

THE EPIDEMIOLOGY AND PUBLIC HEALTH IMPLICATIONS OF
SALMONELLOSIS IN DAIRY CATTLE

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Salmonella enterica is an important zoonotic pathogen that is estimated to cause approximately 94 million illnesses and 155,000 deaths annually throughout the world. Dairy cattle are considered a key source of several *Salmonella* serovars that cause human disease, and transmission may occur via either foodborne exposure or direct contact with infected cattle. Several previous studies have described the prevalence of fecal *Salmonella* shedding among asymptomatic dairy cattle, using either cross-sectional or longitudinal designs. However, there is very little in the literature regarding the epidemiology of clinical disease due to *Salmonella* infection (salmonellosis) in cattle. This dissertation has addressed this gap, both in the field and in a hospital setting, and has emphasized the relevance to public health. The research included herein has shed light on a number of facets of *Salmonella* epidemiology in dairy cattle, including (1) incidence of salmonellosis in the northeastern United States, along with the serovars and antimicrobial resistance patterns of isolates shed by cattle with clinical illness; (2) prevalence and risk factors for fecal *Salmonella* shedding among cattle presenting to a teaching hospital; (3) duration of *Salmonella* shedding following clinical disease in cattle; (4) prevalence of *Salmonella* shedding among dairy herds with clinical vs. subclinical infections; and (5) emergence of a previously sporadic pathogen (*Salmonella* Cerro) as a presumptive cause of widespread clinical disease in New York. This work supports the view that dairy cattle with salmonellosis present a greater threat to public health than cattle that are asymptotically shedding

Salmonella organisms; efforts to reduce this threat may thus be facilitated by clinical disease surveillance in dairy cattle populations.

BIOGRAPHICAL SKETCH

Kevin James Cummings was born on September 30, 1970 in Buffalo, New York. He knew by the tender age of four that he wanted to become a veterinarian, primarily as a result of growing up with dogs as an integral part of his childhood, having a remarkable fascination with birds and other wildlife, and being influenced by his animal-loving parents, James and Cathleen. He worked hard in school, played sports as often as he could, and ended up at Kenyon College in Gambier, Ohio. There he got his first taste of research and majored in his favorite subject, biology. He received his Bachelor of Arts with High Honors in 1992. From there, it was off to the College of Veterinary Medicine at Cornell University in Ithaca, New York. Kevin thoroughly enjoyed his years as a veterinary student and also discovered the immense joy of adopting greyhounds. While at Cornell, he had the incredibly good fortune of meeting his future wife, Jodi Korich in the class of 1997, just a few months before receiving his DVM in 1996. After spending a few years in private clinical practice in Virginia, Kevin and Jodi returned to Cornell's College of Veterinary Medicine in 2002 as instructors. Kevin identified his passion for teaching at this point, and his long-term interest in infectious disease epidemiology was soon molded into a focus on veterinary public health. Thus, he decided to assume the life of a student again, this time with the goal of receiving a PhD in epidemiology. It is Kevin's steadfast goal to pursue an academic career as a veterinary epidemiologist, with particular emphasis on the epidemiology of foodborne pathogens.

This dissertation is dedicated to my wife and parents.

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CHAPTER 1: Introduction

Introduction to *Salmonella*

Salmonella is a genus of Gram-negative, facultative anaerobic, rod-shaped bacteria belonging to the family Enterobacteriaceae. The most important species, *Salmonella enterica*, is further divided into six subspecies. Serovars of medical significance belong to *S. enterica* subsp. *enterica*, and most have been given a name (e.g. *Salmonella enterica* subsp. *enterica* serovar Newport, typically abbreviated as *Salmonella* Newport). Over 2,500 *Salmonella* serovars have been identified to date, but relatively few are responsible for a large proportion of clinical infections (Jones *et al.*, 2008). For example, preliminary CDC FoodNet data from 2008 show that 10 serovars accounted for 73% of the laboratory-confirmed cases of human salmonellosis, based on surveillance in 10 states (Centers for Disease Control and Prevention (CDC), 2009c). Though many serovars are capable of causing clinical disease in a wide range of host species (e.g. Enteritidis, Typhimurium, and Newport), others are almost exclusively associated with a single host species (host-restricted serovars, such as Abortusovis in sheep) or have a predilection for a particular host species but can also cause disease in other hosts (host-adapted serovars, such as Dublin in cattle) (Uzzau *et al.*, 2000). *Salmonella* occurs worldwide and is a leading cause of acute bacterial gastroenteritis in people. The prevalence of multidrug resistance among *Salmonella* isolates has increased over the past two decades, causing an increase in treatment failures and hospitalization rates (Helms *et al.*, 2002; Helms *et al.*, 2004; Varma *et al.*, 2005; Varma *et al.*, 2005).

Domestic and wild animals are the natural reservoir for all *Salmonella* serovars except for Typhi and Paratyphi, thus highlighting the zoonotic potential of this pathogen. *Salmonella* can be found in the gastrointestinal tract of a wide range of species, often without evidence of clinical disease: cattle, horses, pigs, dogs, cats,

rodents, poultry, wild birds, reptiles, amphibians, and fish (Sanchez *et al.*, 2002). Prevalence estimates among healthy cattle in the U.S. range from 5-14% (Wells *et al.*, 2001; Huston *et al.*, 2002; Blau *et al.*, 2005; Centers for Epidemiology and Animal Health, 2009). Estimates for healthy dogs (1-36%) and cats (1-18%) vary widely, but the prevalence may be as high as 50% in group-housed cats (Sanchez *et al.*, 2002; Hill *et al.*, 2000; Hackett and Lappin, 2003; Van Immerseel *et al.*, 2004; Sokolow *et al.*, 2005). Poultry are considered a principal reservoir of *Salmonella*, with the prevalence among houses of laying hens throughout the U.S. estimated at 86% (Ebel *et al.*, 1992). Reptiles are another well-known source of salmonellosis, as the prevalence of asymptomatic fecal *Salmonella* shedding approaches 90% (Chiodini and Sundberg, 1981). *Salmonella* can also survive in the environment for prolonged periods of time following fecal shedding.

Salmonella is transmitted primarily via the fecal-oral route, and cases may be sporadic or outbreak-associated. It has been estimated that up to 95% of human infections are the result of foodborne exposure (Mead *et al.*, 1999). This may occur when there is fecal contamination of foods derived from infected animals (raw/undercooked eggs, poultry, beef, pork, milk) or alternatively when there is fecal contamination of crops. A number of large, multistate outbreaks over the last decade have been traced to a diverse array of food products, including ground beef (CDC, 2002; CDC, 2006), unpasteurized milk (CDC, 2003), frozen poultry pot pies (CDC, 2008a), peppers (CDC, 2008b), tomatoes (CDC, 2007b), cantaloupes, peanut butter (CDC, 2007a; CDC, 2009a), and cereal. *Salmonella* transmission can also occur by direct contact with the feces of an infected animal. Farmers, veterinarians, pet owners, and those who interact with animals in public settings (such as open farms, petting zoos, and county/state fairs) are all at risk for salmonellosis through direct exposure (CDC, 2001; CDC, 2005; CDC, 2007c; CDC, 2009b; CDC, 2010a; CDC, 2010b).

Person-to-person spread by the fecal-oral route is also possible, particularly when diarrhea is present.

Infections in people may be asymptomatic or result in acute disease ranging from self-limiting to fatal. *Salmonella* causes an estimated 1.4 million illnesses, 16,000 hospitalizations, and between 400 and 600 deaths annually in the United States alone (Mead *et al.*, 1999; Voetsch *et al.*, 2004). Worldwide, it was recently estimated that *Salmonella* results in 93.8 million cases of gastroenteritis and 155,000 deaths annually (Majowicz *et al.*, 2010). The incubation period is 6-72 hours, and symptoms include diarrhea, fever, anorexia, dehydration, headache, abdominal pain, nausea, vomiting, and malaise (Heymann, 2008). Clinical disease generally resolves within 3-7 days. However, *Salmonella* can also produce invasive infections that lead to sepsis and in some cases death. Bacteremia can occur and lead to localized infections including osteomyelitis, septic arthritis, endocarditis, meningitis, cholecystitis, pyelonephritis, and pneumonia (Heymann, 2008). Young children, the elderly, and immunocompromised individuals are especially susceptible to severe disease. Preliminary CDC FoodNet data from 2008 show that *Salmonella* accounted for 40% of all laboratory-confirmed cases of infections caused by enteric pathogens typically transmitted through food; the three most common serovars were Enteritidis (20% of the *Salmonella* isolates), Typhimurium (16%), and Newport (10%) (CDC, 2009c). The fact that the estimated incidence of *Salmonella* infections has not changed significantly compared with the preceding three years (CDC, 2009c) underscores the need for further advancements in food safety. Disease occurrence appears to be affected by seasonal factors, as the peak incidence of human salmonellosis has been reported by numerous sources to be during the summer months (D'Souza *et al.*, 2004; Gradel *et al.*, 2007; Naumova *et al.*, 2007; Oloya *et al.*, 2007). This is often blamed on such factors as hygiene issues associated with outdoor cooking, increased

recreational water use, and a greater tendency to travel abroad. However, fecal *Salmonella* shedding and salmonellosis among veterinary species have also been found to reach a peak during the summer and early fall (Wells *et al.*, 2001; Smith *et al.*, 1978; Carter *et al.*, 1986; Kabagambe *et al.*, 2000; Fossler *et al.*, 2005; Pangloli *et al.*, 2008). This seasonal association may be related to temperature and/or moisture conditions that prevail in the warmer months, but whether these conditions are impacting the bacteria or the host species is unclear.

The role of dairy cattle

Dairy cattle are considered a key source of several *Salmonella* serovars that are a threat to human health, including multidrug-resistant (MDR) *S.* Newport and *S.* Typhimurium (Gupta *et al.*, 2003; Dechet *et al.*, 2006; Varma *et al.*, 2006; Karon *et al.*, 2007). Fecal contamination of beef carcasses at the time of slaughter is thought to represent the predominant source of foodborne transmission. According to the 1996 USDA National Animal Health Monitoring System (NAHMS) Dairy report, 14.9% of culled dairy cows were shedding *Salmonella* at livestock markets, and 66.0% of markets had at least one cow shedding *Salmonella* (Wells *et al.*, 2001).

Contamination of crops, either by manure used as fertilizer or by irrigation water that has been contaminated by manure run-off, is another important source of transmission (Islam *et al.*, 2004; Sivapalasingam *et al.*, 2004). Milk and other dairy products pose less of a public health threat because of commercial pasteurization, although some subsets of the population continue to consume dairy products that are not pasteurized. Those who work or otherwise interact with livestock are also at risk of infection via direct exposure when cattle are shedding *Salmonella*. Reported risk factors for MDR

S. Newport infections in people include direct exposure to a dairy farm (Gupta *et al.*, 2003; Karon *et al.*, 2007), contact with dairy cattle (Karon *et al.*, 2007), consumption of poorly cooked ground beef (Varma *et al.*, 2006), and consumption of unpasteurized dairy products (Karon *et al.*, 2007). Consumption of poorly cooked ground beef and unpasteurized dairy products have also been found to be risk factors for MDR *S. Typhimurium* infections in people (Dechet *et al.*, 2006; Cody *et al.*, 1999; Villar *et al.*, 1999). Numerous multistate outbreaks over the last several years have been linked to cattle, including a 2002 outbreak of MDR *S. Newport* involving five northeastern states (undercooked ground beef) (CDC, 2002), a 2002-2003 outbreak of *S. Typhimurium* involving four midwestern states (unpasteurized milk) (CDC, 2003), a 2003-2004 outbreak of MDR *S. Typhimurium* involving nine northeastern states (undercooked ground beef) (Dechet *et al.* 2006), and a 2004 outbreak of *S. Typhimurium* involving nine states and the District of Columbia (undercooked ground beef) (CDC, 2006).

Introduction of *Salmonella* onto a dairy farm can occur through a variety of routes, including purchased cattle, contaminated feed or water, wild animals such as rodents and birds, human traffic, and insects (Sanchez *et al.*, 2002; Bender, 1994; Evans and Davies, 1996; Nielsen *et al.*, 2007). Clinical signs of bovine salmonellosis may include diarrhea, fever, anorexia, dehydration, decreased milk production, abortion, and evidence of endotoxemia, although many infections remain asymptomatic (Divers and Peek, 2008). Infected cattle can shed copious numbers of *Salmonella* organisms in their feces; the concentration of *Salmonella* within the manure of an infected cow ranges from 10^2 to 10^7 organisms per gram of feces (You *et al.*, 2006). Considering that adult dairy cattle generate approximately 70 kg of manure per day (You *et al.*, 2006), this translates into a daily environmental contamination of between 7×10^6 and 7×10^{11} *Salmonella* organisms. Widespread contamination of the

dairy farm environment can thus result from *Salmonella* shedding, and the organism can survive for prolonged periods in suitable conditions outside a host (You *et al.*, 2006; Wray and Wray, 2000). Fecal *Salmonella* shedding can also augment the risk of within-herd transmission and inadvertent spread to other herds. In addition to impacting the health and productivity of dairy cattle, these factors lead to an increased risk of zoonotic transmission.

Dissertation objectives

Although the prevalence of fecal *Salmonella* shedding among asymptomatic dairy cattle has been estimated in a number of studies (Wells *et al.*, 2001; Huston *et al.*, 2002; Blau *et al.*, 2005; Fossler *et al.*, 2004), the epidemiology of clinical outbreaks of bovine salmonellosis and the associated public health implications are not well understood. The overarching goal of this dissertation was to address this gap by meeting the following broad objectives:

- 1) Estimate the animal- and herd-level incidence of salmonellosis among dairy herds in the northeastern United States,
- 2) Describe the serovars and antimicrobial resistance profiles of isolates obtained from incident cases,
- 3) Determine the duration of fecal *Salmonella* shedding among dairy cattle following laboratory-confirmed clinical disease,
- 4) Estimate the prevalence of fecal *Salmonella* shedding among bovine patients at a veterinary medical teaching hospital,
- 5) Identify risk factors for *Salmonella* shedding and hospital contamination within the population of bovine patients,

- 6) Characterize the serovars of isolates obtained from hospitalized cattle,
- 7) Determine the effect of clinical outbreaks of salmonellosis on the prevalence of asymptomatic fecal *Salmonella* shedding within dairy herds in New York, and
- 8) Describe the serovars and antimicrobial resistance profiles of isolates obtained from dairy herds with clinical vs. subclinical infections.

REFERENCES

Bender JB. Reducing the risk of *Salmonella* spread and practical control measures in dairy herds. *Bovine Pract* 1994; 28:62-5.

Blau DM, McCluskey BJ, Ladely SR, *et al.* *Salmonella* in dairy operations in the United States: prevalence and antimicrobial drug susceptibility. *J Food Prot* 2005; 68:696-702.

Carter JD, Hird DW, Farver TB, *et al.* Salmonellosis in hospitalized horses: seasonality and case fatality rates. *J Am Vet Med Assoc* 1986; 188:163-7.

Centers for Disease Control and Prevention (CDC). Multistate outbreak of human *Salmonella* Typhimurium infections associated with aquatic frogs - United States, 2009. *MMWR Morb Mortal Wkly Rep* 2010a; 58:1433-6.

Centers for Disease Control and Prevention (CDC). Multistate outbreak of human *Salmonella* Typhimurium infections associated with pet turtle exposure --- United States, 2008. *MMWR Morb Mortal Wkly Rep* 2010b; 59:191-6.

Centers for Disease Control and Prevention (CDC). Multistate outbreak of *Salmonella* infections associated with peanut butter and peanut butter-containing products--United States, 2008-2009. *MMWR Morb Mortal Wkly Rep* 2009a; 58:85-90.

Centers for Disease Control and Prevention (CDC). Multistate outbreaks of *Salmonella* infections associated with live poultry--United States, 2007. *MMWR Morb Mortal Wkly Rep* 2009b; 58:25-9.

Centers for Disease Control and Prevention (CDC). Preliminary FoodNet Data on the incidence of infection with pathogens transmitted commonly through food--10 States, 2008. *MMWR Morb Mortal Wkly Rep* 2009c; 58:333-7.

Centers for Disease Control and Prevention (CDC). Multistate outbreak of *Salmonella* infections associated with frozen pot pies--United States, 2007. *MMWR Morb Mortal Wkly Rep* 2008a; 57:1277-80.

Centers for Disease Control and Prevention (CDC). Outbreak of *Salmonella* serotype Saintpaul infections associated with multiple raw produce items--United States, 2008. *MMWR Morb Mortal Wkly Rep* 2008b; 57:929-34.

Centers for Disease Control and Prevention (CDC). Multistate outbreak of *Salmonella* serotype Tennessee infections associated with peanut butter--United States, 2006-2007. MMWR Morb Mortal Wkly Rep 2007a; 56:521-4.

Centers for Disease Control and Prevention (CDC). Multistate outbreaks of *Salmonella* infections associated with raw tomatoes eaten in restaurants--United States, 2005-2006. MMWR Morb Mortal Wkly Rep 2007b; 56:909-11.

Centers for Disease Control and Prevention (CDC). Three outbreaks of salmonellosis associated with baby poultry from three hatcheries--United States, 2006. MMWR Morb Mortal Wkly Rep 2007c; 56:273-6.

Centers for Disease Control and Prevention (CDC). Multistate outbreak of *Salmonella* Typhimurium infections associated with eating ground beef--United States, 2004. MMWR Morb Mortal Wkly Rep 2006; 55:180-2.

Centers for Disease Control and Prevention (CDC). Outbreak of multidrug-resistant *Salmonella* Typhimurium associated with rodents purchased at retail pet stores--United States, December 2003-October 2004. MMWR Morb Mortal Wkly Rep 2005; 54:429-33.

Centers for Disease Control and Prevention (CDC). Multistate outbreak of *Salmonella* serotype Typhimurium infections associated with drinking unpasteurized milk--Illinois, Indiana, Ohio, and Tennessee, 2002-2003. MMWR Morb Mortal Wkly Rep 2003; 52:613-5.

Centers for Disease Control and Prevention (CDC). Outbreak of multidrug-resistant *Salmonella* Newport--United States, January-April 2002. MMWR Morb Mortal Wkly Rep 2002; 51:545-8.

Centers for Disease Control and Prevention (CDC). Outbreaks of multidrug-resistant *Salmonella* Typhimurium associated with veterinary facilities--Idaho, Minnesota, and Washington, 1999. MMWR Morb Mortal Wkly Rep 2001; 50:701-4.

