PREVALENCE OF *YERSINIA ENTEROCOLITICA* ACROSS PHASES OF SWINE PRODUCTION

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Abstract The objective of this study was to describe the prevalence of *Yersinia enterocolitica* (YE) in different swine production phases. In this cross-sectional study, individual pigs on eight farrow-to-finish farms were sampled for YE by collection of both feces and oral-pharyngeal swabs. Samples were cultured with a three-week cold enrichment followed by culture onto selective media. Presumptive YE isolates were confirmed as YE and assayed for the presence of the *ail* gene using a multiplex PCR. A pig was considered positive if either the fecal sample or oral-pharyngeal swab tested positive. Of the 2321 pigs sampled, 141 (6.1%) were YE positive and of those, 109 were *ail* positive (77.3% of YE isolates). There was a consistent trend of increasing prevalence with maturity. These results represent the first on-farm description of YE in US herds providing first step for future studies to understand the epidemiology of YE in US market swine.

Introduction *Yersinia enterocolitica* is a food borne pathogen which causes an estimated 96,000 human illnesses in the United States each year (Mead et al., 1999). While the bacterium has been found in a variety of food and environmental samples, swine are the only food animals that regularly harbor pathogenic *Y. enterocolitica* (Kapperud, 1991). Thus swine and pork products have been implicated as the primary reservoir of pathogenic *Y. enterocolitica*. Recent outbreaks in the US have been related to the preparation and consumption of chitterlings (pig intestines). (Anonymous, 1990; Anonymous, 2003) Of particular food safety concern is the ability for *Y. enterocolitica* to grow at refrigeration temperatures and survive repeated freezing and thawing.(Toora et al., 1992)

Previous studies of *Y. enterocolitica* have investigated swine at harvest (Hanna et al., 1980; Harmon et al., 1984) but little research has focused on the epidemiology of *Y. enterocolitica* at the farm level. Understanding the on-farm epidemiology of *Y. enterocolitica* is the first step towards identifying risk factors and potential interventions in swine production that may decrease the risk of product contamination during harvest and processing. The objective of this study was to describe the prevalence of *Y. enterocolitica* in different production phases on swine farms.

Methods A cross-sectional study to survey individual pigs for the presence of *Y. enterocolitica* was undertaken on eight farrow-to-finish swine operations in Ohio. On each farm, during a one-time visit, gestating sows (G), farrowing sows (S), their suckling piglets (P), nursery pigs (N), and finishing pigs (F) were cultured for the presence of *Y. enterocolitica*. When possible, the youngest (1) and oldest animals (2) within each phase were sampled. The number of pigs sampled in each production phase was calculated in order to estimate prevalence with 95% confidence and a ±10% confidence interval based on previous estimates in the literature of 25% prevalence in swine at harvest. This sampling scheme resulted in the sampling of 2321 pigs from May to August 2003.

Fecal samples (10g) and oral-pharyngeal swabs were collected and tested for each animal with the exceptions of 1) sows in the farrowing room where oral-pharyngeal swabs were not collected and 2) in the cases where 10g of feces could not be collected (i.e. piglets under 10 weeks of age) rectal swabs were used in place of a fecal sample. Animals were considered positive if either the fecal or oral-pharyngeal sample tested positive for *Y. enterocolitica*. Samples were cultured according to the gold standard method of three-week cold enrichment in phosphate buffered saline (PBS) (Aleksic et al., 1999). Briefly, swabs were placed into 10mL of PBS and fecal samples were diluted at a 1:10 ratio with PBS (EMD Chemicals Inc.). Swab and fecal samples were incubated for 3 weeks at 4°C. Following enrichment, 10µL of the inoculated PBS was streaked onto Yersinia Selective Agar (CIN) plates (Becton Dickinson and Company). Plates were incubated at 25°C for 48 hours before being examined for colonies resembling *Y. enterocolitica*. Colonies having morphologies typical of *Y. enterocolitica* were tested on Kligler’s Iron Agar (KIA) (Becton Dickinson and Company) slants and urease broth (Becton Dickinson and Company). Colonies that produced an alkaline slant with an acid butt on the KIA within 24 hours and an alkaline urease broth reaction within 48 hours were classified as presumptive *Y. enterocolitica*. Presumptive *Y.
enterocolitica were stored at -80°C until PCR could be performed. All presumptive isolates were confirmed as Y. enterocolitica (based on 16S RNA target sequence) and assayed for the presence of the ail gene (a virulence gene associated with human clinical disease isolates) with the use of a multiplex PCR. Y. enterocolitica isolates that harbored the ail gene were classified as potentially pathogenic. The multiplex PCR setup used was based on that of Wannet et al. (Wannet et al., 2001). To amplify the 330 base pair product of the Y. enterocolitica 16 RNA gene, primers Y1 (5’-AAT ACC GCA TAA CGT CTT CG-3’) and Y2 (5’-CTT CTT CTG CGA GTA ACG TC-3’), were used. Primers A1 (5’-TTA ATG TGT ACG CTG CGA GTG-3’) and A2 (5’-GGA GTA TTC ATA TGA AGC GTC-3’) were used to amplify the ail gene, resulting in a PCR product of 425 base pairs. Frozen isolates were streaked for isolation on Iso-Sensitest agar plates (Oxoid). One isolated colony was picked with a sterile inoculating needle into 50µL sterile water. A 30µL total volume reaction was performed with Qiaqen Multiplex PCR Kit following manufacturer’s recommended protocol including individual primer concentrations of 3.2 µM and 5uL of diluted colony. PCR amplification conditions were an initial denature at 94°C for 15 minuets followed by 36 cycles of denaturing for 30 seconds at 94°C annealing at 62°C for 90 seconds and extension at 72°C for 90 seconds. A final extension of 10 minutes at 72°C was used after the 36 cycles. PCR products were visualized on the 4% E-Gel 48 Gel system (Invitrogen).

