# Association between Salmonella sp. and Yersinia enterocolitica infection in swine

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## Abstract
Swine are known reservoirs for both Salmonella and Yersinia enterocolitica. Both are foodborne pathogens and can result in zoonotic disease if contamination of pork products occurs during harvest. The epidemiology of Y. enterocolitica and Salmonella in swine is not well understood. Previous reports from experimental studies in mice suggest that, via quorum-sensing, Salmonella detects Y. enterocolitica signals, increasing Salmonella colonization. The objective of this study is to determine if there was an association between fecal shedding of Salmonella and Y. enterocolitica in naturally infected swine. DNA was extracted from 1232 fecal samples collected from finishing pigs at commercial farms. The Salmonella status of the samples was known from previous culture results. All positive samples and a random selection of negative samples were included in the study. High throughput duplex real-time PCR reactions were conducted to detect the presence or absence of Y. enterocolitica. TaqMan® assays targeted the Y. enterocolitica ail gene and a Yersinia specific region of the 16S rRNA gene. The prevalence rate of Y. enterocolitica in Salmonella positive versus Salmonella negative fecal samples was 3.9% and 7.5%, respectively. Based on cross-sectional sampling, and the status of an individual fecal sample, Salmonella positive pigs were less likely to be Y. enterocolitica positive. At the pig level, there was no significant association between Salmonella and Yersinia enterocolitica status.



## Introduction
Salmonella and Yersinia enterocolitica are both important foodborne pathogens of which swine are a reservoir. There are limited epidemiological investigations regarding co-infections with foodborne pathogens in swine. Yet, there are suggestions that that Y. enterocolitica infection may be associated with Salmonella infection.

Quorum sensing has been established as a form of communication between cells and a method by which bacteria can regulate their expression and colonization factors. Bacteria use LuxR-type transcription factors to detect self-produced N-acylhomoserine lactones (AHLs) in order to gain information about their own population density (Asad et al., 2008). Salmonella cannot synthesize its own AHLs, but does encode a LuxR-type AHL receptor, SdiA. It has been demonstrated that Salmonella can detect AHLs from other bacteria species, including Y. enteroclitica in a mouse model (Dyszel et al., 2009). Furthermore, Salmonella was seen to colonize the small intestines and Peyer's patches in higher number when the mice were co-infected with Y. enterocolitica. Given this prior research, our study was designed to explore a possible association between Salmonella sp. and Y. enterocolitica in the fecal shedding of swine. We hypothesized that swine shedding Salmonella in their feces would be more likely to be Y. enterocolitica positive as compared to swine from which Salmonella was not cultured from their feces.

## Material and Methods
Sampling: Fecal samples were collected from various commercial finishing-stage swine operations in the state of Michigan between June 2008 and April 2010. The fecal samples were collected as a component of an on-going project investigating risk factors for Salmonella shedding in swine. Pigs were sampled 8 times at 2 week intervals from 10-26 weeks of age. All Salmonella culture positive fecal samples (n=383) and a random sample of culture negative fecal samples (n=849) were selected for inclusion in this study. These samples originated from 535 individual pigs. The fecal samples (250 mg) were stored at -80°C prior to inclusion in this study. In addition, since the Salmonella status of the pig during the entire finishing phase was known, pigs that were represented by the 1,232 samples (n= were categorized as "ever culture positive" meaning they had at least one fecal positive sample during finishing or "always culture negative" during the finishing phase.

DNA Extraction: DNA extraction was performed using the QIAamp DNA Stool Mini Kit (Qiagen, Inc.) following the manufacturer's protocol under "Stool Pathogen Detection" with slight modification. A vacuum manifold was utilized to remove the lysate and wash buffers from the spin columns instead of the centrifugation method as stated in the instructional booklet. In addition, only 75µl of AE buffer was used during the elution step.