Centers for Epidemiology and Animal Health. National Animal Health Monitoring System (NAHMS) Dairy 2007: *Salmonella* and *Campylobacter* on U.S. Dairy Operations, 1996-2007. United States Department of Agriculture, Fort Collins, CO. 2009.

Chiodini RJ and Sundberg JP. Salmonellosis in reptiles: a review. Am J Epidemiol 1981; 113:494-9.

- Cody SH, Abbott SL, Marfin AA, *et al.* Two outbreaks of multidrug-resistant *Salmonella* serotype Typhimurium DT104 infections linked to raw-milk cheese in Northern California. *JAMA* 1999; 281:1805-10.
- Dechet AM, Scallan E, Gensheimer K, *et al.* Outbreak of multidrug-resistant *Salmonella enterica* serotype Typhimurium Definitive Type 104 infection linked to commercial ground beef, northeastern United States, 2003-2004. *Clin Infect Dis* 2006; 42:747-52.
- Divers TJ and Peek SF. *Rebhun's diseases of dairy cattle*. St. Louis: Saunders Elsevier, 2008.
- D'Souza RM, Becker NG, Hall G, *et al.* Does ambient temperature affect foodborne disease? *Epidemiology* 2004; 15:86-92.
- Ebel ED, David MJ, and Mason J. Occurrence of *Salmonella* Enteritidis in the U.S. commercial egg industry: report on a national spent hen survey. *Avian Dis* 1992; 36:646-54.
- Evans S and Davies R. Case control study of multiple-resistant *Salmonella* Typhimurium DT104 infection of cattle in Great Britain. *Vet Rec* 1996; 139:557-558.
- Fossler CP, Wells SJ, Kaneene JB, *et al.* Prevalence of *Salmonella* spp on conventional and organic dairy farms. *J Am Vet Med Assoc* 2004; 225:567-573.
- Fossler CP, Wells SJ, Kaneene JB, *et al.* Cattle and environmental sample-level factors associated with the presence of *Salmonella* in a multi-state study of conventional and organic dairy farms. *Prev Vet Med* 2005; 67:39-53.
- Gradel KO, Dethlefsen C, Schonheyder HC, *et al.* Severity of infection and seasonal variation of non-typhoid *Salmonella* occurrence in humans. *Epidemiol Infect* 2007; 135:93-9.
- Gupta A, Fontana J, Crowe C, *et al.* Emergence of multidrug-resistant *Salmonella enterica* serotype Newport infections resistant to expanded-spectrum cephalosporins in the United States. *J Infect Dis* 2003; 188:1707-1716.
- Hackett T and Lappin MR. Prevalence of enteric pathogens in dogs of north-central Colorado. *J Am Anim Hosp Assoc* 2003; 39:52-6.

Helms M, Simonsen J, Molbak K. Quinolone resistance is associated with increased risk of invasive illness or death during infection with *Salmonella* serotype Typhimurium. *J Infect Dis* 2004; 190:1652-1654.

Helms M, Vastrup P, Gerner-Smidt P, *et al.* Excess mortality associated with antimicrobial drug-resistant *Salmonella* Typhimurium. *Emerg Infect Dis* 2002; 8:490-495.

Heymann DL. *Control of Communicable Diseases Manual*. Washington, DC: American Public Health Association, 2008.

Hill SL, Cheney JM, Taton-Allen GF, *et al.* Prevalence of enteric zoonotic organisms in cats. *J Am Vet Med Assoc* 2000; 216:687-92.

Huston CL, Wittum TE, Love BC, *et al.* Prevalence of fecal shedding of *Salmonella* spp in dairy herds. *J Am Vet Med Assoc* 2002; 220:645-649.

Islam M, Morgan J, Doyle MP, *et al.* Persistence of *Salmonella enterica* serovar Typhimurium on lettuce and parsley and in soils on which they were grown in fields treated with contaminated manure composts or irrigation water. *Foodborne Pathog Dis* 2004; 1:27-35.

Jones TF, Ingram LA, Cieslak PR, *et al.* Salmonellosis outcomes differ substantially by serotype. *J Infect Dis* 2008; 198:109-14.

Kabagambe EK, Wells SJ, Garber LP, *et al.* Risk factors for fecal shedding of *Salmonella* in 91 US dairy herds in 1996. *Prev Vet Med* 2000; 43:177-194.

Karon AE, Archer JR, Sotir MJ, *et al.* Human multidrug-resistant *Salmonella* Newport infections, Wisconsin, 2003-2005. *Emerg Infect Dis* 2007; 13:1777-1780.

Majowicz SE, Musto J, Scallan E, *et al.* The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clin Infect Dis* 2010; 50:882-9.

Mead PS, Slutsker L, Dietz V, *et al.* Food-related illness and death in the United States. *Emerg Infect Dis* 1999; 5:607-625.

Naumova EN, Jagai JS, Matyas B, *et al.* Seasonality in six enterically transmitted diseases and ambient temperature. *Epidemiol Infect* 2007; 135:281-92.

Nielsen LR, Warnick LD, Greiner M. Risk factors for changing test classification in the Danish surveillance program for *Salmonella* in dairy herds. J Dairy Sci 2007; 90:2815-2825.

Oloya J, Theis M, Doetkott D, *et al.* Evaluation of *Salmonella* occurrence in domestic animals and humans in North Dakota (2000-2005). Foodborne Pathog Dis 2007; 4:551-63.

Pangloli P, Dje Y, Ahmed O, *et al.* Seasonal incidence and molecular characterization of *Salmonella* from dairy cows, calves, and farm environment. Foodborne Pathog Dis 2008; 5: 87-96.

Sanchez S, Hofacre CL, Lee MD, *et al.* Animal sources of salmonellosis in humans. J Am Vet Med Assoc 2002; 221:492-497.

Sivapalasingam S, Friedman CR, Cohen L, *et al.* Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. J Food Prot 2004; 67:2342-2353.

Smith BP, Reina-Guerra M, and Hardy AJ. Prevalence and epizootiology of equine salmonellosis. J Am Vet Med Assoc 1978; 172: 353-356.

Sokolow SH, Rand C, Marks SL, *et al.* Epidemiologic evaluation of diarrhea in dogs in an animal shelter. Am J Vet Res 2005; 66:1018-24.

Uzzau S, Brown DJ, Wallis T, *et al.* Host adapted serotypes of *Salmonella enterica*. Epidemiol Infect 2000; 125:229-55.

Van Immerseel F, Pasmans F, De Buck J, *et al.* Cats as a risk for transmission of antimicrobial drug-resistant *Salmonella*. Emerg Infect Dis 2004; 10:2169-74.

Varma JK, Greene KD, Ovitt J, *et al.* Hospitalization and antimicrobial resistance in *Salmonella* outbreaks, 1984-2002. Emerg Infect Dis 2005a; 11:943-946.

Varma JK, Marcus R, Stenzel SA, *et al.* Highly resistant *Salmonella* Newport-MDRampC transmitted through the domestic US food supply: a FoodNet case-control study of sporadic *Salmonella* Newport infections, 2002-2003. J Infect Dis 2006; 194:222-230.

Varma JK, Molbak K, Barrett TJ, *et al.* Antimicrobial-resistant nontyphoidal *Salmonella* is associated with excess bloodstream infections and hospitalizations. *J Infect Dis* 2005b; 191:554-561.

Villar RG, Macek MD, Simons S, *et al.* Investigation of multidrug-resistant *Salmonella* serotype Typhimurium DT104 infections linked to raw-milk cheese in Washington State. *JAMA* 1999; 281:1811-6.

Voetsch AC, Van Gilder TJ, Angulo FJ, *et al.* FoodNet estimate of the burden of illness caused by nontyphoidal *Salmonella* infections in the United States. *Clin Infect Dis* 2004; 38 Suppl 3:S127-34.

Wells SJ, Fedorka-Cray PJ, Dargatz DA, *et al.* Fecal shedding of *Salmonella* spp. by dairy cows on farm and at cull cow markets. *J Food Prot* 2001; 64:3-11.

Wray C and Wray A. *Salmonella* in domestic animals. Oxford ; New York: CABI Pub., 2000.

You Y, Rankin SC, Aceto HW, *et al.* Survival of *Salmonella enterica* serovar Newport in manure and manure-amended soils. *Appl Environ Microbiol* 2006; 72:5777-5783.

CHAPTER 2: The incidence of salmonellosis among dairy herds in the northeastern
United States*

*Cummings KJ, Warnick LD, Alexander KA, Cripps CJ, Gröhn YT, McDonough PL, Nydam DV, Reed KE. The incidence of salmonellosis among dairy herds in the northeastern United States. *Journal of Dairy Science* 2009; 92: 3766–3774.

ABSTRACT

The objectives of this study were to estimate the incidence of salmonellosis among a large sample of dairy herds in the northeastern United States (both at the animal level and the herd level), to describe the serotypes and antimicrobial resistance profiles of the positive samples, and to determine whether various herd-level factors were important predictors of incidence. Participating veterinarians enrolled 831 dairy herds and submitted fecal samples from 2,565 female dairy cattle for *Salmonella* culture due to suspicion of clinical disease. Estimates of animal-level incidence rates were calculated for each age group as the number of cases per animal time at risk, and an estimate of herd-level incidence rate was calculated as the number of positive herds per herd time at risk. Descriptive analysis of serotype data and level of antimicrobial resistance was performed, and Poisson regression analysis was used to study associations between the within-herd incidence of salmonellosis and certain predictor variables (herd size, housing type, vaccination status, and prior history of *Salmonella* infection). *Salmonella* was isolated from 576 (22.5%) samples representing 93 herds. The animal-level incidence rates for pre-weaned female calves, heifers, and adult cows were 8.1, 0.04, and 1.8 cases per 1,000 animal-years, respectively. The herd-level incidence rate was 8.6 positive herds per 100 herd-years. *S. Newport* was the predominant serotype, accounting for 41% of the cases, followed by *S. Typhimurium*. Over 68% of all isolates were resistant to five or more antimicrobial agents. Herd size was the only significant predictor of the incidence of salmonellosis in a multivariable model; herds with at least 400 female dairy cattle had a higher incidence rate than smaller herds. Our results shed light on the impact of salmonellosis on the dairy industry in the northeastern U.S., and they help clarify the role of dairy cattle as a source of *Salmonella* serotypes which are also important human pathogens.

INTRODUCTION

Salmonella is a zoonotic enteric pathogen that can cause significant disease in both calves and adult cattle. Clinical signs of bovine salmonellosis may include diarrhea, fever, anorexia, dehydration, decreased milk production, abortion, and evidence of endotoxemia, although many infections remain subclinical (Divers and Peek, 2008). It can be a costly disease for dairy producers on account of treatment expenses, mortality, reduced milk yield, and weight loss/decreased weight gain within the herd (Peters, 1985; Huston *et al.*, 2002). *Salmonella* also poses a considerable threat to public health, resulting in approximately 1.4 million illnesses, 16,000 hospitalizations, and between 400 and 600 deaths annually in the U.S. alone (Mead *et al.*, 1999; Voetsch *et al.*, 2004).

Infected cattle can shed the organism while ill and following clinical recovery, and asymptomatic shedders never show signs at all. Widespread environmental contamination can result from *Salmonella* shedding, and the organism can survive for prolonged periods in suitable conditions outside a host (Wray and Wray, 2000). Fecal *Salmonella* shedding can also augment the risk of within-herd transmission and inadvertent spread to other herds. In addition to having implications for the health and productivity of dairy cattle, these factors lead to an increased risk of zoonotic transmission. Foodborne exposure may occur when there is fecal contamination of beef carcasses at slaughter, contamination of crops by manure fertilizer, and contamination of water by manure run-off (Wells *et al.*, 2001; Islam *et al.*, 2004; Sivapalasingam *et al.*, 2004). People can also become infected via direct contact; those who work or otherwise interact with livestock are at particular risk when cattle are shedding *Salmonella*. Fecal shedding often persists well beyond the clinical

outbreak in the herd (Clegg *et al.*, 1983; Giles *et al.*, 1989; Gay and Hunsaker, 1993), underscoring the difficulty in recognizing high-risk herds.

Introduction of *Salmonella* onto a dairy farm can occur through a variety of routes, including purchased cattle, contaminated feed or water, wild animals such as rodents and birds, and human traffic (Bender, 1994; Evans and Davies, 1996; Sanchez *et al.*, 2002; Nielsen *et al.*, 2007). Therefore, the presence of *Salmonella* on a farm is not an unexpected finding. In fact, one study involving 110 dairy farms in four states found that over 90% of the farms had at least one *Salmonella*-positive culture obtained (fecal and/or environmental) during the course of five sampling visits over a one year period (Fossler *et al.*, 2004). The USDA National Animal Health Monitoring System (NAHMS) Dairy 2002 study, based on a single sampling visit to five herds in each of 21 states, found that 31% of herds yielded at least one *Salmonella*-positive fecal culture (Blau *et al.*, 2005).

Although a number of studies have examined the prevalence of fecal *Salmonella* shedding on dairy farms (Wells *et al.*, 2001; Huston *et al.*, 2002; Fossler *et al.*, 2004; Blau *et al.*, 2005), there is very little information available regarding the occurrence of clinical disease associated with *Salmonella* infections in cattle. Knowledge of the distribution and frequency of salmonellosis, both among herds and among age groups within a herd, would be important for producers and veterinarians alike. An understanding of the full impact of *Salmonella* on the health of dairy cattle could lead to more focused strategies for preventing the introduction and spread of this pathogen on the farm, with obvious public health benefits as well. Furthermore, information regarding which serotypes are typically associated with clinical disease in cattle would shed light on the risk posed by certain types of *Salmonella* on the dairy farm; this would also add to our collective knowledge of the role played by dairy cattle as a potential source of *Salmonella* infection for people. It would be especially helpful

to gain information on whether clinically affected cattle are shedding the serotypes that are also important human pathogens, such as Newport and Typhimurium, as this could facilitate the recognition of herds that present the greatest threat to public health. Thus, the objectives of this study were to estimate the animal- and herd-level incidence of salmonellosis among a large sample of dairy herds in the northeastern U.S., to describe the serotypes and antimicrobial resistance profiles of the positive samples, and to ascertain whether certain herd-level factors (herd size, housing type, vaccination status, and prior history of *Salmonella* infection) were important predictors of incidence.

MATERIALS AND METHODS

Study Design

Veterinary practices serving New York, Pennsylvania, Vermont, Massachusetts, and Connecticut were enrolled between February and September, 2004, with the goal of selecting practices that were known to provide clinical service to a large number of dairy herds. Practices were identified through our personal contacts and via an announcement of the study at a regional veterinary continuing education meeting. Participating veterinarians were asked to enroll all of their dairy herd clients with at least 30 dairy cattle for whom they provided routine clinical service. Veterinarians were instructed to educate their clients regarding the clinical signs of salmonellosis in cattle, including diarrhea with blood, mucus, or a foul odor, fever of at least 103°F (39.4°C), depression, and decreased appetite, as well as sudden death in the absence of specific clinical signs. Herd owners were asked to contact

their veterinarian if any of their cattle displayed these signs or if unusually high mortality among cattle with diarrhea was noted. At enrollment, veterinarians collected information on herd size, type of housing, and vaccination protocols. Throughout the duration of the study, project personnel would obtain updated information on numbers of cattle within each herd; the targeted interval between herd updates was three months.

Veterinarians submitted fecal samples from suspected clinical cases to the Animal Health Diagnostic Center (AHDC) at Cornell University for *Salmonella* culture, from the time of herd enrollment through September, 2005. In order to encourage herd owners and veterinarians to submit samples from every clinical suspect animal, all shipping and laboratory costs were covered by the study. In addition, no costs were incurred by herd owners for the testing of calves and weaned heifers for other enteric pathogens, if requested by their veterinarian. Herd owners were also allowed to submit *Salmonella* fecal culture samples from animals with compatible clinical signs, provided this was under the supervision of their veterinarian.

Sample Collection and Processing

Fecal samples were generally collected via rectal retrieval, with a new glove being used to collect each sample. Approximately 10 g of fecal matter was placed into a Para-Pak bottle (modified Cary-Blair medium; Meridian Bioscience Inc., Cincinnati, OH) and sealed. For small calves, a BBL culture swab (Amies medium; Becton Dickinson and Company, Franklin Lakes, NJ) was occasionally used for sample collection. All samples were either delivered by the participating veterinarian or shipped to the Animal Health Diagnostic Center for bacteriologic culture, and standard culture methods were used to isolate *Salmonella* from feces. Individual fecal swabs

from sample bottles were enriched in 10 ml of Tetrathionate broth (Difco, Detroit, MI) containing 0.2 ml of iodine solution; the mixture was incubated at 42°C for 18–24 hours. After incubation, the sample-broth mixture was streaked onto Brilliant Green with novobiocin (BGN; Becton Dickinson and Company, Franklin Lakes, NJ) and Xylose Lysine Tergitol 4 (XLT-4) selective media, and both plates were incubated at 37°C for 18–24 hours. Red bacterial colonies (lactose non-fermenting) on BGN and black colonies (H₂S-producing) on XLT-4 were inoculated into Kligler Iron Agar (KIA) slants and then incubated at 37°C for 18–24 hours. XLT-4 plates without suspected colonies were re-incubated at 37°C for an additional 18–24 hours before checking again for characteristic black colonies. Colonies on KIA slants which exhibited the biochemical properties of *Salmonella* were then serogrouped by slide agglutination using standard protocols. Those colonies that were positive by slide agglutination were then identified as *Salmonella* using the Sensititre Automated Microbiology System's A80 panel (TREK Diagnostic Systems Inc., Cleveland, OH). Confirmed *Salmonella* isolates were sent to the USDA National Veterinary Services Laboratories (NVSL) in Ames, Iowa for serotyping using standard protocols.

Antimicrobial susceptibility of *Salmonella* isolates was determined by use of the broth dilution method. Minimal inhibitory concentrations (MIC) were established for each isolate against a panel of 11 antimicrobial agents (ampicillin, ceftiofur, chlortetracycline, enrofloxacin, florfenicol, gentamicin, neomycin, oxytetracycline, spectinomycin, sulfadimethoxine, and trimethoprim/sulfamethoxazole; Sensititre, TREK Diagnostic Systems Inc.). Clinical and Laboratory Standards Institute (CLSI) guidelines were used to interpret MIC values when available (CLSI, 2008). Isolates were classified as being resistant or susceptible to each agent; those isolates with intermediate susceptibility were categorized as being susceptible. Quality control was performed weekly using four strains of bacteria: *Escherichia coli* ATCC 25922,

Staphylococcus aureus 29213, *Enterococcus faecalis* 29212, and *Pseudomonas aeruginosa* 27853. The MIC ranges for quality control recommended by the CLSI were used, and results were accepted if the MIC values were within expected ranges for these bacterial strains.

