30 semen samples were negative. It is unclear why one of the studs, despite similar PCR results, has not cultured Serratia in extended semen over 15 years in production. As a result of the valuable information from this study, a real time PCR is being pursued to provide better sensitivity, specificity, and quantitative information that may help answer further questions and help establish intervention strategies to mitigate the devastating effects of Serratia contamination on fertility.

Acknowledgments

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