

The Prevalence of Pathogenic *Yersinia enterocolitica* Positive Swine Herds

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1. ABSTRACT

In order to estimate the prevalence of swine herds infected with pathogenic *Yersinia enterocolitica*, one hundred three lots of market swine were randomly selected at slaughter over six, one month intervals. Pigs within each lot were sampled by swabbing the oral-pharyngeal surface, post-stunning and -exsanguination but pre-scalding.

Ninety-five lots (92.2%) contained at least one pig infected with *Yersinia enterocolitica*. Pathogenic strains were defined as those harboring the *ail* gene which has been identified in *Yersinia enterocolitica* that cause human clinical disease. Identification of those strains harboring the *ail* gene was accomplished using a polymerase chain reaction (PCR) technique. Twenty-nine lots (28.2%) contained at least one pig from which a pathogenic (*ail* containing) *Yersinia enterocolitica* was isolates identified, 89.7% were serotype O:5 and 3.7% were serotype O:3. The results from this study will aid the design of future epidemiological investigations concerning on-farm prevalence and associated risk factors for pathogenic *Yersinia enterocolitica*. Additionally, the results support the hypothesis that swine are a significant potential reservoir for human infections by *Yersinia enterocolitica*.

2. INTRODUCTION

During the last decade the adequacy of safety of meat and poultry products has emerged as a major public health issue. Food safety recently culminated at the forefront of public concern as a result of the outbreak of *Escherichia coli* O157:H7 associated with the consumption of improperly handled ground beef¹³. Currently, there is a national reevaluation of procedures that take meat from farms to the consumer's table. Based upon recommendations of the National Research Council^{25,26,42,47} a risk based system of monitoring meat and poultry processing is to be implemented. In addition to programs at the processing and slaughter plants, development of pre-harvest or production methods to reduce potential foodborne pathogens from food producing animals is suggested³⁴.

In production environments, control of potential foodborne pathogens that do not pose a threat to the health or production of the food producing animal, but that are a potential threat to the consumer, is a change from traditional disease control efforts, and very little

information is available in this area. In order to identify control-management strategies applicable to animal production systems, identification of both the prevalence of carrier animals and affected production units are required.

Foodborne microbial threats are likely to become an increasing problem for several reasons:³⁰ The food chain, from the animal production site through home preparation, has many points at which food safety can be compromised. It has been suggested that the advent of large scale farming operations has coincided with increasing incidence of human illnesses. New food processing and storage techniques such as vacuum packaging and increased shelf life due to improved preservation techniques allow for decreased perception of risks of spoilage and provide opportunities for certain potential pathogens to be given an environmental niche not previously available.

Changes in human demographics occurring over the last several decades have likely increased the incidence of foodborne illness. There is an increase in the proportion of our population that is more susceptible to foodborne disease due to immunocompromise as a consequence of being very young (especially within lower socioeconomic classes), or because of aging, infection with HIV or from receiving immunosuppressive drugs associated with medical treatments.^{14,30} Additionally, it is becoming more common for children to attend daycare facilities and for the elderly to be housed in nursing homes, creating a greater potential for outbreaks due to a concentrated population of susceptible individuals.¹⁴ Altered behaviors and attitudes on the part of the consumer are involved particularly as a consequence of changing family roles of women and a decrease in food handling and preparation instruction in high schools^{14,30}. The globalization of the food industry made possible by refrigerated freighters has increased the risk of infectious agents entering countries^{14,30}.

Yersinia enterocolitica (Ye) is a foodborne pathogen estimated²⁰ to cause annually 3,000 to 20,000 cases of human disease in the United States. The most prevalent clinical signs in human beings (children are the most frequently affected) are abdominal pain and fever; diarrhea, nausea and vomiting may also occur. The disease can range in severity from a self limiting gastroenteritis to potentially fatal septicemia. Pseudoappendicular syndrome has been reported,^{8,53} resulting in unnecessary surgical interventions. Post-infection manifestations include reactive arthritis, erythema nodosum, and Reiter's Syndrome; auto-immune related disorders resulting from host defenses to Ye. Grave's disease (hyperthyroidism) may also be a complication associated with yersiniosis. People afflicted with Grave's disease often have high serum titers to Ye.¹¹ More recently,^{2,3,61} it is apparent that human beings can be asymptomatic but bacteremic because clinically normal blood donors have supplied Ye infected blood that resulted in fatal septicemias in the transfused patients. In these episodes, four of the six donors reported a diarrheal illness within 30 days of donation.³ While the incidence of human disease attributable to *Yersinia enterocolitica* in the U.S. is less than other major microbiological foodborne disease agents, certain biological characteristics of *Yersinia enterocolitica* in association with changing human population demographics and behaviors strongly suggest *Yersinia enterocolitica* as an emerging microbial threat.