Data Analysis

All animals were classified as pre-weaned female calves, pre-weaned male calves, heifers (from weaning age, i.e. approximately eight weeks, to calving age), adult cows, or adult bulls. Estimates of herd size for incidence calculations were based on the initial total at enrollment and the subsequent updates obtained throughout the study. For each time interval between updates, the number of cattle at risk in each age group was calculated as the average of the number of animals reported at the start and at the end of the interval. In doing this, we accounted for changes in the size of the population at risk due to death, sale, or purchase. Animal time at risk for each age group was calculated by multiplying each herd's number of animals at risk by the time interval between updates, then summing these across all herds; cases were not excluded from the animal time at risk calculations following diagnosis, due to their negligible contribution to the total time at risk. Herd time at risk was calculated by summing the total enrollment times for all negative herds and the time between enrollment and onset of the first case for each positive herd. Incident cases were defined as clinically ill cattle that were identified as having signs consistent with salmonellosis and that tested positive for *Salmonella* via bacteriologic culture. Repeated positive samples from the same animal were excluded from incidence calculations. A herd was considered *Salmonella*-positive if at least one animal with compatible clinical signs tested positive via bacteriologic culture; such an animal had

to have been diagnosed at least two weeks beyond herd enrollment in order for it to be viewed as an incident case. Estimates of animal-level incidence rates (incidence densities) were calculated for each age group as the number of cases per animal time at risk, and an estimate of herd-level incidence rate was calculated as the number of positive herds per herd time at risk.

In order to check for selection bias related to herd enrollment, estimates of animal-level incidence rates were also calculated separately for herds within veterinary practices that enrolled more than 50% of their dairy clients and for herds within practices enrolling fewer than 50%. Our concern was that low-enrollment practices may have been selectively recruiting study herds with either a known history of salmonellosis or a current outbreak of diarrhea, rather than a representative sample of their client herds.

Descriptive analysis of serotype data and level of antimicrobial resistance was performed, including the distribution of serotypes and multidrug-resistant (MDR) isolates by animal age group and by herd. The proportion of MDR isolates by serotype was also determined. Bivariable analysis using the chi-squared test was utilized to determine whether age group or serotype was significantly associated with multidrug resistance (MDR vs. not MDR). Separate logistic regression models were used to further investigate any associations with multidrug resistance while controlling for herd as a random effect, using MDR status as the dichotomous outcome variable. In this study, multidrug resistance was defined as having *in vitro* resistance to five or more antimicrobial agents.

Poisson regression analysis was performed to study associations between the within-herd incidence of salmonellosis and various predictor variables (herd size, housing type, vaccination status, and prior history of *Salmonella* infection), controlling for herd as a random effect. A backward stepwise approach was used to identify a

final multivariable model, and p-values < 0.05 were considered significant. Incidence density ratios and their corresponding 95% confidence intervals were determined. All data analysis was performed in SAS (version 9.1; SAS Institute Inc., Cary, NC), and the generalized estimating equations (GEE) method was used for the regression models via PROC GENMOD.

RESULTS

A total of 35 veterinary practices participated in this study, enrolling 831 herds with 327,686 female dairy cattle (pre-weaned female calves, heifers, and adult cows). Median herd size was 180 female dairy cattle (range: 20–5241). Free-stall housing was found on 454 (54.6%) farms (this was the exclusive form of housing on 418 of these farms), while barns with individual stalls for each cow (either tie-stall or stanchion) were utilized on 355 (42.7%) farms; the remaining 2.7% of farms used some other form of housing. Use of a commercial gram-negative vaccine was reported among 337 (40.6%) herds (ENDOVAC-Bovi, IMMVAC, Inc., Columbia, MO; J-VAC, Merial, Duluth, GA; J-5 BACTERIN, Pfizer Animal Health, New York, NY). There were 59 (7.1%) herds that had reportedly experienced at least one *Salmonella* case during the previous 12 months.

During the study period, fecal samples from 2,565 female dairy cattle on 412 farms were submitted for *Salmonella* culture due to suspicion of clinical disease. Of these, *Salmonella* was isolated from 576 (22.5%) samples representing 93 herds. Sixteen herds accounted for 72% of the positive samples, while 40 herds had only one laboratory-confirmed case of salmonellosis each. Twelve of the 40 herds had their single case diagnosed during the two weeks immediately following herd enrollment,

and those animals were not regarded as incident cases in any of the calculations. The herd-level incidence rate was 8.6 positive herds per 100 herd-years.

Fecal samples were submitted from 866 pre-weaned female calves, 168 heifers, and 1531 adult cows for *Salmonella* culture. The organism was isolated from 152 (17.6%) female calves, seven (4.2%) heifers, and 417 (27.2%) cows. The animal-level incidence rates for pre-weaned female calves, heifers, and adult cows were 8.1, 0.04, and 1.8 cases per 1,000 animal-years, respectively. For herds within high-enrollment practices, the animal-level incidence rates for pre-weaned female calves, heifers, and adult cows were 9.9, 0.09, and 2.0 cases per 1,000 animal-years, while herds within low-enrollment practices had incidence rates of 7.4, 0.03, and 1.8 cases per 1,000 animal-years, respectively. The within-herd incidence rates among all positive herds ranged from 0.2 to 119.3 cases per 1,000 animal-years, with a median of 4.8 cases per 1,000 animal-years (Figure 2.1).

The predominant serotype was Newport, accounting for 41% of the cases, followed by Typhimurium (including the Copenhagen variant), Infantis, 4,5,12:i:-, Agona, Muenster, and Kentucky. These seven serotypes comprised 87% of the total, and 11 other serotypes made up the remainder (Table 2.1). The serotype most frequently isolated from calves was Typhimurium (40.1%, 61/152), while Newport was the primary serotype among cows (46.8%, 195/417). The most common serotypes were also widespread among farms, with Typhimurium being isolated from 36 herds and Newport from 30 herds (Table 2.2). There were 71 (76.3%) herds that had only one serotype identified during the study period, 18 (19.4%) herds had two serotypes, and four (4.3%) had three serotypes.

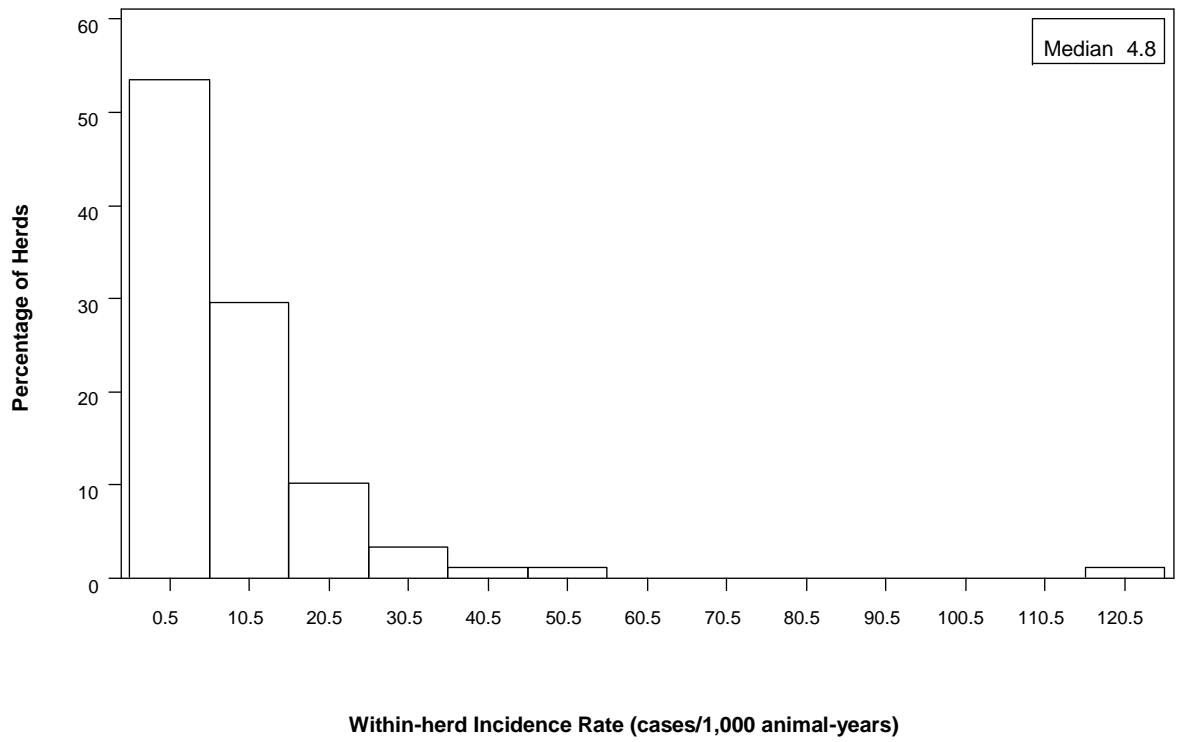


Figure 2.1: Within-herd incidence rates among all positive herds

Table 2.1: Serotypes isolated from female dairy cattle with salmonellosis in the northeastern United States

<i>Serotype</i>	<i>No. of Isolates (%)</i>
4,5,12:i:-	35 (6.1%)
Adelaide	1 (0.2%)
Agona	30 (5.2%)
Anatum	9 (1.6%)
Bardo	5 (0.9%)
Cerro	1 (0.2%)
Infantis	47 (8.2%)
Kentucky	20 (3.5%)
Mbandaka	12 (2.1%)
Montevideo	5 (0.9%)
Muenchen	4 (0.7%)
Muenster	23 (4.0%)
Newport	236 (41.0%)
Ohio	11 (1.9%)
Oranienburg	3 (0.5%)
Senftenberg	1 (0.2%)
Thompson	2 (0.3%)
Typhimurium	49 (8.5%)
Typhimurium (Copenhagen)	61 (10.6%)
Unknown	21 (3.6%)

Table 2.2: Distribution of the most common *Salmonella* serotypes by dairy herd

<i>Serotype</i>	<i>No. of Dairy Herds</i>	<i>% of Positive Herds</i>
4,5,12:i:-	4	4.3%
Agona	8	8.6%
Infantis	7	7.5%
Kentucky	2	2.2%
Muenster	6	6.5%
Newport	30	32.3%
Typhimurium	27	29.0%
Typhimurium (Copenhagen)	11	11.8%

A total of 395 (68.6%) isolates were multidrug-resistant. Only 114 (19.8%) isolates were pan-susceptible, not displaying resistance to any of the 11 antimicrobial drugs in our panel. Resistance to individual antimicrobial agents (Table 2.3) ranged from 0% (enrofloxacin) to 79.3% of all isolates (sulfadimethoxine). Chi-squared testing revealed that the proportion of multidrug resistance was significantly higher ($P = 0.006$) among isolates obtained from calves (77.0%, 117/152) than among those from cows (65.2%, 272/417). Calf isolates were also more likely to be MDR than cow isolates in a logistic regression model which controlled for herd as a random effect ($P = 0.01$). In addition, six of the seven isolates from heifers were multidrug-resistant. Cattle harboring MDR isolates represented 60 (64.5%) of the *Salmonella*-positive herds in our study.

There was considerable variation in antimicrobial resistance across serotypes (Table 2.4). For instance, 97.0% (229/236) of the Newport isolates were multidrug-resistant, while only 2.1% (1/47) of the Infantis isolates were MDR. Chi-squared testing showed that the proportion of multidrug resistance was significantly higher ($P < 0.0001$) among serotypes that are also the most important human pathogens (Newport and Typhimurium; 89.0%, 308/346) than among all other serotypes (37.4%, 86/230). Newport and Typhimurium isolates were also more likely to be MDR than other serotypes in a logistic regression model controlling for herd as a random effect ($P < 0.0001$).

Using a multivariable Poisson regression model, it was found that herd size was the only significant predictor of *Salmonella* incidence (Table 2.5). Larger herds with at least 400 female dairy cattle had a higher incidence rate than smaller herds with fewer than 100 female dairy cattle (IDR, 4.7; $P = 0.004$). The incidence rates among the three smaller herd size categories (200-399, 100-199, and <100 female dairy cattle) did not differ significantly. There was not a significant association

Table 2.3: Resistance to individual antimicrobial agents among all *Salmonella* isolates and the most common serotypes

Antimicrobial Agent	All Isolates (%)	4,5,12:i- (%)	Agona (%)	Infantis (%)	Kentucky (%)	Muenster (%)	Newport (%)	Typhimurium (%)	Typhimurium (Copenhagen) (%)
Ampicillin	68.8	60.0	83.3	2.1	5.0	8.7	97.0	38.8	100.0
Ceftiofur	60.4	57.1	80.0	2.1	5.0	8.7	97.0	10.2	42.6
Chlortetracycline	66.8	62.9	80.0	4.3	0	8.7	96.6	30.6	91.8
Enrofloxacin	0	0	0	0	0	0	0	0	0
Florfenicol	63.7	57.1	83.3	2.1	5.0	8.7	93.2	34.7	72.1
Gentamicin	2.3	2.9	0	2.1	0	0	0	2.0	0
Neomycin	42.2	5.7	73.3	2.1	5.0	0	55.5	22.4	78.7
Oxytetracycline	68.9	62.9	83.3	4.3	5.0	8.7	97.5	38.8	96.7
Spectinomycin	16.1	8.6	10.0	2.1	0	8.7	2.1	36.7	85.2
Sulfadimethoxine	79.3	68.6	100.0	6.4	65.0	60.9	97.0	65.3	100.0
Trimethoprim/ sulfamethoxazole	5.2	0	63.3	2.1	0	4.3	1.3	2.0	9.8

Table 2.4: Multidrug resistance (*in vitro* resistance to ≥ 5 antimicrobial agents) among the most common *Salmonella* serotypes

<i>Serotype</i>	<i>% MDR</i>
4,5,12:i:-	60.0% (21/35)
Agona	83.3% (25/30)
Infantis	2.1% (1/47)
Kentucky	5.0% (1/20)
Muenster	8.7% (2/23)
Newport	97.0% (229/236)
Typhimurium	38.8% (19/49)
Typhimurium (Copenhagen)	98.4% (60/61)

Table 2.5: Multivariable Poisson regression analysis of herd-level factors for association with *Salmonella* incidence rate (incidence density)

<i>Variable</i>	<i>Incidence Density Ratio</i>	<i>95% Confidence Interval</i>	<i>p-value</i>
<i>Herd size</i>			
≥ 400	4.7	(1.6, 13.3)	0.004
200-399	1.4	(0.5, 4.2)	0.6
100-199	1.4	(0.5, 4.0)	0.6
< 100	1.0	---	---

between *Salmonella* incidence and either housing type, commercial gram-negative vaccine use, or a history of at least one *Salmonella* case during the previous 12 months, when these were included with herd size in a multivariable model.

DISCUSSION

Several studies have described the prevalence of fecal *Salmonella* shedding among dairy cattle, using either cross-sectional or longitudinal designs. To our

knowledge, however, no studies have investigated the incidence of clinical disease associated with *Salmonella* infections in cattle. This study had the particular advantage of involving a very large number of female cattle (over 327,000) from numerous dairy herds (831) throughout the northeastern U.S. These herds were characterized by a wide range of sizes and management types representative of the dairy industry in this area of the country. Although it was our goal for participating veterinarians to enroll all of their dairy herd clients with at least 30 dairy cattle for whom they provided routine clinical service, not all such herds were enrolled. However, our comparable animal-level incidence rates for herds within high-enrollment and low-enrollment practices would suggest that selection bias related to herd enrollment was not present in this study. In fact, the incidence rates for all animal age groups were actually lower among practices that were apparently more selective in enrolling herds.

It is conceivable that the incidence of salmonellosis among dairy herds was underestimated if clinically affected cattle went undetected by herd owners. Furthermore, fecal culture does not have perfect sensitivity for detecting the presence of *Salmonella*, and we recognize that some positive cattle were presumably missed by culturing. On the other hand, it is also plausible that some animals with a positive *Salmonella* culture result and compatible clinical signs were actually symptomatic because of another primary disease process; this would lead to an overestimation of the incidence of salmonellosis. Alternatively, it is possible that some cases assumed to be incident were in fact ongoing at the start of the study, also leading to an overestimation of incidence rates. However, we believe that we eliminated this possibility by requiring that only cases diagnosed at least two weeks beyond herd enrollment be considered for incidence calculations.

Laboratory-confirmed salmonellosis was found in 11% (93/831) of the dairy herds monitored for approximately one year over the course of the study. The herd-level incidence rate was around nine positive herds per 100 herd-years. However, only 17% (16/93) of the positive study herds accounted for over 70% of the clinical *Salmonella* cases. The median within-herd incidence rate among positive herds was approximately five cases per 1,000 animal-years, with a maximum of almost 120 cases per 1,000 animal-years. These results suggest a wide disparity in the incidence of salmonellosis among dairy herds in the northeastern U.S. While most farms did not experience any clinical disease due to *Salmonella* infection during the study period, a subset of the positive farms had a very high incidence of salmonellosis. Clustering of disease among herds is consistent with the prevalence study which found that 25% of the enrolled dairy farms accounted for over 75% of the *Salmonella*-positive fecal and environmental samples (Fossler *et al.*, 2004). It seems likely that the most efficient approach to controlling *Salmonella* at the farm level would be to focus our efforts on addressing biosecurity and hygiene practices among the relatively few herds with high frequency of disease, as well as preventing pathogen spread from such herds to those that remain uninfected.

Over 22% (576/2,565) of the fecal samples from clinical suspect animals were positive for *Salmonella*. The animal-level incidence rate for pre-weaned female calves was 8.1 cases per 1,000 animal-years, while that for adult cows was 1.8 cases per 1,000 animal-years. Heifers in this study rarely developed salmonellosis. Both humoral and cellular immune mechanisms play a role in resistance to *Salmonella* (Lindberg and Robertsson, 1983), and calves may be at a greater risk of infection than adults due to their more naïve immune system. In addition, concurrent infection with multiple enteric pathogens (*E. coli*, Rotavirus, Coronavirus, and/or *Cryptosporidium*) is a common scenario in calves (Divers and Peek, 2008), further compromising their

immune status. It is also possible that a relative lack of intestinal microflora in young calves contributes to their susceptibility; such microflora is believed to offer a degree of protection against colonization by pathogenic enteric bacteria. There are very few studies which compare *Salmonella* prevalence among age groups of cattle, with mixed results regarding whether pre-weaned calves (Warnick *et al.*, 2003) or cows (Fossler *et al.*, 2005) have a higher prevalence of fecal *Salmonella* shedding. Calf isolates in this study were also more likely to be multidrug-resistant than isolates from cows, a trend which has been observed with *E. coli* as well (Sato *et al.*, 2005; Cho *et al.*, 2007).

