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Prevalence, Serotype, Virulence Characteristics, Clonality, and Antibiotic Susceptibility of Pathogenic *Yersinia enterocolitica* from Swine Feces

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14 Prevalence, Serotype, Virulence Characteristics, Clonality, and Antibiotic Susceptibility of Pathogenic *Yersinia enterocolitica* from Swine Feces

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14.1 Introduction

Enteroinvasive pathogenic strains of *Yersinia enterocolitica* are recognized as major human pathogens that cause 96,000 cases of human disease annually in the United States (Scallen et al. 2011). Ninety percent of those cases are the result of foodborne transmission (Centers for Disease Control and Prevention (CDC) 2006; Nesbakken 2005; Scallen et al. 2011). Swine are identified as important reservoirs of *Y. enterocolitica* serotypes (O:3, O:5, O:8, O:9) that are associated with human illness (Fredriksson-Ahomaa et al. 2011). Pathogenic *Y. enterocolitica* carriage in swine ranges from 35 to 70% of herds and 4.5 to 100% of individual pigs (Ortiz Martinez et al. 2009). *Y. enterocolitica* is transmitted among swine by the oral–fecal route and is found on the surface of freshly slaughtered pig carcasses. This is likely the result of the spread of feces or contamination from the oral cavity during the slaughtering process (Laukkanen et al. 2009). In the United States, few studies on the prevalence of *Y. enterocolitica* in swine have been conducted (Bhaduri 2001; Bhaduri and Wesley 2006; CDC 2006, 2011).

Since pork safety begins on the farm, producers and practitioners play a critical role in providing safe products. Therefore, an investigation was conducted as a part of the U.S. Department of Agriculture (USDA) National Animal Health Monitoring System (NAHMS) Swine 2000 Study to measure the distribution of pathogenic *Y. enterocolitica* in finisher pigs and thus to identify potential control factors to reduce the public health risk factors associated with this pathogen. This study represented 92% of the U.S. hog inventory and 75% of its operations from the top 15 hog-producing states. Since *Y. enterocolitica* is a commensal organism of swine, fecal samples were tested for its occurrence. Regulatory policy states that isolation of a pathogen is an essential requirement in pathogen monitoring programs. Thus, enrichment, isolation, and verification by a fluorogenic PCR assay were used to monitor the prevalence of pathogenic *Y. enterocolitica*. The pathogenic potential of the individual isolates was assessed by serotyping, screening for the presence of the plasmid for *Yersinia* virulence (pYV), and testing for virulence-associated determinants, which were then correlated with genotype, expression of YopE protein, and antibiotic susceptibility. The information generated will be useful for the identification of on-farm management and processing practices leading

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to *Y. enterocolitica* contamination. Modification of such practices would ultimately result in reducing *Y. enterocolitica* transmission from pork products to humans.

14.2 Materials and Methods

14.2.1 Study Design and Sample Collection

On-farm sampling collections were conducted by USDA-Animal and Plant Health Inspection Service (APHIS) federal and state field veterinarians from September 6 through December 6, 2000 and January 3 through March 20, 2001 as previously described (Bhaduri and Wesley 2006; Bhaduri et al. 2005, 2009).

14.2.2 Isolation of Pathogenic *Yersinia enterocolitica*

The overall process for isolation and characterization of presumptive *Y. enterocolitica* isolates is depicted in Fig. 14.1. Identification of CIN$^+$ presumptive clones as *ail* positive pathogenic *Y. enterocolitica* by fluorogenic 5′ nuclease PCR assay targeting the chromosomal virulence *ail* gene (*a*ttachment *i*nvasion *l*ocus); storage of *ail*$^+$ *Y. enterocolitica* isolates; pYV screening in *ail*-positive *Y. enterocolitica*; determination of pYV-associated virulence determinants; detection of YopE in cell lysates by sodium dodecyl phosphate-polyacrylamide gel electrophoresis (SDS-PAGE); and Western blotting were carried out as previously described (Bhaduri and Wesley 2006; Bhaduri et al. 2005).

