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YERSINIA PREVALENCE IN ANTIBIOTIC FREE AND CONVENTIONALLY REARED SWINE

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Summary: These preliminary results compare the on-farm prevalence and frequency of carcass contamination with *Yersinia enterocolitica (YE)* for pigs reared under conventional and antibiotic free production systems. At the time of submission, results are available for 6 herds. In 5 of 6 herds, we were able to isolate *YE* from at least one pig on-farm. The overall individual pig prevalence was 13.3% on conventional farms 59.3% on ABF farms. *YE* was isolated from 2 of 191 carcass swabs. Although the preliminary nature of these results limits interpretation, it supports previous results that indicate swine serve as a reservoir for *YE*.

Introduction: *Yersinia enterocolitica* is a food-borne pathogen causing an estimated 96,000 Americans to become ill each year (Mead, 1999). Swine are considered the primary reservoir of pathogenic YE. In a previous survey of US market pigs (Funk et al., 1998), over 28% of herds have been estimated to be positive for pathogenic (*ail* gene-harboring) *YE*. As part of multi-state study to determine the prevalence, antimicrobial resistance and genotypic diversity of three major foodborne pathogens in swine: *Salmonella*, *Campylobacter* and *Yersinia*, the goal of the present study was to compare the prevalence of *YE* in market swine reared under conventional and antibiotic-free (ABF) production.

Methods: A total of 60 farms were selected for entry into the project, 30 conventionally reared (antibiotics included in the feed) and 30 antibiotic free (no antibiotics included in the feed) during the growing phase. The farms were equivalently distributed in 3 regions of the United States. Within each region, 10 conventional and 10 antibiotic free herds were recruited. A questionnaire was administered in person to record farm antibiotic use and management practices. A schematic of sampling protocol is shown in Table 1. On-farm sampling consisted of 10 g of feces obtained from each of 30 pigs (or the entire marketing if less than 30) on farm that were destined for harvest within 48 hours. Sampling of carcasses at the slaughter plant was conducted in the following manner. Carcasses were sampled at each of 3 points during the slaughter process; pre-evisceration, post-evisceration and post-chilling. Of the 30 pigs sampled on farm, 10 carcasses were sampled at pre-evisceration, 10 at post-evisceration and 10 post-chilling. One swab was conducted per carcass at pre-evisceration and post-evisceration, and 2 swabs were obtained from carcasses post-chill. The swabbing technique at pre- and post evisceration consisted of a bung-to-jowl swipe along the evisceration incision with a 7.62 X 12.70 cm sterile cellulose sponge (Solar-cult cellulose sponge, Solar Biologicals, Ogdensberg, NY, USA) dampened with 20ml of buffered peptone water. At post-chilling, the same protocol was conducted on one side of the carcass. On the other side of the same carcass, a modification of the USDA sampling method for HACCP monitoring (Anonymous, 1996) was conducted. Briefly, the same locations were swabbed (belly, bung, jowl), but with the 7.62 X 12.7 cm sponge using a template with 3 times the area of the standard protocol. Swabs were placed in Whirl-pak bags (Nasco, Ft. Atkinson, WI, USA) and transported to the lab on ice. Swabs were divided into 3 equal portions using sterile technique, with each 1/3 swab portion placed in a sterile Whirl-pak. One sub-sample of the swab was cultured for *YE*. The other swabs were forwarded to collaborating investigators for isolation of other foodborne pathogens. Feces and swabs were cultured utilizing cold enrichment in

phosphate buffered saline for 21 days followed by plating on cefsulodin-Irgasan-novobiocin agar (*Yersinia* Selective Agar, CIN, Becton Dickinson and Company, Franklin Lakes, NJ, USA). Colonies with morphology typical of *YE* were then biochemically screened by subculture onto Kligler's Iron Agar and urea broth (Becton Dickinson and Company). Isolates biochemically typical of *YE* were then further characterized by presence or absence of the *ail* gene by PCR and by serotyping (O1, O3, O5, O8, O9, *Yersinia* antisera, Denka Seiken, Tokyo, Japan). Antimicrobial resistance was determined against 17 antimicrobials (Sensitire, Trek Diagnostics, Cleveland, Ohio, USA).

Results: At the time of submission, 6 farms (4 conventional and 2 ABF) have complete results through presumptive identification of *YE* by biochemical testing (Table 2). Five herds (3 conventional and 2 antibiotic free) have had at least one *YE* isolate from fecal samples. One herd had 2 positive swab samples. The prevalence of pigs positive for *YE* as determined by fecal culture on farm was 13.3% on conventional farms and 59.3% on ABF farms. Serotype distribution, PCR analysis for detection of the presence or absence of the *ail* gene and antimicrobial resistance are pending. The preliminary nature of this data precludes us from forming conclusions from these results. These data do support previous investigations that suggest swine can serve as a reservoir of *YE*.

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Table 1. *Schematic of target sampling scheme for each participating pig herd.*

		Slaughter Plant Sample Scheme			
	On Farm	**Pre-evisceration**	**Post-evisceration**	**Post-Chill**	
		bung to jowl	bung to jowl	bung to jowl	Modified USDA
Pigs 1-30	30 fecal				
Pigs 1-10		10 swabs			
Pigs 11-20			10 swabs		
Pigs 21-30				10 swabs	10 swabs

Table 2. *Prevalence of YE in herds with biochemical results at time of submission.*

Farm	Conventional or Antibiotic Free*	Fecal	Pre-evisceration	Post-evisceration	Post -chill	
					Bung/Jowl	Mod. USDA
1	C	0/28	0/9	0/5	ND	0/12
2	C	9/35	0/10	0/10	ND	0/10
3	ABF	15/22	0/10	0/10	0/10	0/10
4	C	5/22	0/10	1/10	0/10	1/10
5	C	1/28	0/10	0/10	0/10	0/10
6	ABF	1/5	0/5	0/5	ND	0/5
Total		31/140	0/54	1/50	0/30	1/57

**C=conventional, ABF=antibiotic free*