Newport was certainly the major serotype in this study, accounting for over 40% of the isolates, followed by Typhimurium at nearly 20%; both were also widespread among farms. Dairy cattle represent an important source of *Salmonella* serotypes that are a threat to human health. CDC FoodNet data from 2007 show that Newport and Typhimurium were two of the three most common *Salmonella* serotypes isolated from people with laboratory-confirmed foodborne infection, accounting for over 26% of the human cases (Centers for Disease Control and Prevention (CDC), 2008b). The predominance of *S. Newport* in this study is particularly noteworthy because it is generally multidrug-resistant in cattle and is becoming an increasingly important human pathogen. According to the CDC, the annual incidence of *S. Newport* infections among people in the U.S. has increased by more than 40% over the last decade (Centers for Disease Control and Prevention (CDC), 2007). Multidrug resistance is also on the rise; the prevalence of the most common MDR *S. Newport* phenotype (Newport-MDRampC, resistant to at least nine antimicrobial agents) increased from 1% of human Newport isolates tested by the National Antimicrobial Resistance Monitoring System (NARMS) in 1998 to 21% of isolates tested in 2003. Reported risk factors for Newport-MDRampC infection in people include direct exposure to a dairy farm (Gupta *et al.*, 2003), consumption of uncooked ground beef

(Varma *et al.*, 2006), and consumption of unpasteurized dairy products (Centers for Disease Control and Prevention (CDC), 2008a); these examples illustrate the key role that dairy cattle play as a source of MDR *S. Newport*.

In contrast, the serotypes most commonly isolated in studies of fecal *Salmonella* shedding among clinically healthy cattle differ from those that most frequently cause human disease. According to the 1996 National Animal Health Monitoring System (NAHMS) report, *S. Montevideo* (21%) was the most prevalent serotype isolated from healthy lactating cows, and neither *Newport* nor *Typhimurium* were among the 10 most common serotypes isolated (Wells *et al.*, 2001). The 2002 NAHMS study found *S. Meleagridis* (24%) to be the most prevalent serotype, while *Newport* and *Typhimurium* accounted for only 3% and 10% of all isolates, respectively (Blau *et al.*, 2005). Clearly there is a great diversity in *Salmonella* serotypes shed by dairy cattle. Though many serotypes may be shed by apparently healthy cattle, our results suggest that *Newport* and *Typhimurium* are two that pose a higher risk to their health and welfare. Furthermore, this study supports the view that clinically affected cattle present the greatest threat to public health, as they are often shedding serotypes that are also important human pathogens. This would be in contrast with other foodborne zoonotic pathogens, such as *Campylobacter jejuni* and *E. coli* O157:H7, which occur widely in adult cattle without accompanying clinical disease (Cho *et al.*, 2006; Kwan *et al.*, 2008).

Interestingly, the *Dublin* serotype was not isolated from any of the cattle in this study, which may simply reflect the fact that this serotype was not common among northeastern dairy farms during the study period. Alternatively, this may be related to our emphasis on enteric disease in the case definition provided to herd owners. The predominant clinical sign among cattle infected with *Dublin* is respiratory disease rather than diarrhea (Divers and Peek, 2008), and we may have missed such cases as a

result. Furthermore, optimal culture specimens for diagnosing *S. Dublin* infections are a trans-tracheal aspirate and/or blood culture rather than a fecal sample.

Herd size was a significant predictor of the incidence of salmonellosis in a multivariable Poisson regression model. The association between large herd size and fecal *Salmonella* shedding has been reported in numerous studies (Kabagambe *et al.*, 2000; Warnick *et al.*, 2001; Wells *et al.*, 2001; Huston *et al.*, 2002; Fossler *et al.*, 2004; Blau *et al.*, 2005; Fossler *et al.*, 2005; Davison *et al.*, 2006). Larger herds may have a greater likelihood of purchasing cattle from outside sources, with the accompanying risk of introducing *Salmonella* via a subclinical shedder that has been stressed by transport. High cattle density may also be a feature of larger herds and could promote *Salmonella* transmission; animal crowding not only enhances contact among cattle but also may encourage stressful group dynamics. Alternatively, large herd size may simply equate to a higher number of susceptible animals within the herd. Finally, larger herds may be characterized by management practices which somehow play a role in increasing the incidence of salmonellosis. Herd size is a risk factor that does not easily lend itself to practical intervention due to the management trends and economic constraints that prevail in the modern dairy industry. However, it is possible that certain attributes of larger herds that contribute to their higher *Salmonella* incidence could in fact be modified to reduce the impact of this disease.

The use of free-stall housing was not a significant predictor of *Salmonella* incidence in this study, after adjusting for herd size. One study found free-stall housing to be associated with increased odds of fecal *Salmonella* shedding in a multivariable model that adjusted for herd size (Fossler *et al.*, 2005), while another found a similar association in a univariable analysis but not in a model that controlled for the size of the herd (Huston *et al.*, 2002). Free-stall housing, associated primarily with large herds, presents considerable challenges when combating manure-

transmitted pathogens. Freedom of movement in free-stall barns allows cattle to have direct contact with manure from other members of the herd, and it facilitates fecal contamination of common feed and water sources. It is conceivable that this form of housing promotes fecal shedding of *Salmonella* among dairy cattle in general, including those that are clinically normal, but has a reduced impact on the incidence of salmonellosis.

Neither the use of a commercial gram-negative vaccine nor an owner-reported history of *Salmonella* during the previous 12 months was significantly associated with the incidence of salmonellosis in a multivariable Poisson regression model. Other researchers have similarly found that vaccine use (either a gram-negative vaccine or a *Salmonella* bacterin) and a history of salmonellosis within the past year were not associated with the prevalence of fecal *Salmonella* shedding, when adjusting for herd size in a multivariable model (Huston *et al.*, 2002). A previous history of salmonellosis was unassociated with fecal *Salmonella* shedding in another study as well (Vanselow *et al.*, 2007). While we did not find a relationship between immunization practices and the incidence of salmonellosis, it is important to bear in mind that observational studies are not the ideal approach for assessing vaccine efficacy. The lack of an association between salmonellosis and a prior history of *Salmonella* on the farm was surprising to us, despite the aforementioned prevalence studies. It would seem intuitive for past isolation of the organism to be predictive of future clinical disease, but such was not the case in this study.

CONCLUSIONS

Although fecal *Salmonella* shedding has been found to be common at the herd level (Fossler *et al.*, 2004; Blau *et al.*, 2005), the incidence of laboratory-confirmed clinical disease due to *Salmonella* was comparatively low in this study. Among the positive herds, there was a wide disparity in the incidence of salmonellosis. The animal-level incidence rate was highest for pre-weaned female calves and lowest for heifers. *S. Newport* and *Typhimurium* accounted for about 60% of the isolates and were widespread among farms; these are also two of the most common *Salmonella* serotypes isolated from people with foodborne infections. Herds with at least 400 female dairy cattle had a higher incidence of salmonellosis than smaller herds, though additional research is needed to clarify the relationship between large herd size and *Salmonella*.

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REFERENCES

- Bender JB. Reducing the risk of *Salmonella* spread and practical control measures in dairy herds. *Bovine Pract* 1994; 28:62-65.
- Blau DM, McCluskey BJ, Ladely SR, *et al.* *Salmonella* in dairy operations in the United States: prevalence and antimicrobial drug susceptibility. *J Food Prot* 2005; 68:696-702.
- Centers for Disease Control and Prevention (CDC). Outbreak of multidrug-resistant *Salmonella enterica* serotype Newport infections associated with consumption of unpasteurized Mexican-style aged cheese--Illinois, March 2006-April 2007. *MMWR Morb Mortal Wkly Rep* 2008a; 57:432-435.
- Centers for Disease Control and Prevention (CDC). Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food--10 states, 2006. *MMWR Morb Mortal Wkly Rep* 2007; 56:336-339.
- Centers for Disease Control and Prevention (CDC). Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food--10 states, 2007. *MMWR Morb Mortal Wkly Rep* 2008b; 57:366-370.
- Cho S, Bender JB, Diez-Gonzalez F, *et al.* Prevalence and characterization of *Escherichia coli* O157 isolates from Minnesota dairy farms and county fairs. *J Food Prot* 2006; 69:252-259.
- Cho S, Fossler CP, Diez-Gonzalez F, *et al.* Antimicrobial susceptibility of shiga toxin-producing *Escherichia coli* isolated from organic dairy farms, conventional dairy farms, and county fairs in Minnesota. *Foodborne Pathog Dis* 2007; 4:178-186.
- Clegg FG, Chiejina SN, Duncan AL, *et al.* Outbreaks of *Salmonella* Newport infection in dairy herds and their relationship to management and contamination of the environment. *Vet Rec* 1983; 112:580-584.
- Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard - Third Edition*. CLSI document M31-A3. Wayne, PA: Clinical and Laboratory Standards Institute. 2008.
- Davison HC, Sayers AR, Smith RP, *et al.* Risk factors associated with the *Salmonella* status of dairy farms in England and Wales. *Vet Rec* 2006; 159:871-880.

Divers TJ and Peek SF. *Rebhun's diseases of dairy cattle*. St. Louis: Saunders Elsevier, 2008.

Evans S and Davies R. Case control study of multiple-resistant *Salmonella* Typhimurium DT104 infection of cattle in Great Britain. *Vet Rec* 1996; 139:557-558.

Fossler CP, Wells SJ, Kaneene JB, *et al.* Herd-level factors associated with isolation of *Salmonella* in a multi-state study of conventional and organic dairy farms I. *Salmonella* shedding in cows. *Prev Vet Med* 2005; 70:257-277.

Fossler CP, Wells SJ, Kaneene JB, *et al.* Prevalence of *Salmonella* spp on conventional and organic dairy farms. *J Am Vet Med Assoc* 2004; 225:567-573.

Fossler CP, Wells SJ, Kaneene JB, *et al.* Cattle and environmental sample-level factors associated with the presence of *Salmonella* in a multi-state study of conventional and organic dairy farms. *Prev Vet Med* 2005; 67:39-53.

Gay JM and Hunsaker ME. Isolation of multiple *Salmonella* serovars from a dairy two years after a clinical salmonellosis outbreak. *J Am Vet Med Assoc* 1993; 203:1314-1320.

Giles N, Hopper SA, and Wray C. Persistence of *S. Typhimurium* in a large dairy herd. *Epidemiol Infect* 1989; 103:235-241.

Gupta A, Fontana J, Crowe C, *et al.* Emergence of multidrug-resistant *Salmonella enterica* serotype Newport infections resistant to expanded-spectrum cephalosporins in the United States. *J Infect Dis* 2003; 188:1707-1716.

Huston CL, Wittum TE, Love BC, *et al.* Prevalence of fecal shedding of *Salmonella* spp in dairy herds. *J Am Vet Med Assoc* 2002; 220:645-649.

Islam M, Morgan J, Doyle MP, *et al.* Persistence of *Salmonella enterica* serovar Typhimurium on lettuce and parsley and in soils on which they were grown in fields treated with contaminated manure composts or irrigation water. *Foodborne Pathog Dis* 2004; 1:27-35.

Kabagambe EK, Wells SJ, Garber LP, *et al.* Risk factors for fecal shedding of *Salmonella* in 91 US dairy herds in 1996. *Prev Vet Med* 2000; 43:177-194.

- Kwan PS, Birtles A, Bolton FJ, *et al.* Longitudinal study of the molecular epidemiology of *Campylobacter jejuni* in cattle on dairy farms. *Appl Environ Microbiol* 2008; 74:3626-3633.
- Lindberg AA and Robertsson JA. *Salmonella* Typhimurium infection in calves: Cell-mediated and humoral immune reactions before and after challenge with live virulent bacteria in calves given live or inactivated vaccines. *Infect Immun* 1983; 41:751-757.
- Mead PS, Slutsker L, Dietz V, *et al.* Food-related illness and death in the United States. *Emerg Infect Dis* 1999; 5:607-625.
- Nielsen LR, Warnick LD, Greiner M. Risk factors for changing test classification in the Danish surveillance program for *Salmonella* in dairy herds. *J Dairy Sci* 2007; 90:2815-2825.
- Peters AR. An estimation of the economic impact of an outbreak of *Salmonella* Dublin in a calf rearing unit. *Vet Rec* 1985; 117:667-668.
- Sanchez S, Hofacre CL, Lee MD, *et al.* Animal sources of salmonellosis in humans. *J Am Vet Med Assoc* 2002; 221:492-497.
- Sato K, Bartlett PC, and Saeed MA. Antimicrobial susceptibility of *Escherichia coli* isolates from dairy farms using organic versus conventional production methods. *J Am Vet Med Assoc* 2005; 226:589-594.
- Sivapalasingam S, Friedman CR, Cohen L, *et al.* Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *J Food Prot* 2004; 67:2342-2353.
- Vanselow BA, Hornitzky MA, Walker KH, *et al.* *Salmonella* and on-farm risk factors in healthy slaughter-age cattle and sheep in eastern Australia. *Aust Vet J* 2007; 85:498-502.
- Varma JK, Marcus R, Stenzel SA, *et al.* Highly resistant *Salmonella* Newport-MDRampC transmitted through the domestic US food supply: a FoodNet case-control study of sporadic *Salmonella* Newport infections, 2002-2003. *J Infect Dis* 2006; 194:222-230.
- Voetsch AC, Van Gilder TJ, Angulo FJ, *et al.* FoodNet estimate of the burden of illness caused by nontyphoidal *Salmonella* infections in the United States. *Clin Infect Dis* 2004; 38 Suppl 3:S127-34.

Warnick LD, Crofton LM, Pelzer KD, *et al.* Risk factors for clinical salmonellosis in Virginia, USA cattle herds. *Prev Vet Med* 2001; 49:259-275.

Warnick LD, Kanistanon K, McDonough PL, *et al.* Effect of previous antimicrobial treatment on fecal shedding of *Salmonella enterica* subsp. *enterica* serogroup B in New York dairy herds with recent clinical salmonellosis. *Prev Vet Med* 2003; 56:285-297.

Wells SJ, Fedorka-Cray PJ, Dargatz DA, *et al.* Fecal shedding of *Salmonella* spp. by dairy cows on farm and at cull cow markets. *J Food Prot* 2001; 64:3-11.

Wray C and Wray A. *Salmonella* in domestic animals. Oxford ; New York: CABI Pub., 2000.

CHAPTER 3: The duration of fecal *Salmonella* shedding following clinical disease
among dairy cattle in the northeastern USA*

*Cummings KJ, Warnick LD, Alexander KA, Cripps CJ, Gröhn YT, James KL, McDonough PL, Reed KE. The duration of fecal *Salmonella* shedding following clinical disease among dairy cattle in the northeastern USA. *Preventive Veterinary Medicine* 2009; 92: 134–139.

Abstract

The objectives of this study were to determine the duration of fecal *Salmonella* shedding among dairy cattle in the northeastern United States following laboratory-confirmed clinical disease and to evaluate whether age group or serotype was associated with either shedding period or mortality. Study farms included 22 dairy herds that had at least two previous salmonellosis cases confirmed by fecal culture. Veterinarians continued to submit culture samples from clinical suspects following herd enrollment, and fecal samples from positive cattle were collected monthly until three sequential negative results were obtained or until loss to follow-up. There were 357 culture-positive clinical cases that each involved a single serotype during the shedding period. The Kaplan-Meier median duration of fecal *Salmonella* shedding was 50 days, and the maximum was 391 days. *S. Newport* was the predominant serotype, accounting for 51% of the cases. Age group and serotype were not significant predictors of *Salmonella* shedding duration in a Cox proportional hazards model, when stratifying by herd. However, the proportion of adult cows shedding for at least two consecutive monthly samples was significantly greater than the proportion of female calves shedding for this duration (Fisher's exact test p-value < 0.01). Age group was also associated with mortality in this study; calves with salmonellosis were more likely to die than cows as estimated by a logistic regression model which controlled for herd as a random effect (p-value = 0.04).

1. Introduction

Salmonella is a zoonotic enteric pathogen with significant public health implications, resulting in approximately 1.4 million illnesses, 16,000 hospitalizations, and between 400 and 600 deaths annually in the U.S. alone (Mead *et al.*, 1999; Voetsch *et al.*, 2004). Though primarily a cause of self-limiting acute enteritis (diarrhea, abdominal pain, and fever, with a typical duration of four to seven days), *Salmonella* can produce invasive infections that lead to sepsis and death. People generally acquire salmonellosis through foodborne exposure, although direct contact with infected animals is another possible route (Mead *et al.*, 1999; L Plym and Wierup, 2006). Preliminary CDC FoodNet data from 2007 show that *Salmonella* accounted for 38% of all laboratory-confirmed cases of foodborne infections, based on surveillance in 10 states (Centers for Disease Control and Prevention (CDC), 2008b). Dairy cattle are considered an important source of *Salmonella* serotypes that are a threat to human health. Fecal contamination of beef carcasses at the time of slaughter is thought to represent the predominant route of transmission. According to the 1996 USDA National Animal Health Monitoring System (NAHMS) report, 15% of culled dairy cows were shedding *Salmonella* at livestock markets, and 66% of markets had at least one cow shedding *Salmonella* (Wells *et al.*, 2001). Contamination of crops by manure used as fertilizer, as well as water contamination by manure run-off, are additional sources of transmission (Islam *et al.*, 2004; Sivapalasingam *et al.*, 2004). Those who work or otherwise interact with livestock are also at risk of infection via direct exposure when cattle are shedding *Salmonella*.

Introduction of *Salmonella* onto a dairy farm can occur through a variety of routes, including purchased cattle, contaminated feed or water, wild animals such as rodents and birds, and human traffic (Bender, 1994; Evans and Davies, 1996; Sanchez

et al., 2002; Nielsen *et al.*, 2007). Thus, the presence of *Salmonella* on a farm is not an unexpected finding. In fact, one study involving 110 dairy farms in four states found that over 90% of the farms had at least one *Salmonella*-positive culture obtained (fecal and/or environmental) during the course of five sampling visits over a one year period (Fossler *et al.*, 2004). The NAHMS Dairy 2002 study, based on a single sampling visit to five herds in each of 21 states, found that 31% of herds yielded at least one *Salmonella*-positive fecal culture (Blau *et al.*, 2005). Considering these high herd-level prevalence values, it would seem logical that fecal shedders within a herd represent a potential point of intervention to mitigate public health risk.