Results Of the 2321 pigs sampled, 141(6.1%) tested positive and of those, 109 were ail positive (77.3 % of Y. enterocolitica isolates). On all farms, there was a consistent trend of increasing prevalence as pigs mature (Figure 1). Less than 1% of suckling piglets tested positive for Y. enterocolitica. Only 1.4% (0.7 % ail positive) of nursery pigs tested positive but 13.9% (11.2 % ail positive) of finishing pigs harbored Y. enterocolitica. An interesting finding was that while gestating sows had the second highest prevalence of Y. enterocolitica at 9.7% (6.8 % ail positive), Y. enterocolitica was never detected from sows in the farrowing phase.

Discussion These results represent the first on-farm description of Y. enterocolitica in US swine herds. Overall, the prevalence of Y. enterocolitica was lower than previous reports based on cross-sectional surveys at harvest (Funk et al., 1998). One potential explanation for this finding may be due to the fact the previous sampling was done at harvest thus only sampling older animals whereas our sampling included the younger, lower prevalence, animals. Another possible explanation for the lower prevalence is sampling occurring predominantly in the summer months. Previous investigators have suggested that Y. enterocolitica prevalence is lower in summer months (Toma et al., 1975; Tsubokura et al., 1976; Funk et al., 1998). Based on limited experimental data, the dogma has been that pigs are infected early in life and maintain infection in the tonsils for long periods of time, perhaps for life (Nielsen et al., 1996). The low prevalence in farrowing sows, as well as suckling and nursery pigs, suggests that sows may not be a source of Y. enterocolitica infection in growing pigs. An additional potential explanation for the lower overall prevalence is a decreased sensitivity of our microbiological methods in farrowing sows and pre-weaning piglets. The differential sensitivity for farrowing sows may be attributable to 1) farrowing sows were only sampled using feces, and gestation sows were sampled both with oral swabs and feces and 2) sows in gestation tend to have feces with a higher dry matter content relative to sows in farrowing, which may impact detection because we determine the amount of feces to culture based on weight. For piglets, only rectal swabs were sampled instead of a larger 10g fecal weight. Sample weight has been shown to impact sensitivity of culture methods for Salmonella (Funk et al., 2001). Interestingly, this same pattern of high gestation prevalence but low prevalence in farrowing and in suckling piglets has been reported for Salmonella (Funk et al., 2001).

The consistent increase in prevalence of Y. enterocolitica as pigs reach market weight is of great importance from a food safety standpoint. The two groups of animals with the highest prevalence, gestating sows and finishing pigs, are the two groups most proximal to entering the food supply. These data provide the important first step for designing future studies to understand the epidemiology of Y. enterocolitica in US swine. Future warranted studies include following individual pigs from birth to harvest, evaluating sensitivity of the microbiological tests for different age groups, and evaluation of seasonal variability of prevalence. Regulatory based monitoring of food borne pathogens at harvest and processing is likely to increase the interest in controlling food borne pathogens on-farms. Understanding the epidemiology of Y. enterocolitica will provide veterinarians the tools to help coordinate on-farm controls in order to help preserve a safe pork chain.
With safe pork accountability tracing back to the farm level, veterinarians serve a crucial role to their clients by helping to assure the quality of the product being supplied to abattoirs.

References