Ye is very tolerant to low temperatures and can continue to grow at temperatures as low as 10°C.³⁵ Ye have been isolated from vacuum packaged meats²² and can survive for extended periods in frozen food, even with repeated freezing and thawing.⁵⁹

Of concern is the shifting of the predominant serotypes of Ye associated with human disease in the United States from serotype O:8 and O:5,²⁷ to serotype O:3.^{39,40} In Belgium, Canada, Japan, and other countries where *Yersinia enterocolitica* is a primary foodborne pathogen rivaling Salmonella, Shigella, and Campylobacter, Ye serotype O:3 is the predominant serotype.^{4-6,43,50,55,58,60} Yersiniosis caused by serotype O:3 has been identified as an emerging disease in U.S. children⁴⁰ and in indigent populations.⁸

Despite the fact that *Yersinia enterocolitica* can be isolated from soil and water^{31,32,52,56}, flies²¹ and many species of mammals and birds²⁰ those causing human illness. Swine are the only species from which Ye strains pathogenic to humans have been frequently isolated.^{6,7,9,10,12,15-18,23,24,27,28,36,37,44-46,48,49,51,54,62} In Belgium⁵⁷ and the United States^{1,39}, consumption of improperly prepared pork products has been directly related to outbreaks of human yersiniosis. Human and porcine Ye isolates have been demonstrated³³ to harbor virulence plasmids and have chromosomal restriction endonuclease analysis patterns that are identical.

3. OBJECTIVES

In order to be able to address the potential emerging threat of Ye as a primary foodborne pathogen, baseline information is needed to conduct risk assessment of the U.S. swine population as a reservoir for human yersiniosis infection. The goal of this study was to identify the prevalence of farms whose market pig population harbors pathogenic strains of *Yersinia enterocolitica*. This data can then be applied to investigations of the epidemiology of Ye on farms and indeed throughout the food production cycle. This epidemiological information may lead to the ability to identify control points that can be implemented in management programs to control transmission of Ye to humans from pork products.

4. PROCEDURES

Sampling Procedures

Oral-pharyngeal (tonsillar surface) swabbing was conducted at slaughter in order to sample numerous herds that would represent a wide geographic area. Swabbing was accomplished post-stunning and -exsanguination, but prior to scalding. The number of animals as well as number of lots selected for sampling was based on calculations to establish appropriate statistical power. Previous U.S. studies^{18,24,39} estimated a 50% prevalence rate of Ye from pork products. Based on this estimation, 96 lots were the sample size targeted. The average farm size in the Illinois region of the Midwest is approximately 500 sows²⁹, with the number of finishing pigs on inventory estimated at approximately 4400. Assuming a prevalence of 50% within the finishing population, 45

animals per lot were targeted for sampling. To facilitate specimen handling at the laboratory, six sampling periods were scheduled. For each period random lots of pigs were selected for sampling using random numbers generated by a computer program (Epistat, T.L. Gustafson, Richardson, TX).

At the time of sampling, the lot number, other lot identification, number of pigs in the lot, average weight of pigs in the lot, time of weigh-in, and time sampled were recorded.

Specimen Collection

The oral cavity (including tonsillar area) was exposed using an oral speculum. Oral specula were disinfected between samplings with dilute Nolvasan (Fort Dodge Laboratories, Fort Dodge, IA) in hot water followed by two hot water rinses.

Specimen Handling/Isolation

Pharyngeal-tonsillar swabs were placed in Amies transport medium (Starswab, Starplex Scientific, Ontario, Canada) immediately after sampling. Swabs from each lot were placed in a plastic bag at ambient temperature, labeled with the lot number and sealed until the end of each collection visit. At the completion of sampling, swabs were inoculated into 10ml of cold phosphate buffered saline(PBS) and transported to the laboratory on ice. At the laboratory, PBS inocula were cold enriched at 4oC for two weeks. The PBS inocula were then subcultured onto Cefsulodin-Irgasan-novobiocin agar (CIN, Yersinia Selective Agar, Difco, Detroit, MI) and incubated at 25oC for 48 hours. Colonies that were morphologically characteristic of *Yersinia* species were selected for further identification.