Clinical signs of salmonellosis in cattle may include diarrhea, fever lasting one to seven days, anorexia, dehydration, decreased milk production, abortion, and evidence of endotoxemia (Divers and Peek, 2008). Infected cattle can shed the organism while ill and following clinical recovery, and asymptomatic shedders never show signs at all. Once a cow has become infected, the duration and magnitude of fecal shedding are important determinants of public health risk. Widespread environmental contamination can result from *Salmonella* shedding, and the organism can survive for prolonged periods in suitable conditions outside a host (Wray and Wray, 2000; You *et al.*, 2006). Fecal shedding also increases the risk of within-herd transmission, potentially serving as a source of infection for other animals in the herd. Finally, the shedding of *Salmonella* on one farm increases the probability of inadvertent transmission to other herds, perpetuating the cycle of infection. All of the above lead to an increased risk of zoonotic transmission. In addition to its public health consequences, salmonellosis can be a costly disease for dairy producers on account of mortality, treatment expenses, reduced milk yield, and weight loss within the herd (Peters, 1985; Huston *et al.*, 2002).

The objectives of this study were to determine the duration of fecal *Salmonella* shedding among dairy cattle in the northeastern United States following laboratory-confirmed clinical disease and to ascertain whether age group or serotype was predictive of either shedding period or mortality.

2. Materials and Methods

2.1. Study design

This study was based on data collected prospectively as part of a larger project to estimate the incidence of salmonellosis among dairy cattle from 831 herds in the northeastern USA (Cummings *et al.*, 2009). In that project, *Salmonella* culture was performed on fecal samples obtained from dairy cattle with compatible clinical signs (including diarrhea with blood, mucus, or a foul odor, fever of at least 103°F, depression, and decreased appetite) between February, 2004 and September, 2005. The current study included 22 herds that had at least two laboratory-confirmed *Salmonella* cases during that time span; these herds all had on-farm individual cow milk production records as well. Herds were enrolled as they became eligible at the time of the second positive *Salmonella* culture, with dates ranging from June, 2004 to March, 2005. Following herd enrollment, veterinarians continued to submit culture samples from suspected salmonellosis cases, using the same clinical case guidelines. If an animal had a positive fecal *Salmonella* culture on initial testing, subsequent fecal samples were then collected by project personnel at approximately monthly intervals until three sequential negative results were obtained or until the animal was lost to follow-up due to death, sale, or lost animal identification. All animals were classified as pre-weaned female calves, pre-weaned male calves, heifers (from weaning to

calving age), or adult cows based on their age at the time of laboratory-confirmed clinical salmonellosis.

2.2. Sample collection and processing

Fecal samples were collected via rectal retrieval, with a new glove being used to collect each sample. Approximately 10 g of fecal matter was placed into a Para-Pak bottle (Meridian Bioscience Inc., Cincinnati, OH) and sealed. All samples were shipped to the Animal Health Diagnostic Center (AHDC) at Cornell University for bacteriologic culture. Standard culture methods were used to isolate *Salmonella* from feces. Individual fecal swabs from sample bottles were enriched in 10 ml of Tetrathionate broth (Difco, Detroit, MI) containing 0.2 ml of iodine solution; the mixture was incubated at 42°C for 18–24 hours. After incubation, the sample-broth mixture was streaked onto Brilliant Green agar with novobiocin (BGN; Becton Dickinson and Company, Franklin Lakes, NJ) and Xylose Lysine Tergitol 4 (XLT-4) selective media, and both plates were incubated at 37°C for 18–24 hours. Red colonies (lactose non-fermenting bacteria) on BGN and black colonies (H₂S-producing bacteria) on XLT-4 were inoculated into Kligler Iron Agar (KIA) slants and then incubated at 37°C for 18–24 hours. XLT-4 plates without suspected colonies were re-incubated at 37°C for an additional 18–24 hours before checking again for characteristic black colonies. Colonies on KIA slants which exhibited the biochemical properties of *Salmonella* were then serogrouped by slide agglutination using standard protocols. Those colonies that were positive by slide agglutination were then identified as *Salmonella* using the Sensititre Automated Microbiology System's A80 panel (TREK Diagnostic Systems Inc., Cleveland, OH). Confirmed *Salmonella* isolates were sent to the USDA, APHIS National Veterinary Services Laboratories

(NVSL) in Ames, Iowa for serotyping using established procedures for cell wall (O) and flagellar (H) antigen identification (Edwards and Ewing, 1972).

2.3. Data analysis

For each clinical case, the duration of shedding was defined as the interval between the sampling date of the first laboratory-confirmed *Salmonella* positive and the sampling date of the last laboratory-confirmed positive before three consecutive negative samples were obtained, with the addition of 15 days to account for shedding during half the sampling interval. Animals lost to follow-up before three consecutive negative samples were right-censored. Estimates of shedding duration were determined by age group and by serotype. Survival analysis techniques, including the Kaplan-Meier method and Cox proportional hazards model, were used to test the association between age group, serotype, and the dependent variable, duration of *Salmonella* shedding in days. The proportional hazards model was stratified by herd in order to control for any herd-level confounding factors. Model fit was assessed by plotting the martingale and deviance residuals. A sensitivity analysis was performed to test the effect of our assumption that censoring times were independent of the duration of fecal *Salmonella* shedding among cattle in this study. Thus, we repeated the model under two extreme scenarios: that all censored cattle reached the endpoint of *Salmonella* shedding at the time of censoring, and alternatively that censored cattle remained censored but had a censoring time equal to the longest period of *Salmonella* shedding in the study. We then compared the parameter estimates and hazard ratios from our original model with those of the two hypothetical models. In addition to our survival analysis methods, Fisher's exact test was used to compare age groups by proportion shedding *Salmonella* for at least two consecutive monthly samples.

Bivariable analysis using the chi-squared test was utilized to determine whether age group or serotype was significantly associated with mortality. A logistic regression model was used to further investigate associations with mortality while controlling for herd as a random effect, with vital status serving as the dichotomous outcome variable. The generalized estimating equations (GEE) method was employed, and a backward stepwise approach was used to identify a final model. All data analysis was performed in SAS (version 9.1; SAS Institute Inc., Cary, NC), and p-values < 0.05 were considered significant.

3. Results

3.1. Descriptive statistics

Median herd size was 553 adult cattle (range: 245–1516). The median number of culture-positive clinical cases per herd was eight (range: 2–121). There were 357 clinical cases that each involved a single serotype during the period of shedding. Only eight clinical cases were culture-positive for multiple serotypes during the shedding period, and these were excluded from the statistical analysis. Of the cattle that yielded at least two positive samples, 92% (86/94) had the same serotype isolated from all of them. Overall, the Kaplan-Meier median duration of fecal *Salmonella* shedding was 50 days, and the maximum was 391 days. A total of 183 animals (51%) were lost to follow-up before three sequential negative results were obtained, of which 179 were either adult cows (135) or female calves (44); all of these cases were therefore right-censored. Among the censored adults, 59 (44%) were lost to follow-up due to death, 69 (51%) due to sale, and the remaining 7 (5%) due to unknown reasons. Among the

censored female calves, however, 33 (75%) were lost to follow-up due to death, while sale and unknown reasons only accounted for one (2%) and 10 (23%), respectively.

Of the 357 culture-positive clinical cases, 77% were adult cows and 21% were female calves, with the rest being either heifers, male calves, or of unknown age group. The Kaplan-Meier median duration of shedding among adult cows was 51 days, while the maximum was 391 days. In contrast, the Kaplan-Meier median duration of shedding among female calves was 45 days, with a maximum of only 72 days.

The predominant serotype was Newport, accounting for 51% of the cases, followed by Infantis, 4,5,12:i:-, Typhimurium, and Kentucky; these five serotypes comprised 83% of the total (Table 3.1). Ten other serotypes made up the remainder and were treated as one serotype category (“Other”) in the analysis. The Kaplan-Meier median duration of shedding was highest among those cattle infected with the Kentucky serotype (105 days), while the maximum was seen in a cow infected with Newport (391 days).

Table 3.1: Duration of fecal *Salmonella* shedding by serotype among 357 culture-positive clinical cases from 22 dairy herds in the northeastern USA

<i>Serotype</i>	<i>% (N)</i>	<i>Kaplan-Meier Median Duration of Shedding (days)</i>	<i>Max Observed Duration of Shedding (days)</i>
4,5,12:i:-	9% (32)	16	183
Infantis	10% (37)	16	84
Kentucky	5% (17)	105	245
Newport	51% (182)	50	391
Typhimurium	8% (30)	66	66
Other*	17% (59)	69	388

* Agona (4), Anatum (9), Bardo (3), Cerro (2), Mbandaka (10), Montevideo (1), Muenchen (3), Muenster (15), Ohio (10), Oranienburg (2)

3.2. Risk factor analysis

Examination of the residual plots revealed adequate fit of the Cox proportional hazards model. The two hypothetical models for our sensitivity analysis assumed (1) that censored cattle reached the endpoint of *Salmonella* shedding at the time of censoring and (2) that censored cattle had a censoring time of 391 days. The parameter estimates and hazard ratios from these models and our true model were all comparable (the hazard ratios from hypothetical model 1, hypothetical model 2, and our true model, respectively, were as follows: Cow [0.7, 1.3, 0.8], 4,5,12:i:- [1.4, 3.0, 2.4], Infantis [1.5, 0.9, 1.7], Kentucky [1.2, 1.1, 0.8], Newport [0.7, 1.8, 1.0], and Typhimurium [1.1, 1.9, 1.2]). Age group and serotype were not significant predictors of *Salmonella* shedding duration in our model, when stratifying by herd (Table 3.2).

Table 3.2: Association between fecal *Salmonella* shedding duration and age group/serotype among dairy cattle in the northeastern USA, when stratifying by herd in a Cox proportional hazards model

<i>Variable</i>	<i>Hazard Ratio</i>	<i>95% Confidence Interval</i>
• <i>Age Group</i>		
Cow	0.8	(0.5, 1.2)
Calf	1.0	---
• <i>Serotype</i>		
4,5,12:i:-	2.4	(0.3, 17.2)
Infantis	1.7	(0.2, 12.6)
Kentucky	0.8	(0.1, 7.0)
Newport	1.0	(0.4, 2.7)
Typhimurium	1.2	(0.3, 4.8)
Other	1.0	---

Likelihood Ratio Chi-square = 8.3 (df = 6)

However, the proportion of adult cows shedding for at least two consecutive monthly samples was significantly greater than the proportion of female calves shedding for this duration (Fisher's exact test p -value < 0.01). Only 8% of calves had an observed duration of *Salmonella* shedding greater than 30 days, and none reached a shedding time of three months. In contrast, 22% of cows had an observed shedding duration greater than 30 days, and 7% of cows had an observed duration in excess of three months. Figure 3.1 is a Kaplan-Meier graph of survivorship function which illustrates the difference in shedding duration between these age groups after about 30 days.

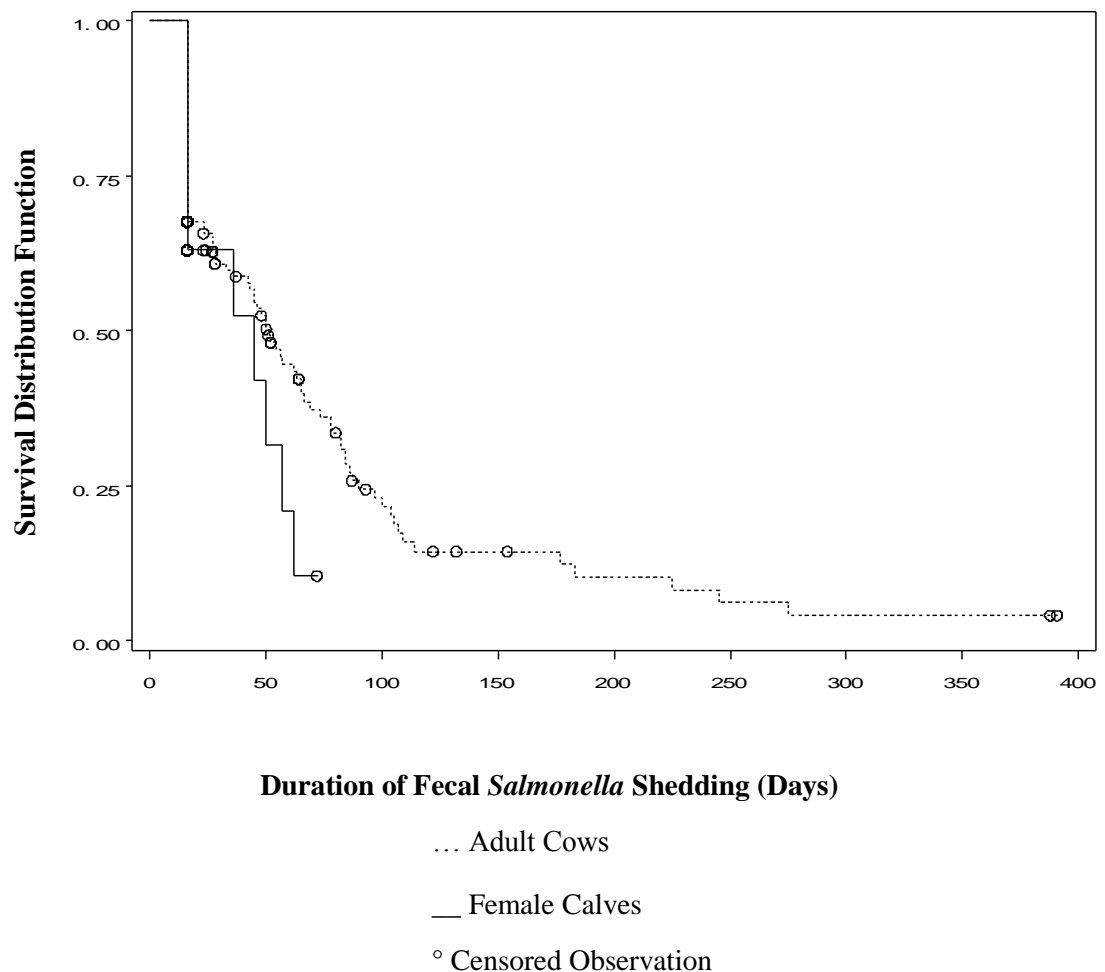


Figure 3.1: Kaplan-Meier survival curves for duration of fecal *Salmonella* shedding among adult cows and female calves from 22 dairy herds in the northeastern USA

Chi-squared testing revealed that mortality was significantly higher (p-value < 0.01) among female calves (45%, 33/73) than among adult cows (22%, 59/269). Calves with salmonellosis were also more likely to die than cows in a logistic regression model which controlled for herd as a random effect (p-value = 0.04, Table 3.3). The serotype of the *Salmonella* isolate was not significantly associated with mortality in this study.

Table 3.3: Association between mortality and age group among dairy cattle with salmonellosis in the northeastern USA, when controlling for herd as a random effect in a logistic regression model

<i>Variable</i>	<i>Odds Ratio</i>	<i>95% Confidence Interval</i>	<i>p-value</i>
• <i>Age Group</i>			
Calf	2.9	(1.04, 8.4)	0.04
Cow	1.0	---	---

Deviance = 384 (df = 340)

4. Discussion

A number of studies have described the prevalence and/or risk factors for fecal *Salmonella* shedding among dairy cattle (Kabagambe *et al.*, 2000; Warnick *et al.*, 2001; Huston *et al.*, 2002; Fossler *et al.*, 2004), but few have examined the duration of shedding in either subclinical or clinical cases. This study had the particular advantage of involving a large number of clinically affected animals from multiple herds. Over 350 cattle with laboratory-confirmed salmonellosis were tested via fecal culture at approximately monthly intervals until three sequential negative results were obtained or until loss to follow-up.

Although we obtained data on a large number of cattle, the relatively small number of enrolled herds (22) may have introduced a degree of selection bias into this study. These herds were all located in either New York (15) or Vermont (7) and may not be representative of all dairy operations in the northeastern USA. These herds were also quite homogeneous with respect to a number of important covariates of interest in this study, thus precluding analysis; for example, all 22 herds utilized free-stall housing, and only one used a flush water system to remove manure from alleys. It is possible that additional selection bias may have arisen from our requirement that study herds had to have had at least two previous laboratory-confirmed *Salmonella* cases in order to be eligible. Perhaps the pattern of *Salmonella* shedding is different among those herds with sporadic cases of disease in individual animals, although we found no significant correlation between the number of cases per herd and the maximum duration of fecal *Salmonella* shedding within that herd. Finally, the issue of informative censoring must be addressed, particularly in light of the high percentage of censored cases in this study. It is conceivable that those cattle that were right-censored due to death or sale would have had an extended duration of fecal *Salmonella* shedding on account of disease severity. However, our sensitivity analysis led us to decide that even if our assumption of independent censoring was not met, we would have reached the same conclusions. Furthermore, loss of an animal due to death or culling is, in practical terms, a means of bringing an end to that animal's shedding of *Salmonella* into the dairy farm environment. One could argue that these outcomes be considered as legitimate endpoints of fecal *Salmonella* shedding duration on the dairy farm.

In this study, fecal *Salmonella* shedding exceeded one year in two animals, with the maximum duration (391 days) being seen in an adult cow infected with the Newport serotype. This is more than twice the maximum shedding time (190 days)

reported in a study involving two dairy herds that had experienced clinical outbreaks of *Salmonella* Newport (Cobbold *et al.*, 2006). Prolonged periods of shedding could lead to extensive environmental contamination and an increased risk of within-herd transmission and spread to other herds. As evidenced in this study, shedding often persists well beyond the typical duration of clinical signs in cattle with salmonellosis. Furthermore, extended survival of the organism in the environment is possible; one study found that *S. Newport* could survive in manure for at least six months over the course of winter (Clegg *et al.*, 1983). Despite the fact that multiple *Salmonella* serotypes are common in the dairy farm environment (Wells *et al.*, 2001; Edrington *et al.*, 2004), 92% of the cattle with multiple positive samples in this study had the same serotype isolated from all of them. This is consistent with true bacterial colonization rather than organisms simply passing through the gastrointestinal tract. However, we cannot rule out either re-infection from the environment or a pass-through effect as contributing to repeated positive samples in some cattle.

Adult cows tended to shed *Salmonella* in their feces longer than calves did, in part because affected calves often died early in the course of their disease. In fact, the odds of mortality were significantly higher among calves in a logistic regression model controlling for herd as a random effect. Of the 34 calves lost to follow-up for known reasons, death was the cause in 33 of them. Dairy herd outbreaks that involve calves represent an important concern for producers because of high case fatality. On the other hand, infected cows are more likely to have an extended duration of shedding, which can result in significant public health consequences in addition to potential transmission to other cattle.