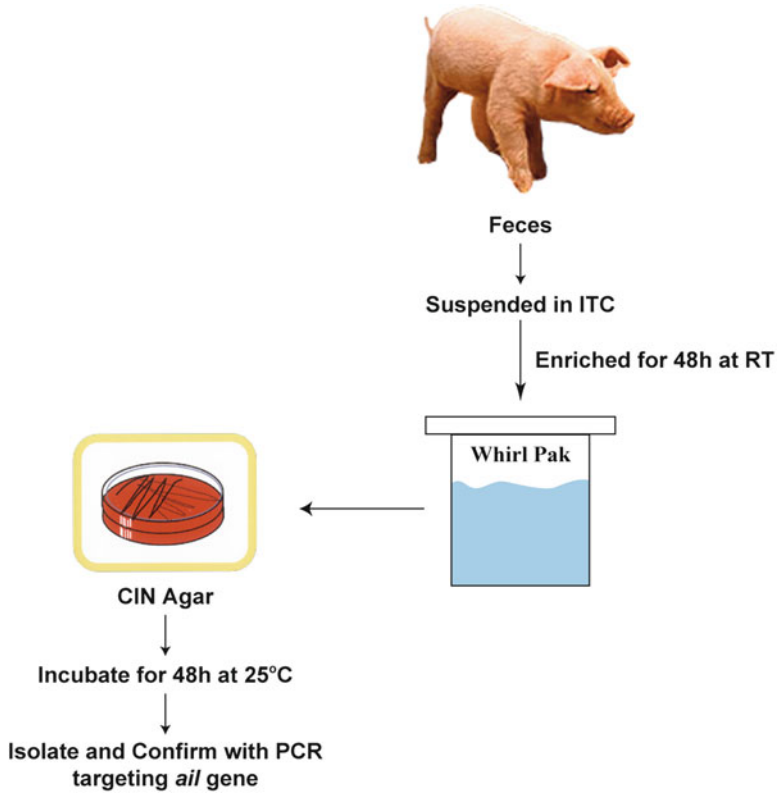

Fig. 14.1 Flow chart for isolation and characterization of presumptive *Yersinia enterocolitica* isolates

14.2.3 Assessment of the Pathogenic Potential of the Isolates

The *ail*-positive *Y. enterocolitica* isolates were serotyped as previously described (Bhaduri and Wesley 2006); Pulsed-Field Gel Electrophoresis (PFGE) and cluster analysis were performed as described (Bhaduri et al. 2009) and antimicrobial susceptibility testing was determined as described (Bhaduri et al. 2009).

14.3 Results and Discussion

14.3.1 Prevalence

A total of 2,793 swine fecal samples from 77 production sites were surveyed for the presence of pathogenic *Y. enterocolitica* over a period of 27 weeks (September 6, 2000 to December 6, 2001 and January 3 through March 20, 2001). The farms were located in 2 eastern and 13 mid-western states. Figure 14.2 shows the states that participated in the swine study. A fluorogenic 5′ nuclease PCR assay (Bhaduri et al. 2005) detected the chromosomal *ail* gene in 345 of 2,793 enrichments. Samples were used for the presumptive isolation of *Y. enterocolitica* on selective CIN agar, and CIN$^+$ presumptive colonies were then identified as pathogenic *Y. enterocolitica* by a fluorogenic 5′ nuclease PCR assay targeting the chromosomal *ail* gene. A total of 106 (3.8%) CIN$^+$ (*ail*-positive) *Y. enterocolitica* colonies were isolated from 2,793 total fecal samples and evaluated for pathogenic potential. In this study, prevalence based on fecal samples was higher (3.8%) than the recent 0.5–1.4% estimate for pigs surveyed in Europe (Fredriksson-Ahomaa et al. 2011; Laukkanen et al. 2009;