Identification

Colonies selected from CIN agar were subcultured onto sheep blood agar (Remel, Lenexa, KS) and incubated in a microaerophilic environment at 35oC overnight. The isolate was then subcultured onto Kligler's Iron Agar (KIA) slants (Remel, Lenexa, KS) and incubated aerobically at 35oC. Isolates producing an alkaline slant with acid butt by 48 hours of incubation were then subcultured onto Christensen's Urea Agar slants (Remel, Lenexa, Kansas) and incubated at 35oC. An isolant that produced an alkaline reaction before 24 hours of incubation was identified as Ye.

All isolates were stored on Brain Heart Infusion agar slants (Remel, Lenexa, KS) at 25oC.

All colonies selected from CIN agar were evaluated for pathogenicity with a polymerase chain reaction assay (PCR). Primers to direct DNA amplification were selected based on previous work by Kwaga38 (5'-GAACTCGATGATAACTGGG-3') and Feng19 (5'-GGAGTATTCATATGAAGCGTC-3'). Primers were synthesized at the University of Illinois Genetic Engineering Facility. The expected product of amplification of the target sequence with these primers was 305 base pair long. To release chromosomal DNA from the bacteria, a crude lysate was prepared by boiling CIN isolates in 50 microliters (ul) of water for 5 minutes followed by plunging into an ice bath. Ten ul of the crude lysate were amplified in an 100ul reaction mixture containing 1X reaction buffer (50 mM KCl, 10mM Tris-hydrochloride [pH 8.3], 1.5mM MgCl₂); 200uM each dATP, dCTP, dGTP, dTTP(Perkin Elmer Cetus, Foster City, CA); 2.5U *Taq* polymerase (Life Technologies,

Gibco BRL, Gaithersburg, MD). The samples were subjected to 35 cycles in a GeneAmp PCR System 9600 (Perkin Elmer, Foster City, California). The parameters for the amplification cycles were as follows: denaturation for 1 minute at 97°C for the first three cycles, followed by denaturation at 94°C, annealing of primers for 1 minute at 65°C, and primer extension for 1 minute at 72°C. A negative (#9271) and positive (#8081C) control (provided by Dr. V.L. Miller, University of California, Los Angeles) were included with each test run. The PCR amplified products were analyzed by agarose gel electrophoresis and visualized by staining with ethidium bromide and exposure to ultraviolet light.⁴¹

All isolates that were *ail* positive by PCR were verified as Ye using the API20E System (bioMérieux Vitek, Inc., Hazelwood, MO).

Isolates identified as pathogenic (*ail* harboring) were further identified by serotyping using a slide agglutination technique against anti-sera for antigens O:3, O:5, O:8, and O:9 (Denka Seiken Co., Ltd., Tokyo, Japan).

5. RESULTS

One hundred three lots and 3,375 individual pigs were surveyed for carriage of pathogenic *Yersinia enterocolitica*. Of these, 95 lots (92.2%) were found to contain pigs carrying *Yersinia enterocolitica*. In twenty-nine lots (29/103 or 28.2%), at least one pig was found to be harboring *Yersinia enterocolitica* considered to be pathogenic by presence of the *ail* gene.

There was a significant difference between collection periods in the isolation rate of pathogenic *Yersinia enterocolitica* (Figures 1 and 2). In Figure 1, the proportion of lots that were positive for pathogenic *Yersinia enterocolitica* varied from 0% in collections 3 and 4 to 92% in collection 2. In Figure 2, collection two is shown to have a much higher percentage of pathogenic *Yersinia enterocolitica* per lot than the other five collection periods.

The isolation rate of pathogenic *Yersinia enterocolitica* per lot varied widely, ranging from 0 to 11 isolates per lot (0-45.8% of pigs sampled per lot) with nearly half of positive lots (14/29 or 48.1%) having one or two pathogenic isolates.

The majority of pathogenic isolates were identified serotype O:5 (96/107 or 89.7%), with 3.7% serotype O:3 (4/107). None of the lot characteristics recorded at slaughter (average pig weight, time between weigh-in and slaughter, distance travelled to slaughter, or farm or buying station of origin) were found to be significantly associated with infection with pathogenic *Yersinia enterocolitica*.

The results support the hypothesis that pigs are a significant potential reservoir for human infections by *Yersinia enterocolitica*. The lack of association of transportation and handling differences with carriage of pathogenic *Yersinia enterocolitica* among herds may indicate that on-farm management practices could be potentially involved with *Yersinia enterocolitica* infection. The results from this study will aid the design of future

epidemiological investigations concerning risk factors for pathogenic *Yersinia enterocolitica* carriage in swine.

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