Serotype was not a significant predictor of *Salmonella* shedding duration in this study, according to a Cox proportional hazards model. It may be that host (immune status) and environmental factors (herd management and hygiene practices)

play a more prominent role in determining the length of shedding. Alternatively, other pathogen factors such as dose of inoculum may have a significant effect on shedding duration. One study found that periparturient cows and cows designated as sick by farm personnel were more likely than other cattle to be shedding *Salmonella* in their feces (Fossler *et al.*, 2005). According to another study, cows in early lactation (≤ 60 DIM) were more likely to be shedding *Salmonella* than cows in late lactation (Fitzgerald *et al.*, 2003). Therefore, it seems logical that physiologic stress and concurrent illness could predispose to prolonged fecal shedding among dairy cattle. A number of studies have reported large herd size as a risk factor for fecal *Salmonella* shedding in dairy herds (Kabagambe *et al.*, 2000; Huston *et al.*, 2002; Blau *et al.*, 2005; Fossler *et al.*, 2005). Large herds tend to be housed in free-stalls, which present considerable challenges when combating manure-transmitted pathogens, and they may have a greater likelihood of purchasing cattle from outside sources. It is conceivable that these factors could lead to increased duration of fecal *Salmonella* shedding among individual cattle as well. Manure management could also play a role in shedding; one study found that farms where manure was removed from alleys via a flush water system were more likely to have *Salmonella* shedders than farms that employed a different system (Kabagambe *et al.*, 2000). Again, it is possible that a similar mechanism could also lead to prolonged shedding among individual animals.

Newport was clearly the major serotype in this study, accounting for over half the cases. This is particularly noteworthy since Newport is also an increasingly important human pathogen and has generally been multidrug-resistant among cattle in recent years (Cummings *et al.*, 2009). According to CDC FoodNet data from 2006, the annual incidence of foodborne *S.* Newport infections in the U.S. had increased by 42% over the average annual incidence for 1996–1998 (Centers for Disease Control and Prevention (CDC), 2007). Multidrug resistance is also on the rise; the prevalence

of the most common MDR *S. Newport* phenotype increased from 1% of human Newport isolates tested by the National Antimicrobial Resistance Monitoring System (NARMS) in 1998 to 21% of isolates tested in 2003. This phenotype, Newport-MDRampC, is characterized by resistance to at least nine antimicrobial agents (ampicillin, amoxicillin-clavulanic acid, cefoxitin, ceftiofur, cephalothin, chloramphenicol, streptomycin, sulfamethoxazole/sulfisoxazole, and tetracycline). It also displays decreased susceptibility to ceftriaxone, a crucial drug used for treating invasive *Salmonella* infections in children. This serotype undoubtedly represents an important threat to public health. Reported risk factors for Newport-MDRampC infection in people include direct exposure to a dairy farm (Gupta *et al.*, 2003), consumption of uncooked ground beef (Varma *et al.*, 2006), and consumption of unpasteurized dairy products (Centers for Disease Control and Prevention (CDC), 2008a); these examples illustrate the key role that dairy cattle play as a source of MDR *Salmonella Newport*.

5. Conclusion

In this study, adult cattle with clinical salmonellosis tended to shed the organism in their feces longer than calves did, partly because calves often died early in the course of disease. Newport was the predominant serotype observed, accounting for over half the cases. The duration of fecal *Salmonella* shedding may exceed one year in some animals, and shedding frequently persists well beyond the typical length of clinical signs in cattle with salmonellosis. Additional work is needed to determine whether various herd-level covariates, such as housing type and manure management system, are significantly associated with the duration of fecal *Salmonella* shedding

among cattle. A large sample of herds with diverse production methods would be required for such a study. It would also be valuable to examine other animal-level factors, including stage of lactation and concurrent disease, as potential predictors of *Salmonella* shedding duration.

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REFERENCES

- Bender JB. Reducing the risk of *Salmonella* spread and practical control measures in dairy herds. *Bovine Pract* 1994; 28:62-65.
- Blau DM, McCluskey BJ, Ladely SR, *et al.* *Salmonella* in dairy operations in the United States: prevalence and antimicrobial drug susceptibility. *J Food Prot* 2005; 68:696-702.
- Centers for Disease Control and Prevention (CDC). Outbreak of multidrug-resistant *Salmonella enterica* serotype Newport infections associated with consumption of unpasteurized Mexican-style aged cheese--Illinois, March 2006-April 2007. *MMWR Morb Mortal Wkly Rep* 2008a; 57:432-435.
- Centers for Disease Control and Prevention (CDC). Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food--10 states, 2006. *MMWR Morb Mortal Wkly Rep* 2007; 56:336-339.
- Centers for Disease Control and Prevention (CDC). Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food--10 states, 2007. *MMWR Morb Mortal Wkly Rep* 2008b; 57:366-370.
- Clegg FG, Chiejina SN, Duncan AL, *et al.* Outbreaks of *Salmonella* Newport infection in dairy herds and their relationship to management and contamination of the environment. *Vet Rec* 1983; 112:580-584.
- Cobbold RN, Rice DH, Davis MA, *et al.* Long-term persistence of multi-drug-resistant *Salmonella enterica* serovar Newport in two dairy herds. *J Am Vet Med Assoc* 2006; 228, 585-591.
- Cummings KJ, Warnick LD, Alexander KA, *et al.* The incidence of salmonellosis among dairy herds in the northeastern United States. *J Dairy Sci* 2009; 92:3766-3774.
- Divers TJ and Peek SF. *Rebhun's diseases of dairy cattle*. St. Louis: Saunders Elsevier, 2008.
- Edrington TS, Hume ME, Loofer ML, *et al.* Variation in the faecal shedding of *Salmonella* and *E. coli* O157:H7 in lactating dairy cattle and examination of salmonella genotypes using pulsed-field gel electrophoresis. *Lett Appl Microbiol* 2004; 38, 366-372.

Edwards PR and Ewing WH. Identification of Enterobacteriaceae. Burgess Pub. Co, Minneapolis, 1972.

Evans S and Davies R. Case control study of multiple-resistant *Salmonella* Typhimurium DT104 infection of cattle in Great Britain. *Vet Rec* 1996; 139:557-558.

Fitzgerald AC, Edrington TS, Loofer ML, *et al.* Antimicrobial susceptibility and factors affecting the shedding of *E. coli* O157:H7 and *Salmonella* in dairy cattle. *Lett Appl Microbiol* 2003; 37, 392-398.

Fossler CP, Wells SJ, Kaneene JB, *et al.* Prevalence of *Salmonella* spp on conventional and organic dairy farms. *J Am Vet Med Assoc* 2004; 225:567-573.

Fossler CP, Wells SJ, Kaneene JB, *et al.* Cattle and environmental sample-level factors associated with the presence of *Salmonella* in a multi-state study of conventional and organic dairy farms. *Prev Vet Med* 2005; 67:39-53.

Gupta A, Fontana J, Crowe C, *et al.* Emergence of multidrug-resistant *Salmonella enterica* serotype Newport infections resistant to expanded-spectrum cephalosporins in the United States. *J Infect Dis* 2003; 188:1707-1716.

Huston CL, Wittum TE, Love BC, *et al.* Prevalence of fecal shedding of *Salmonella* spp in dairy herds. *J Am Vet Med Assoc* 2002; 220:645-649.

Islam M, Morgan J, Doyle MP, *et al.* Persistence of *Salmonella enterica* serovar Typhimurium on lettuce and parsley and in soils on which they were grown in fields treated with contaminated manure composts or irrigation water. *Foodborne Pathog Dis* 2004; 1:27-35.

Kabagambe EK, Wells SJ, Garber LP, *et al.* Risk factors for fecal shedding of *Salmonella* in 91 US dairy herds in 1996. *Prev Vet Med* 2000; 43:177-194.

L Plym F and Wierup M. *Salmonella* contamination: a significant challenge to the global marketing of animal food products. *Rev Sci Tech* 2006; 25:541-554.

Mead PS, Slutsker L, Dietz V, *et al.* Food-related illness and death in the United States. *Emerg Infect Dis* 1999; 5:607-625.

Nielsen LR, Warnick LD, Greiner M. Risk factors for changing test classification in the Danish surveillance program for *Salmonella* in dairy herds. *J Dairy Sci* 2007; 90:2815-2825.

Peters AR. An estimation of the economic impact of an outbreak of *Salmonella* Dublin in a calf rearing unit. *Vet Rec* 1985; 117:667-668.

Sanchez S, Hofacre CL, Lee MD, *et al.* Animal sources of salmonellosis in humans. *J Am Vet Med Assoc* 2002; 221:492-497.

Sivapalasingam S, Friedman CR, Cohen L, *et al.* Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *J Food Prot* 2004; 67:2342-2353.

Varma JK, Marcus R, Stenzel SA, *et al.* Highly resistant *Salmonella* Newport-MDRampC transmitted through the domestic US food supply: a FoodNet case-control study of sporadic *Salmonella* Newport infections, 2002-2003. *J Infect Dis* 2006; 194:222-230.

Voetsch AC, Van Gilder TJ, Angulo FJ, *et al.* FoodNet estimate of the burden of illness caused by nontyphoidal *Salmonella* infections in the United States. *Clin Infect Dis* 2004; 38 Suppl 3:S127-34.

Warnick LD, Crofton LM, Pelzer KD, *et al.* Risk factors for clinical salmonellosis in Virginia, USA cattle herds. *Prev Vet Med* 2001; 49:259-275.

Wells SJ, Fedorka-Cray PJ, Dargatz DA, *et al.* Fecal shedding of *Salmonella* spp. by dairy cows on farm and at cull cow markets. *J Food Prot* 2001; 64:3-11.

Wray C and Wray A. *Salmonella* in domestic animals. Oxford ; New York: CABI Pub., 2000.

You Y, Rankin SC, Aceto HW, *et al.* Survival of *Salmonella enterica* serovar Newport in manure and manure-amended soils. *Appl Environ Microbiol* 2006; 72:5777-5783.

CHAPTER 4: Fecal shedding of *Salmonella* spp among cattle admitted to a veterinary medical teaching hospital*

*Cummings KJ, Divers TJ, McDonough PL, Warnick LD. Fecal shedding of *Salmonella* spp among cattle admitted to a veterinary medical teaching hospital. *Journal of the American Veterinary Medical Association* 2009; 234: 1578–1585.

Objective—To estimate the prevalence of fecal shedding of *Salmonella* spp among bovine patients at a veterinary teaching hospital, to identify risk factors for fecal *Salmonella* shedding, and to characterize the serotypes.

Design— Retrospective cohort study.

Sample Population—5,398 hospitalized cattle.

Procedures—Data were collected for all cattle admitted during an 11-year period.

Fecal shedding of *Salmonella* spp was determined by means of standard bacteriologic culture. Multivariable logistic regression models were used to identify risk factors for fecal *Salmonella* shedding among patients.

Results—The prevalence of fecal *Salmonella* shedding among clinical suspects was 6.5% (50/768), whereas that among nonsuspects tested through routine surveillance was 2.5% (50/2,020). Among clinical suspect calves, those admitted in the fall were more likely to be shedding *Salmonella* spp (odds ratio [OR] = 5.9), as were those with septicemia (OR = 3.3) or an umbilical hernia (OR = 8.6). Among clinical suspect adult cattle, those with enteritis (OR = 9.9) or metritis (OR = 5.2) were more likely to be shedding *Salmonella* spp. Among nonsuspect cattle, none of the variables were significant predictors of shedding status. Twenty-one serotypes were detected during the study period, with the most common being *Salmonella enterica* serotypes Typhimurium (33%), Newport (23%), and Agona (12%).

Conclusions and Clinical Relevance—Seasonal and disease risk factors for fecal shedding of *Salmonella* spp were evident among clinical suspect cattle admitted to a veterinary teaching hospital. In contrast, lack of significant associations among nonsuspect cattle would suggest that targeted screening within this population is not warranted.

ABBREVIATIONS

BGN	Brilliant Green with novobiocin
CI	Confidence interval
LDA	Left displacement of the abomasum
MDR	Multidrug-resistant
OR	Odds ratio
RDA	Right displacement of the abomasum
XLT-4	Xylose-lysine-tergitol 4

Salmonella enterica is a zoonotic pathogen that is an important cause of disease in cattle. Clinical signs of bovine salmonellosis may include diarrhea, fever, anorexia, dehydration, decreased milk production, abortion, and evidence of endotoxemia, although many infections remain subclinical (Divers and Peek, 2008). Infected cattle can shed *Salmonella* organisms for variable periods and intermittently following clinically apparent or subclinical infections. Cattle in a hospital setting are likely to be at particular risk of developing salmonellosis due to concurrent disease, stress associated with transport and an unfamiliar environment, and factors related to their care (including drug administration and surgery), all of which may affect normal intestinal physiology and immunity (Divers and Peek, 2008).

This study originated in an attempt to heighten biosecurity efforts at our veterinary medical teaching hospital. The possible introduction of *Salmonella* spp into a large animal hospital via an incoming patient is a tremendous concern because of the threat of nosocomial infections. An outbreak of salmonellosis in a veterinary medical teaching hospital would have a number of serious consequences, including the risk of zoonotic transmission, morbidity and mortality among patients, economic costs

associated with lost revenue and targeted hygiene efforts, and a decrease in caseload available for teaching purposes (Tillotson *et al.*, 1997; Schott *et al.*, 2001; Ernst *et al.*, 2004).

A number of studies (Ernst *et al.*, 2004; Hird *et al.*, 1986; Traub-Dargatz *et al.*, 1990; Kim *et al.*, 2001; House *et al.*, 1999; Alinovi *et al.*, 2003) have been published on salmonellosis in equine hospital patients. Risk factors for fecal shedding of *Salmonella* spp among hospitalized horses include admission because of colic (Hird *et al.*, 1986), diarrhea at the time of admission or shortly after arrival (Traub-Dargatz *et al.*, 1990; Kim *et al.*, 2001), antimicrobial drug administration (Ernst *et al.*, 2004; Hird *et al.*, 1986; House *et al.*, 1999), nasogastric intubation (Hird *et al.*, 1986), fever (Traub-Dargatz *et al.*, 1990), change of diet while hospitalized (Traub-Dargatz *et al.*, 1990), and an increase in the mean daily ambient temperature (House *et al.*, 1999). In other studies, investigators have failed to detect a significant association between shedding of *Salmonella* spp among horses and reason for admission (Traub-Dargatz *et al.*, 1990), antimicrobial use (Traub-Dargatz *et al.*, 1990; Alinovi *et al.*, 2003), and nasogastric intubation (Alinovi *et al.*, 2003). Foals with gastrointestinal tract disease in 1 study (Ernst *et al.*, 2004) were more likely to be shedding *Salmonella* than were adults with gastrointestinal tract disease, but age was not associated with *Salmonella* shedding among horses in 2 other studies (Hird *et al.*, 1986; Traub-Dargatz *et al.*, 1990).

In contrast, little has been published on *Salmonella* infections among hospitalized cattle. In the study reported here, our objectives were to estimate the prevalence of fecal shedding of *Salmonella* spp among bovine patients, to identify possible risk factors for *Salmonella* shedding and hospital contamination within this population, and

to characterize the serotypes of the positive samples. We were particularly interested in determining whether specific characteristics of cattle admitted to a veterinary medical teaching hospital were associated with an increased risk of excreting *Salmonella* organisms in their feces and thus serving as a potential source of zoonotic and nosocomial infection.

Materials and Methods

Study design—Data were collected retrospectively for all cattle admitted to the Cornell University Equine and Farm Animal Hospital between Jan 1, 1996 and June 1, 2007, using the hospital's computerized medical records database. Variables collected for each patient included age, breed, sex, state of origin, date of admission, diagnoses, and, when performed, results of bacteriologic culture for *Salmonella* spp.

Detection of *Salmonella* organisms in the feces was conducted via 1 of 2 tests. The most commonly used test was a *Salmonella* surveillance culture, intended to be performed on samples obtained from all admitted cattle that did not have clinical signs consistent with salmonellosis. This test was primarily a biosecurity measure aimed at identifying subclinical shedders that posed a threat to the hospital environment. The second test was for cattle admitted with a combination of signs typical of salmonellosis (eg, diarrhea, fever, or dehydration) or for cattle originating from a farm with a history of endemic or epidemic salmonellosis (as determined on the basis of information voluntarily provided by the referring veterinarian or by recollection of the hospital clinician regarding previous admission of confirmed *Salmonella*-positive cattle from that farm). These patients were tested with a specific *Salmonella* culture or a broad enteric bacteriology culture panel that included *Salmonella*, depending on the age of the patient (calf vs adult) and preference of the attending clinician. The specific

Salmonella culture was more commonly selected for samples from adult cattle, whereas the enteric culture panel was generally used for samples from calves because *Escherichia coli* is a common bacterial pathogen within this age group.

Sample collection—For the purpose of *Salmonella* testing, clinicians and students in the veterinary medical teaching hospital were asked to routinely collect at least 10 g of fecal matter from each patient on the day of admission by use of an examination glove. The sample was packaged in a plastic specimen cup with a tight lid and then transported directly to the Cornell Animal Health Diagnostic Center for bacteriologic culture. Fecal samples collected after 5:00 PM were refrigerated overnight at 4°C prior to submission.

Microbiologic procedures for *Salmonella* detection—Personnel at the Animal Health Diagnostic Center used standard bacteriologic culture methods to isolate *Salmonella* organisms from fecal samples. *Salmonella* culture procedures for both the surveillance and clinical suspect samples included selective enrichment as an initial step. Fecal swab specimens from each sample container were added to 10 mL of tetrathionate broth^a (10% [wt:vol]) containing 0.2 mL of iodine solution, and the mixture was incubated at 42°C for 18 to 24 hours. After incubation, the sample-broth mixture was streaked onto plates consisting of BGN^b and XLT-4 selective media, and both plates were incubated at 37°C for 18 to 24 hours. Red colonies (lactose non-fermenting bacteria) on BGN and black colonies (hydrogen sulfide-producing bacteria) on XLT-4 were inoculated into Kligler iron agar slants, which were then incubated at 37°C for 18 to 24 hours. The XLT-4 plates without suspected colonies were incubated at 37°C for an additional 18 to 24 hours before they were again examined for characteristic black colonies. Colonies on Kligler iron agar slants that

had the biochemical properties of *Salmonella* organisms were then serogrouped by use of slide agglutination with standard protocols. Those colonies that were positive by slide agglutination were subsequently identified as *Salmonella* spp by use of an automated microbial identification system.^c Serotyping of confirmed *Salmonella* isolates was performed at the USDA, APHIS National Veterinary Services Laboratories in Ames, Iowa.