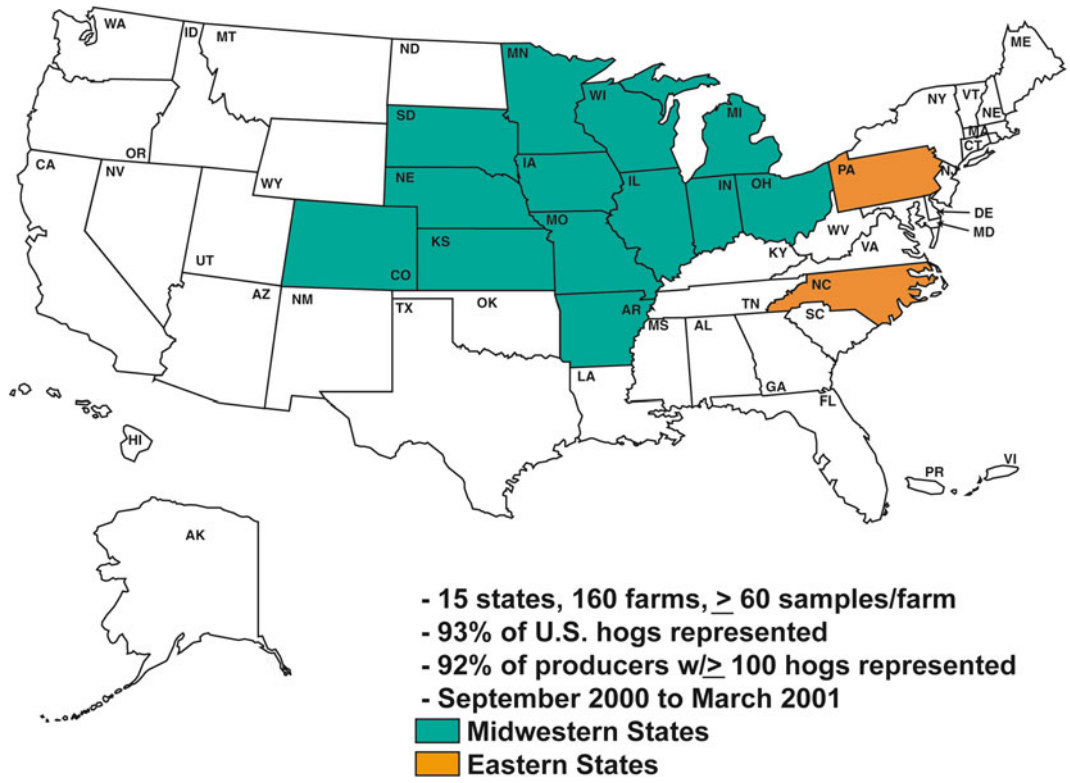

Fig. 14.2 States that participated in the swine study

Ortiz Martinez et al. 2009). Thus, the isolation of pathogenic *Y. enterocolitica* from feces may be more difficult or less sensitive than other samples from swine or it may be that the prevalence is low. In 7 of the 15 participating states, at least one isolate was positive for the chromosomally encoded *ail* sequence, yielding an overall on-farm prevalence of 46.6%. Chi-square analysis (Bhaduri et al. 2005) showed that four states, including Indiana ($n=25$ [23.5%] of 106), Iowa ($n=21$ [19.1%] of 106), Minnesota ($n=18$ [16.8%] of 106), and Nebraska ($n=29$ [27.3%] of 106), had a significantly higher percentage (17–28%) of *ail*-positive *Y. enterocolitica* than the number isolated (1–8%) from Illinois ($n=4$ [3.7%] of 106), Ohio ($n=1$ [0.9%] of 106), and South Dakota ($n=8$ [7.5%] of 106).

14.3.2 Serotyping

Serotyping is a valuable indicator of pathogenic potential, since both serotypes O:3 and O:5 harbor the *ail* gene (Carniel 2006). The *ail*-positive isolates were primarily serotype O:3. The number and distribution of serotypes were statistically analyzed by the chi-square test (Bhaduri and Wesley 2006). Among seven states, 79 (74.2%) of 106 of the isolates were serotype O:3, and 27 (24.5%) of 106 of the isolates were serotype O:5. The O:3 serotype was exclusively distributed in Indiana ($n=24$), Nebraska ($n=28$), and South Dakota ($n=8$). Serotype O:5 was found only in Illinois ($n=4$) and Ohio ($n=1$). Both serotypes were found in Iowa (O:3, $n=12$; O:5, $n=9$) and Minnesota (O:3, $n=6$; O:5, $n=12$). These results agree with previously published results but they differ from the observation that serotype O:5 was predominant in swine (Bhaduri and Wesley 2006). Serotype O:3 strains are regarded as a major cause of human yersiniosis in the United States (CDC 2003). A significant finding of our study is that serogroup O:3 (74.5% of isolates) is the dominant virulence serogroup presently associated with swine in the United States.