Real-time Quantitative PCR: We targeted both the 16S rRNA gene (Sen , 2000) and ail gene (Mäde et. al 2008). Probes were purchased from Applied Biosystems and primers were constructed by the Macromolecular Core of the Research Technology Support Facility at Michigan State University. The forward primer for the 16S rRNA had the sequence 5'CGGCAGCGGGAAGTAGTTT3' and the reverse primer 5'GCCATTACCCCACCTACTAGCTAA3'. These primers amplified a segment of the 16S rRNA gene spanning nucleotides 47 to 247. The TaqMan fluorescent probe had the sequence 5'VIC-AAGGTCCCCCACTTTGGTCCGAAG-TAMRA3'. VIC is the reporter dye and TAMRA (6-carboxytetramethylrhodamine) is the quencher dye. Primers targeting the ail gene had the following sequences. Forward primer 5'GGTTATGCACAAAGCCATGTAAA3' and reverse primer 5'AAACGAACCTATTCCCCAGTT3' with the probe sequence of 5'FAM-AACCTGAAGTACCGTTATGAACTCGATGA-DQ3'. FAM (6-carboxyfluorescein) is the reporter dye and DQ is the quencher dye. TaqMan® assays were run on the AB 7900HT Sequence Detection System. Preliminary tests were run with the primers/probes on Y. enterocolitica strains 8081V and ATCC9610 (which were also utilized as positive controls for all runs). The results garnered showed that this particular set of primers and probes would be efficient and adequate at detecting Y. enterocolitica.

Statistical Analysis: To compare the association between Salmonella and Y. enterocolitica status of a fecal sample, an odds ratio was calculated and p-value determined using the $\chi 2$ statistic. The same method was used to compare the association between Salmonella status of the pig during the finishing phase (ever/never Salmonella positive) and Y. enterocolitica status based on the selected samples.

## Results

A total of 76 samples (6.2%) were positive for Y. enterocolitica by PCR detection of the 16S rRNA target. Of these 73 samples (96.1%) were PCR positive for the ail gene. Six of the samples that tested positive for Y. enterocolitica amplified only one of the genes. (3 detected only ail; 3 detected only 16S rRNA). We considered a sample Y. enterocolitica positive if either the 16SrRNA or ail targets were detected (n=79).

Of Salmonella positive fecal samples, 3.9% were Y. enterocolitica positive (Table 1). Of Salmonella negative samples, 7.5% were Y. enterocolitica positive. Salmonella positive fecal samples were 0.5 times as likely to be Y. enterocolitica positive. (OR 0.5; 95% CI 0.27, 0.87; p<0.01)

Because the Salmonella status at the pig level during the finishing phase was known as a result of the on-going study from which the samples originated, we evaluated the odds for a sample to be Y. enterocolitica positive if the pig had ever been detected as Salmonella positive during the finishing period. Of the 535 total pigs that contributed to the study 22.6% (121/535) had at least one Salmonella positive fecal sample detected during the finishing period, and 11.8% (63/535) had at least one positive Y. enterocolitica sample detected within this study. The proportion of pigs that were ever detected as Salmonella positive that had a positive Y. enterocolitica sample detected in this study was 14.1% and for pigs from which Salmonella was never detected the proportion of pigs with a positive Y. enterocolitica sample was 11.1%. The odds of pig that was Salmonella positive during the finishing period had a Y. enterocolitica positive sample in this study was 1.3 (95% CI 0.67-2.44, p>0.05).

## Discussion

The results of this study do not support our original hypothesis that Salmonella positive swine are more likely to be colonized with Y. enterocolitica.

There are several limitations to this study, not the least of which is that the sampling strategy was a convenience sample of a subset of fecal samples that were selected for another purpose. Therefore, the sampling strategy did not allow longitudinal within pig assessment of Y. enterocolitica status (which was available for Salmonella). Therefore, we have an incomplete picture of the Y. enterocolitica status of these pigs over the same period for which Salmonella status is known. Future evaluations of samples targeted at the specific research question of this investigation are planned.

One of the effects this study could have on food safety is the potential for an alternative way to help decrease the prevalence of Salmonella and other foodborne pathogens on farms. If mechanisms that impact pig susceptibility to infection with foodborne pathogens, such as fomented co-infection via quorum sensing, are demonstrated to be important, it may provide an additional tool for control of foodborne pathogens on farms.

## Conclusion

In this study, there was no evidence that pigs that were Salmonella positive on fecal culture were more likely to be Y. enteroclitica positive. Future studies with appropriate study design are necessary to evaluate this association.

## References

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