In addition to the aforementioned enrichment process, direct culture of all clinical suspect samples (but not surveillance samples) was concurrently performed on solid media, regardless of whether the animal was tested with a specific *Salmonella* culture or an enteric culture panel that included *Salmonella* spp. This was intended to enable clinicians to more rapidly reach a diagnosis when they were suspicious of *Salmonella* infection. Fecal swab specimens from each sample container were directly inoculated onto trypticase soy blood agar with 5% sheep blood, Levine eosin methylene blue agar, and BGN.

Statistical analysis—Data were imported into a commercially available statistical software program^d for variable coding and analysis. Age was converted into a categorical variable (calf, < 1 year old; yearling, ≥ 1 year old but < 2 years old; and adult, ≥ 2 years old). Date of admission was used to create a variable for season (winter, December through February; spring, March through May; summer, June through August; and fall, September through November). Breed and state of origin were converted into dichotomous variables (Holstein vs other and New York vs other, respectively). Several diseases were chosen as diagnosis predictor variables because they represented the bulk of the bovine inpatient caseload at our veterinary medical teaching hospital: ruminal bloat, LDA, RDA (with or without volvulus), traumatic

reticuloperitonitis (ie, hardware disease), enteritis, cecal dilatation-volvulus, fatty liver syndrome, pneumonia, lymphosarcoma, mastitis, metritis, dystocia, umbilical hernia, septicemia, and diseases causing lameness. Diagnoses with an obvious age restriction (such as dystocia and umbilical hernia) were limited to models involving the relevant age group.

The prevalence of fecal *Salmonella* shedding among tested bovine patients was calculated. Cattle tested for *Salmonella* spp > 1 time were considered positive when at least 1 bacteriologic culture of a fecal sample yielded a positive result. Univariable descriptive analysis was performed on all predictor variables. Bivariable analysis using the χ^2 test was used to determine whether each variable was significantly associated with *Salmonella* status (positive vs negative). Multivariable logistic regression models were used to identify possible risk factors for fecal shedding of *Salmonella* spp among hospitalized cattle, with *Salmonella* status used as the dichotomous outcome variable. Separate logistic regression models were used for each age group because some of the risk factors investigated were unique to particular age groups. Initial selection of variables was based on the bivariable analysis screening ($P < 0.25$), and a backward stepwise approach was used to identify a final multivariable model for each age group; values of $P < 0.05$ were considered significant for the final model. Relevant 2-way interaction terms were also investigated for significance within each model. Serotype information on all cattle with positive test results was obtained from the medical records.

Results

During the study period, 5,398 cattle were admitted to our veterinary medical teaching hospital. Of these, 1,438 (26.6%) were calves, 303 (5.6%) were yearlings, and 2,708

(50.2%) were adult cattle; age was not recorded for 949 (17.6%) cattle. A total of 4,790 (88.7%) were females, 4,775 (88.5%) were Holsteins, and 4,364 (80.8%) were from the state of New York. Caseload was highest in the spring (n = 1,565 cattle [29.0%]) and lowest in the fall (1,139 [21.1%]); caseload for summer and winter was 1,396 (25.9%) and 1,298 (24.0%) cattle, respectively. Disease distribution was as follows: LDA (777 cattle [14.4%]), pneumonia (598 [11.1%]), enteritis (486 [9.0%]), RDA (423 [7.8%]), metritis (356 [6.6%]), mastitis (304 [5.6%]), dystocia (190 [3.5%]), fatty liver syndrome (150 [2.8%]), septicemia (138 [2.6%]), umbilical hernia (137 [2.5%]), cecal dilatation-volvulus (98 [1.8%]), traumatic reticuloperitonitis (82 [1.5%]), ruminal bloat (79 [1.5%]), lameness (65 [1.2%]), and lymphosarcoma (44 [0.8%]). Among animals tested with the *Salmonella* surveillance culture, 66 (17.6%) calves and 177 (14.9%) adult cattle had fecal samples collected 2 or more times for bacteriologic culture; among animals tested as clinical salmonellosis suspects, 109 (36.8%) calves and 69 (20.4%) adults had fecal samples collected 2 or more times for bacteriologic culture. Because of the relatively small number of yearling cattle in the study, these animals were excluded from the statistical analysis.

Among all cattle, 2,020 (37.4%) were tested with the *Salmonella* surveillance culture, and the prevalence of fecal shedding of *Salmonella* organisms was 2.5% (50/2,020) within this group. Of the 374 calves tested with the *Salmonella* surveillance culture, 12 (3.2%) had positive results, whereas 27 of 1,185 (2.3%) adult cattle in this group had positive results (Table 4.1). Male cattle, cattle of non-Holstein breeds, and cattle admitted during the summer tended to be more likely to have positive culture results. Among the disease categories, the probability of having a positive *Salmonella* culture result ranged from 0% for lymphsarcoma or lameness to 8.8% for septicemia.

Table 4.1: Results for cattle admitted to a veterinary medical teaching hospital and tested with a *Salmonella* surveillance culture

<i>Variable</i>	<i>No. Admitted</i>	<i>No. Tested</i>	<i>% Tested</i>	<i>No. Positive</i>	<i>% Positive Among Those Tested</i>
<i>Age</i>					
Calf	1,438	374	26.0%	12	3.2%
Yearling	303	113	37.3%	1	0.9%
Adult	2,708	1185	43.8%	27	2.3%
Unknown	949	348	36.7%	10	2.9%
<i>Sex</i>					
Female	4,790	1905	39.8%	45	2.4%
Male	608	115	18.9%	5	4.3%
<i>Breed</i>					
Holstein	4,775	1805	37.8%	42	2.3%
Other breeds	623	215	34.5%	8	3.7%
<i>State of origin</i>					
New York	4,364	1563	35.8%	41	2.6%
Other states	1,034	457	44.6%	9	2.0%
<i>Season</i>					
Summer	1,396	524	37.5%	17	3.2%
Fall	1,139	464	40.7%	12	2.6%
Winter	1,298	477	36.7%	12	2.5%
Spring	1,565	555	35.5%	9	1.6%
<i>Diseases</i>					
Ruminal Bloat	79	41	51.9%	1	2.4%
LDA	777	426	54.8%	11	2.6%
RDA	423	181	42.8%	4	2.2%
Hardware Disease	82	44	53.7%	1	2.3%
Enteritis	486	129	26.5%	5	3.9%
Cecal Dilatation-Volvulus	98	48	49.0%	1	2.1%
Fatty Liver	150	46	30.7%	1	2.2%
Pneumonia	598	197	32.9%	5	2.5%
Lymphosarcoma	44	25	56.8%	0	---
Mastitis	304	157	51.6%	6	3.8%
Metritis	356	174	48.9%	5	2.9%
Dystocia	190	51	26.8%	2	3.9%
Umbilical Hernia	137	90	65.7%	2	2.2%
Septicemia	138	34	24.6%	3	8.8%
Lameness	65	32	49.2%	0	---

However, bivariable analysis via χ^2 testing failed to detect a significant relationship between any of these variables and *Salmonella* surveillance culture status.

A total of 768 (14.2%) cattle were classified as clinical salmonellosis suspects and tested with a specific *Salmonella* culture or an enteric bacteriologic culture panel that included *Salmonella* spp; the prevalence of fecal *Salmonella* shedding was 6.5% (50/768) within this group. Bivariable analysis revealed that the prevalence of fecal shedding of *Salmonella* spp was significantly higher ($P = 0.004$) in calves (9.1% [27/296]) than in adult cattle (3.6% [12/338]; Table 4.2). There was also a significant ($P < 0.001$) difference in the seasonal distribution of cattle that had positive culture

Table 4.2: Results of bivariable analysis of potential risk factors for positive *Salmonella* status among cattle considered clinical suspects for salmonellosis at the time of admission to a veterinary medical teaching hospital (only those variables for which the p-value was < 0.05 are shown)

<i>Variable</i>	<i>No. Positive (%)</i>	<i>No. Negative (%)</i>	<i>p-value</i>
<i>Age*</i>			0.004
Calves	27 (9.1%)	269 (90.9%)	
Adults	12 (3.6%)	326 (96.4%)	
<i>Season</i>			0.0002
Summer	9 (4.6%)	186 (95.4%)	
Fall	23 (13.9%)	142 (86.1%)	
Winter	11 (5.7%)	183 (94.3%)	
Spring	7 (3.3%)	207 (96.7%)	
<i>Sex</i>			0.01
Female	40 (5.8%)	654 (94.2%)	
Male	10 (13.5%)	64 (86.5%)	
<i>Enteritis</i>			0.0003
Yes	14 (15.2%)	78 (84.8%)	
No	36 (5.3%)	640 (94.7%)	
<i>Septicemia</i>			0.003
Yes	8 (19.5%)	33 (80.5%)	
No	42 (5.8%)	685 (94.2%)	

* 11 animals are excluded from this table due to yearling status or unknown age group

results, with the highest proportion in the fall (13.9% [23/165]) and the lowest in the spring (3.3% [7/214]). The prevalence of *Salmonella* shedding was significantly ($P = 0.01$) higher in male cattle (13.5% [10/74]) than in female cattle (5.8% [40/694]). Breed and state of origin were not significantly associated with *Salmonella* status among the clinical suspect cattle. The only diseases significantly associated with *Salmonella* shedding status among clinical suspects were enteritis ($P < 0.001$) and septicemia ($P = 0.003$).

Risk factors for *Salmonella* shedding—Multivariable logistic regression models were developed for calves and adult cattle tested with the *Salmonella* surveillance culture. It was found that none of the study variables were significant predictors of *Salmonella* shedding status. Multivariable logistic regression analysis for clinical suspect cattle was also performed via separate models for each age group. Calves admitted during the fall were significantly ($P = 0.004$) more likely to be shedding *Salmonella* organisms than were calves admitted in the spring (OR, 5.9; 95% CI, 1.8 to 19.6; Table 4.3). Among the disease categories, calves with septicemia (OR, 3.3; 95% CI, 1.1 to 10.4; $P = 0.04$) or an umbilical hernia (OR, 8.6; 95% CI, 1.3 to 56.8; $P = 0.03$) were significantly more likely to be shedding *Salmonella* organisms than were calves without those diseases. Adult cattle admitted during the fall had an increased but non-significant ($P = 0.15$) likelihood of *Salmonella* shedding (OR, 3.7; 95% CI, 0.6 to 22.5; Table 4.4), compared with the likelihood of *Salmonella* shedding among adult cattle admitted in the spring. Regarding the disease categories, adult cattle with enteritis (OR, 9.9; 95% CI, 2.9 to 34.6; $P < 0.001$) or metritis (OR, 5.2; 95% CI, 1.4 to 19.1; $P = 0.01$) were more likely to be shedding *Salmonella* organisms than were adult cattle without those diseases.

Table 4.3: Results of multivariable analysis of potential risk factors for positive *Salmonella* status among calves considered clinical suspects for salmonellosis at the time of admission to a veterinary medical teaching hospital

<i>Variable</i>	<i>Odds Ratio</i>	<i>95% Confidence Interval</i>	<i>p-value</i>
<i>Season</i>			
Summer	1.1	(0.2, 6.6)	0.9
Fall	5.9	(1.8, 19.6)	0.004
Winter	1.4	(0.4, 5.2)	0.6
Spring	1.0	---	---
<i>Septicemia</i>			
Yes	3.3	(1.1, 10.4)	0.04
No	1.0	---	---
<i>Umbilical Hernia</i>			
Yes	8.6	(1.3, 56.8)	0.03
No	1.0	---	---

Table 4.4: Results of multivariable analysis of potential risk factors for positive *Salmonella* status among adult cattle considered clinical suspects for salmonellosis at the time of admission to a veterinary medical teaching hospital

<i>Variable</i>	<i>Odds Ratio</i>	<i>95% Confidence Interval</i>	<i>p-value</i>
<i>Season</i>			
Summer	2.6	(0.4, 14.9)	0.3
Fall	3.7	(0.6, 22.5)	0.15
Winter	1.9	(0.3, 12.3)	0.5
Spring	1.0	---	---
<i>Enteritis</i>			
Yes	9.9	(2.9, 34.6)	0.0003
No	1.0	---	---
<i>Metritis</i>			
Yes	5.2	(1.4, 19.1)	0.01
No	1.0	---	---

Table 4.5: Distribution of *Salmonella enterica* serotypes among cattle admitted to a veterinary medical teaching hospital and tested for fecal shedding of *Salmonella* organisms

<i>Serotype</i>	<i>Salmonella</i> <i>Surveillance</i>	<i>Salmonella</i> <i>Clinical Suspect</i>	Total
Agona	6	6	12
Anatum	0	1	1
Bardo	1	0	1
Brandenburg	1	0	1
Bredeney	1	0	1
Dublin	2	3	5
Enteritidis	1	0	1
Infantis	0	1	1
Johannesburg	1	0	1
Kentucky	1	2	3
Mbandaka	0	1	1
Muenster	1	1	2
Newington	0	1	1
Newport	12	11	23
Nottingham	1	0	1
Oranienburg	3	1	4
Reading	1	0	1
Senftenberg	1	0	1
Tennessee	2	0	2
Thompson	0	1	1
Typhimurium	10	9	19
Typhimurium (Copenhagen)	2	12	14
Unknown	3	0	3
Total	50	50	100

***Salmonella* serotypes**—We identified 21 *Salmonella* serotypes during the study period (Table 4.5), with the most common being *Salmonella enterica* serotype Typhimurium, including the Copenhagen variant (33/100 [33%] isolates), *S enterica* serotype Newport (23 [23%]), and *S enterica* serotype Agona (12 [12%]). Sixteen serotypes were represented among the cattle testing positive via the surveillance culture, with *Salmonella* Typhimurium and *Salmonella* Newport each accounting for 12 of 50 (24%) isolates. Twelve serotypes were detected among the clinical suspects

with positive culture results, with the predominant serotype being *Salmonella* Typhimurium (21/50 [42%] isolates). Among all cattle with positive culture results, a clear temporal pattern was evident for the 2 most common serotypes: 30 of 33 (91%) *Salmonella* Typhimurium isolates were obtained from cattle admitted between 1996 and late 2001, whereas all of the *Salmonella* Newport isolates were obtained from cattle admitted between 2002 and 2007.

Discussion

The prevalence or risk factors (or both) for fecal shedding of *Salmonella* spp among hospitalized horses have been described in several studies that have focused on the general population of equine inpatients (Hird *et al.*, 1986; Traub-Dargatz *et al.*, 1990; House *et al.*, 1999; Alinovi *et al.*, 2003; Carter *et al.*, 1986) or horses specifically examined because of gastrointestinal tract disease (Ernst *et al.*, 2004; Kim *et al.*, 2001). In contrast, few studies have been published on hospitalized cattle. The study reported here had the advantage of the use of data in medical records gathered on bovine patients over a period of > 11 years, and it examined both the broad population of admitted cattle as well as cattle admitted with a combination of clinical signs compatible with a diagnosis of salmonellosis (eg, diarrhea, fever, and dehydration). Data from 5,398 cattle examined at our veterinary medical teaching hospital were available for the study, although slightly more than half (2,788 [51.6%]) of these cattle were tested with some form of *Salmonella* fecal culture. Bacteriologic culture of feces does not have perfect sensitivity for detecting *Salmonella* shedding, and we recognize that some cattle that were shedding *Salmonella* organisms were presumably missed by culturing and thus misclassified during the study. However, negative culture results in a specific animal would suggest that substantial fecal *Salmonella* shedding and hospital contamination were less likely. Furthermore, 37% and 20% of calves and

adult cattle that were tested as clinical suspects, respectively, were cultured for fecal *Salmonella* shedding multiple times during hospitalization.

Among the general population of cattle not suspected of having salmonellosis, the prevalence of fecal shedding of *Salmonella* spp was 2.5%. Those cattle with clinical signs typical of salmonellosis had a 6.5% prevalence of fecal *Salmonella* shedding. The prevalence of 2.5% among the general population is comparable to results obtained by researchers in California (3.5% during a 10-year period) (Ikeda *et al.*, 1986) and Montreal (1.4% during a 1-year period) (Ravary *et al.*, 1998); in both of those studies, investigators estimated the prevalence of *Salmonella* shedding among hospitalized cattle irrespective of clinical signs at the time of admission. Estimates of prevalence for fecal shedding of *Salmonella* spp among the overall population of hospitalized horses have ranged from 3% to 7% (Traub-Dargatz *et al.*, 1990; House *et al.*, 1999; Begg *et al.*, 1988), while estimates for horses examined specifically because of diseases of the gastrointestinal tract have ranged from 9% to 13% (Ernst *et al.*, 2004; Kim *et al.*, 2001; Palmer *et al.*, 1985). Our estimates of *Salmonella* prevalence among hospitalized cattle may be lower than those that would be obtained at other veterinary teaching hospitals because of the relatively low prevalence of *Salmonella* spp among cattle in the state of New York. The prevalence of fecal shedding of *Salmonella* spp among cattle in central New York has been estimated at 1.7% (Fossler *et al.*, 2005), and > 80% of the bovine caseload at our veterinary medical teaching hospital during the study period originated from the state of New York. According to the 2002 USDA National Animal Health Monitoring System report (Blau *et al.*, 2005), the herd-level prevalence of *Salmonella* shedding was significantly lower in the Northeast than in all other geographic regions of the country.

Age group was significantly associated with *Salmonella* prevalence among those cattle admitted to the hospital as clinical suspects. Prevalence of shedding was > 9% in calves and 3.6% in adult cattle. Among nonsuspect animals tested with the *Salmonella* surveillance culture, the prevalence was also higher in calves than in adult cattle, but this was not a significant difference. Foals with gastrointestinal tract disease were more likely to shed *Salmonella* organisms than were adult horses with gastrointestinal tract disease in 1 study (Ernst *et al.*, 2004), but other studies (Hird *et al.*, 1986; Traub-Dargatz *et al.*, 1990) have failed to detect an association between age and shedding among horses. Both humoral and cellular immune mechanisms play a role in resistance to *Salmonella* organisms (Lindberg and Robertsson, 1983), and calves may be at a greater risk of infection than adult cattle because of their more naïve immune system. In addition, concurrent infection with multiple enteric pathogens is a common scenario in calves, further compromising their immune status (Divers and Peek, 2008). It is also possible that a relative lack of intestinal microflora in young calves contributes to their susceptibility; such microflora is believed to offer a degree of protection against colonization by pathogenic enteric bacteria.