14.3.3 Presence of pYV in *ail*-Positive *Y. enterocolitica*

Y. enterocolitica pathogenicity is dependent on chromosomal virulence genes, as well as virulence factors encoded by pYV (Carniel 2006). In the present study, the multiplex PCR assay targeting a key regulatory gene, *virF*, present on the pYV and the chromosomal *ail* gene showed that these *ail*-positive isolates (104) harbored pYV (YEP) and expressed pYV-associated phenotypes. The absence of pYV in two isolates demonstrated the instability of the pYV, which is easily lost on subculture and storage (Bhaduri 2001).

14.3.4 pYV-Associated Virulence Characteristics

One hundred and four YEP^+ isolates expressed pYV-encoded phenotypic characteristics, including colony morphology, crystal violet binding, low calcium response, Congo red uptake, autoagglutination, hydrophobicity, and YopE. These pYV-encoded virulence factors had been correlated with mouse pathogenicity and were used as direct markers for identifying pathogenic isolates of *Y. enterocolitica* among clinical and food sources (Bhaduri 2001).

14.3.5 Genomic Analysis

*Xba*I was the sole enzyme used in this study since it yields the most discriminating macrorestriction fragments for *Y. enterocolitica*. By PFGE, O:3 and O:5 *ail*-positive isolates could be distinguished. However, isolates were highly clonal within a serotype and exhibited minor variations that could not be correlated with geographic origin. Thus, isolates from different farms within the same state or from different states displayed nearly indistinguishable PFGE profiles. That O:3 and O:5 pulsotypes exhibit only minor variations within a serotype, regardless of geographic origin, indicates high clonality

and that the genome of *Y. enterocolitica* is stable, an observation that concurs with others (Fredriksson-Ahomaa et al. 2011).

14.3.6 Antibiotic Susceptibility of *ail*-Positive Isolates

Antibiotic resistance of 106 YEP+ swine fecal isolates was studied to obtain baseline data of resistance patterns. A high degree of antibiotic susceptibility was observed in the sampled population of *ail*-positive *Y. enterocolitica* from swine feces. All of the strains ($n = 106$) were susceptible to amikacin, amoxicillin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, and trimethoprim. Similar patterns of susceptibility were observed among strains isolated from pig tonsils in Switzerland, southern Germany, as well as in human strains (Bucher et al. 2008). Resistance to ampicillin was shown in all of the 106 isolates. Ampicillin resistance due to production of β-lactamases is well described in the literature (Bhaduri et al. 2009). Of the 106 isolates, 87.7% were resistant to cephalothin, and 27.4% were resistant to tetracycline. All of the isolates resistant to tetracycline were also resistant to cephalothin. Higher percentage of resistance to cephalothin (72–100%) was found among four states; moderate resistance (13–69%) to tetracycline was distributed among three states while no isolate from Nebraska was resistant. Likewise, Funk et al. (2000) in screening *ail*-bearing isolates of serotype O:5 from hog tonsils in the Midwest concluded that the majority of isolates were resistant to ampicillin, penicillin, and cephalothin and could not correlate the presence of the *ail* gene with antimicrobial resistance. The presence or absence of the pYV did not have a significant effect on the resistance profile. These overall susceptibility/resistance results are consistent with what others have reported in the literature (Bucher et al. 2008; Funk et al. 2000).

14.4 Conclusion

Porcine isolates of *Y. enterocolitica*, which retained the chromosomal *ail* gene, pYV, and pYV-associated virulence phenotypic characteristics including cytotoxicity factor, YopE, were further analyzed to determine genotype antimicrobial profiles. Macrorestriction patterns demonstrated a high degree of clonality among isolates of the same serotype, regardless of geographic origin indicating stability of the genome. These pathogen isolates were sensitive to 13 of 16 antimicrobials. The results of this study support the hypothesis that swine are a significant potential reservoir for *Y. enterocolitica* strains. The varying presence of *Y. enterocolitica* from site to site suggests that management factors may influence on-farm prevalence of this organism. Modifications of such practices would ultimately reduce *Y. enterocolitica* transmission from pork products to humans. To fully understand the prevalence and risk factors associated with *Y. enterocolitica* infection in swine, additional investigations are needed of on-farm production, as well as postharvest processing systems.

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