Season was also significantly associated with *Salmonella* prevalence among those cattle admitted to the hospital as clinical suspects. Prevalence was highest in the fall (approx 14%) and lowest in the spring (approx 3%). Among nonsuspect cattle tested with the *Salmonella* surveillance culture, prevalence was highest in the summer and lowest in the spring, but these differences were not significant. These results are in agreement with those of 2 studies (Carter *et al.*, 1986; Smith *et al.*, 1978) on hospitalized horses in which investigators evaluated the seasonality of *Salmonella* shedding. In 1 of those studies (Carter *et al.*, 1986), investigators found that the number of cases reached a peak in September and a low point in March, based on data

gathered over a period of 11 years. In the other study (Smith *et al.*, 1978), investigators found the prevalence of shedding during a 1-year period to be highest between late summer and early fall, whereas it was lowest in the spring. It has been reported in 1 study (Fossler *et al.*, 2005) that fecal shedding of *Salmonella* organisms among dairy cattle on farms is more common in the summer and fall, and investigators in other studies (Kabagambe *et al.*, 2000; Wells *et al.*, 2001; Pangloli *et al.*, 2008) have detected an increase in shedding during the summer months on dairy farms and at livestock markets. This seasonal association may be related to temperature or moisture conditions that prevail in the summer and fall months; an increase in mean daily ambient temperature has been reported to be a risk factor for fecal shedding of *Salmonella* spp among hospitalized horses (House *et al.*, 1999). It is possible that the ability of *Salmonella* to thrive and persist in warm, moist environments makes it more likely that various host species will come in contact with this organism and become infected. The effect of heat stress on cattle is another potential explanation, as such physiologic stress could predispose cattle to intestinal colonization by *Salmonella* spp and other pathogenic bacteria. However, cooling cattle by use of water sprinkling does not have a significant effect on the incidence of fecal shedding of *Salmonella* spp (Morrow *et al.*, 2005). Interestingly, the peak incidence of salmonellosis in humans has been reported by numerous sources to be during the summer months (Oloya *et al.*, 2007; Naumova *et al.*, 2007; Gradel *et al.*, 2007; D'Souza *et al.*, 2004), although this is often blamed on such factors as hygiene issues associated with outdoor cooking, increased recreational water use, and a greater tendency to travel abroad.

A number of diseases were examined to determine whether they were associated with an increased likelihood of *Salmonella* shedding among hospitalized cattle. These particular diseases were selected because they represented most of the conditions

affecting cattle admitted to our veterinary medical teaching hospital. Cattle with primary gastrointestinal tract disease (other than enteritis) during the study period were more likely to be tested with the *Salmonella* surveillance culture than were cattle with diseases of other body systems. This may have occurred because per rectal examinations would have been performed on these cattle during the initial physical examination, making it more likely that a surveillance sample was submitted. However, none of the disease variables were significant predictors of *Salmonella* status, even when separate models were used for each age group. Thus, an animal admitted because of dystocia or mastitis, for example, was just as likely to have positive results for the *Salmonella* surveillance culture as an animal admitted because of an LDA or traumatic reticuloperitonitis. Even a diagnosis of enteritis among cattle tested via surveillance culturing (rather than as clinical suspects) was not significantly associated with *Salmonella* status; this was likely related to the mild clinical signs they had, such that they were not considered salmonellosis suspects. These results suggest that among the general population of bovine patients not suspected of having salmonellosis, targeted screening of cattle with some form of primary gastrointestinal tract disease is not warranted. Rather, all such cattle should be tested with equal vigor. These results concur with those of a study (Traub-Dargatz *et al.*, 1990) of an overall population of hospitalized horses in which investigators failed to detect a significant association between *Salmonella* shedding and reason for admission. Similarly, investigators of another study (Alinovi *et al.*, 2003) involving a general population of equine patients concluded that admission because of gastrointestinal tract disease was not significantly associated with *Salmonella* status; in fact, such horses were less likely to have positive culture results than were those admitted because of respiratory tract or neurologic disease. In contrast, admission due to colic was found in 1 study

(Hird *et al.*, 1986) to be a significant risk factor for positive *Salmonella* status among a general population of hospitalized horses.

Cattle with typical signs of salmonellosis (or originating from a farm with a known history of salmonellosis) were tested with either a specific *Salmonella* culture or an enteric bacteriology culture panel that included *Salmonella* spp, as opposed to the surveillance culture performed for biosecurity reasons. Among calves, a diagnosis of septicemia or an umbilical hernia was associated with a greater likelihood of shedding *Salmonella* in the feces. The diagnosis of septicemia is used by clinicians at our veterinary medical teaching hospital to indicate presumed or confirmed disease in multiple organ systems associated with pathogenic bacteria or their toxins in the bloodstream. Because septicemia is common in calves with salmonellosis, an association with *Salmonella* shedding was not unexpected. This propensity for sepsis, combined with the dehydration and electrolyte disorders that accompany profound fluid loss, is the reason that the mortality attributable to *Salmonella* infections is greater in calves than in adult cattle (Divers and Peek, 2008). In fact, salmonellosis in calves may result in a peracute septicemia that causes death before diarrhea even becomes evident (Divers and Peek, 2008). The relationship between *Salmonella* shedding and a diagnosis of umbilical hernia is less clear. Calf hernias in our veterinary hospital are often complicated by an infectious process, either a cellulitis-abscess or an umbilical remnant infection. Perhaps the concomitant infection has an immunosuppressive effect on these calves, thus increasing their susceptibility to *Salmonella* colonization. A diagnosis of enteritis was not associated with a greater likelihood of shedding *Salmonella* organisms among calves in the study reported here. This was not a surprising finding, as salmonellosis is a relatively uncommon diagnosis in young calves admitted to our hospital with diarrhea. More commonly, calves

admitted because of enteritis are infected with *E coli*, rotavirus, coronavirus, *Cryptosporidium* spp, or a combination of these pathogens.

Among adult cattle, a diagnosis of enteritis or metritis was associated with increased odds of shedding *Salmonella* spp. Enteritis is a hallmark finding in adult cattle with salmonellosis, so an association with *Salmonella* shedding was anticipated. Diarrhea in these patients varies in severity, and the feces may contain blood and mucus; the accompanying foul smell has been described as a septic tank odor (Divers and Peek, 2008). This clinical presentation, particularly in conjunction with a fever, should always raise the index of suspicion for a diagnosis of salmonellosis in cattle. The relationship between *Salmonella* shedding and a diagnosis of metritis is not apparent. Metritis results from a bacterial infection of the uterus following parturition, which in some cows can lead to systemic signs of toxemia. It is possible that this infectious process exerts an immunosuppressive effect. Furthermore, these cattle may have some degree of gastrointestinal stasis as a result of their primary disease, and any subsequent alterations in intestinal microflora could offer an advantage to pathogenic enteric bacteria. Metritis also serves as a marker for the postpartum period, which is a time of substantial physiologic stress for dairy cows. Perhaps the combination of postpartum stress and concurrent infection, along with any gastrointestinal stasis that may exist, enhances the susceptibility of these cattle to *Salmonella* colonization. In contrast to our result in calves, a diagnosis of septicemia was not associated with a greater likelihood of shedding *Salmonella* among adult cattle. This is a predictable result because adult cattle with salmonellosis are less likely than calves to develop bacteremia (Smith, 2008), and clinical evidence of multiple organ system involvement is not typical among adult cattle infected with *Salmonella* organisms (Divers and Peek, 2008).

The most commonly detected serotypes in our study were *Salmonella* Typhimurium, *Salmonella* Newport, and *Salmonella* Agona, which accounted for 68% of the isolations. Two studies (Ikeda *et al.*, 1986; Ravary *et al.*, 1998) of *Salmonella* shedding among hospitalized cattle also revealed that *Salmonella* Typhimurium was the most frequently isolated serotype. In addition, these findings are consistent with recent data from the CDC showing that the 3 most common serotypes isolated from clinical bovine samples were *Salmonella* Typhimurium, *Salmonella* Newport, and *Salmonella* Agona, followed by *Salmonella enterica* serotype Dublin and *Salmonella enterica* serotype Montevideo (Centers for Disease Control and Prevention (CDC), 2007b). In the study reported here, *Salmonella* Newport and *Salmonella* Agona isolations were quite evenly distributed between the cattle tested with the surveillance culture and the clinical suspects, but the Copenhagen variant of *Salmonella* Typhimurium was overrepresented within the clinical suspect cattle. This suggests that this *Salmonella* Typhimurium variant may be especially pathogenic in cattle and thus more likely to cause clinical disease, as opposed to being found via routine surveillance in cattle that do not have clinical signs of salmonellosis. The relative dearth of *Salmonella* Dublin isolations in our study reflects the fact that this serotype was not common among dairy farms in the northeastern United States during the study period (Divers and Peek, 2008).

The clear temporal demarcation for the 2 most common serotypes was particularly striking because > 90% of the cattle with *Salmonella* Typhimurium were admitted during the first half of the study period, whereas all of the cattle with *Salmonella* Newport were admitted during the second half. This temporal shift reflects the potential for rapid and widespread emergence of *Salmonella* serotypes, and it seems to coincide with the historical incidence of *Salmonella* infections in people. During the

early 1990s, MDR *Salmonella* Typhimurium definitive phage type 104 emerged across the United States (Glynn *et al.*, 1998). By 1998, 30% of all foodborne *Salmonella* infections in the United States were caused by *Salmonella* Typhimurium (Centers for Disease Control and Prevention (CDC), 1999). In 2000, however, the CDC noted a sharp increase in the incidence of infections attributable to *Salmonella* Newport, primarily as a result of MDR strains (Gupta *et al.*, 2003). *Salmonella* Newport has continued to increase in importance as a pathogen for humans, while *Salmonella* Typhimurium appears to be less prevalent. According to CDC FoodNet data from 2006 (Centers for Disease Control and Prevention (CDC), 2007a), the annual incidence of foodborne infections attributable to *Salmonella* Newport in the United States had increased by 42% over the average annual incidence for 1996 through 1998. Meanwhile, the annual incidence of foodborne infections attributable to *Salmonella* Typhimurium had decreased by 41%, compared with the average annual incidence for the same baseline period (Centers for Disease Control and Prevention (CDC), 2007a). The predominance of *Salmonella* Newport among hospitalized cattle in our study between 2002 and 2007 is noteworthy because dairy cattle are considered an important reservoir for MDR *Salmonella* Newport infections in people (Gupta *et al.*, 2003; Varma *et al.*, 2006; Centers for Disease Control and Prevention (CDC), 2008).

Footnotes

- a. Difco, Detroit, Mich.
- b. Becton-Dickinson and Co, Franklin Lakes, NJ.
- c. Sensititre Automated Microbiology System A80 panel, TREK Diagnostic Systems Inc, Cleveland, Ohio.
- d. SAS, version 9.1, SAS Institute Inc, Cary, NC.

REFERENCES

- Alinovi CA, Ward MP, Couetil LL, *et al.* Risk factors for fecal shedding of *Salmonella* from horses in a veterinary teaching hospital. *Prev Vet Med* 2003; 60:307-17.
- Begg AP, Johnston KG, Hutchins DR, *et al.* Some aspects of the epidemiology of equine salmonellosis. *Aust Vet J* 1988; 65:221-3.
- Blau DM, McCluskey BJ, Ladely SR, *et al.* *Salmonella* in dairy operations in the United States: prevalence and antimicrobial drug susceptibility. *J Food Prot* 2005; 68:696-702.
- Carter JD, Hird DW, Farver TB, *et al.* Salmonellosis in hospitalized horses: seasonality and case fatality rates. *J Am Vet Med Assoc* 1986; 188:163-7.
- Centers for Disease Control and Prevention (CDC). Outbreak of multidrug-resistant *Salmonella enterica* serotype Newport infections associated with consumption of unpasteurized Mexican-style aged cheese--Illinois, March 2006-April 2007. *MMWR Morb Mortal Wkly Rep* 2008; 57:432-5.
- Centers for Disease Control and Prevention (CDC). Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food--10 states, 2006. *MMWR Morb Mortal Wkly Rep* 2007a; 56:336-9.
- Centers for Disease Control and Prevention (CDC). *Salmonella* Surveillance: Annual Summary, 2005. U.S. Department of Health and Human Services, CDC, Atlanta, GA. 2007b.
- Centers for Disease Control and Prevention (CDC). Incidence of foodborne illnesses: preliminary data from the Foodborne Diseases Active Surveillance Network (FoodNet)--United States, 1998. *MMWR Morb Mortal Wkly Rep* 1999; 48:189-94.
- Divers TJ and Peek SF. *Rebhun's diseases of dairy cattle*. St. Louis: Saunders Elsevier, 2008.
- D'Souza RM, Becker NG, Hall G, *et al.* Does ambient temperature affect foodborne disease? *Epidemiology* 2004; 15:86-92.

Ernst NS, Hernandez JA, MacKay RJ, *et al.* Risk factors associated with fecal *Salmonella* shedding among hospitalized horses with signs of gastrointestinal tract disease. *J Am Vet Med Assoc* 2004; 225: 275-281.

Fossler CP, Wells SJ, Kaneene JB, *et al.* Cattle and environmental sample-level factors associated with the presence of *Salmonella* in a multi-state study of conventional and organic dairy farms. *Prev Vet Med* 2005; 67:39-53.

Glynn MK, Bopp C, Dewitt W, *et al.* Emergence of multidrug-resistant *Salmonella enterica* serotype Typhimurium DT104 infections in the United States. *N Engl J Med* 1998; 338:1333-1338.

Gradel KO, Dethlefsen C, Schonheyder HC, *et al.* Severity of infection and seasonal variation of non-typhoid *Salmonella* occurrence in humans. *Epidemiol Infect* 2007; 135:93-9.

Gupta A, Fontana J, Crowe C, *et al.* Emergence of multidrug-resistant *Salmonella enterica* serotype Newport infections resistant to expanded-spectrum cephalosporins in the United States. *J Infect Dis* 2003; 188:1707-1716.

Hird DW, Casebolt DB, Carter JD, *et al.* Risk factors for salmonellosis in hospitalized horses. *J Am Vet Med Assoc* 1986; 188:173-7.

House JK, Mainar-Jaime RC, Smith BP, *et al.* Risk factors for nosocomial *Salmonella* infection among hospitalized horses. *J Am Vet Med Assoc* 1999; 214: 1511-1516.

Ikeda JS, Hirsh DC, Jang SS, *et al.* Characteristics of *Salmonella* isolated from animals at a veterinary medical teaching hospital. *Am J Vet Res* 1986; 47:232-5.

Kabagambe EK, Wells SJ, Garber LP, *et al.* Risk factors for fecal shedding of *Salmonella* in 91 US dairy herds in 1996. *Prev Vet Med* 2000; 43:177-194.

Kim LM, Morley PS, Traub-Dargatz JL, *et al.* Factors associated with *Salmonella* shedding among equine colic patients at a veterinary teaching hospital. *J Am Vet Med Assoc* 2001; 218:740-8.

Lindberg AA and Robertsson JA. *Salmonella* Typhimurium infection in calves: cell-mediated and humoral immune reactions before and after challenge with live virulent bacteria in calves given live or inactivated vaccines. *Infect Immun* 1983; 41:751-7.

- Morrow JL, Mitloehner FM, Johnson AK, *et al.* Effect of water sprinkling on incidence of zoonotic pathogens in feedlot cattle. *J Anim Sci* 2005; 83:1959-66.
- Naumova EN, Jagai JS, Matyas B, *et al.* Seasonality in six enterically transmitted diseases and ambient temperature. *Epidemiol Infect* 2007; 135:281-92.
- Oloya J, Theis M, Doetkott D, *et al.* Evaluation of *Salmonella* occurrence in domestic animals and humans in North Dakota (2000-2005). *Foodborne Pathog Dis* 2007; 4:551-63.
- Palmer JE, Benson CE, Whitlock RH. *Salmonella* shed by horses with colic. *J Am Vet Med Assoc* 1985; 187:256-7.
- Pangloli P, Dje Y, Ahmed O, *et al.* Seasonal incidence and molecular characterization of *Salmonella* from dairy cows, calves, and farm environment. *Foodborne Pathog Dis* 2008; 5: 87-96.
- Ravary B, Fecteau G, Higgins R, *et al.* Prevalence of infections caused by *Salmonella* spp. in cattle and horses at the Veterinary Teaching Hospital of the Faculty of Veterinary Medicine of the University of Montreal. *Can Vet J* 1998; 39:566-72.
- Schott HC, Ewart SL, Walker RD, *et al.* An outbreak of salmonellosis among horses at a veterinary teaching hospital. *J Am Vet Med Assoc* 2001; 218: 1152-9.
- Smith BP. *Large Animal Internal Medicine*. St. Louis: Mosby Elsevier, 2008.
- Smith BP, Reina-Guerra M, and Hardy AJ. Prevalence and epizootiology of equine salmonellosis. *J Am Vet Med Assoc* 1978; 172: 353-356.
- Tillotson K, Savage CJ, Salman MD, *et al.* Outbreak of *Salmonella* Infantis infection in a large animal veterinary teaching hospital. *J Am Vet Med Assoc* 1997; 211: 1554-1557.
- Traub-Dargatz JL, Salman MD, and Jones RL. Epidemiologic study of *Salmonella* shedding in the feces of horses and potential risk factors for development of the infection in hospitalized horses. *J Am Vet Med Assoc* 1990; 196:1617-22.
- Varma JK, Marcus R, Stenzel SA, *et al.* Highly resistant *Salmonella* Newport-MDRampC transmitted through the domestic US food supply: a FoodNet case-control study of sporadic *Salmonella* Newport infections, 2002-2003. *J Infect Dis* 2006; 194:222-230.

Wells SJ, Fedorka-Cray PJ, Dargatz DA, *et al.* Fecal shedding of *Salmonella* spp. by dairy cows on farm and at cull cow markets. J Food Prot 2001; 64:3-11.

CHAPTER 5: Temporal clusters of bovine *Salmonella* cases at a veterinary medical teaching hospital, 1996-2007*

*Cummings KJ, Divers TJ, McDonough PL, Switt AI, Wiedmann M, Warnick LD. Temporal clusters of bovine *Salmonella* cases at a veterinary medical teaching hospital, 1996 – 2007. *Vector-borne and Zoonotic Diseases* (Epub ahead of print).

Abstract

The objectives of this study were to identify and characterize temporal clusters of bovine *Salmonella* cases at a veterinary medical teaching hospital and to determine which clusters were likely to have involved nosocomial transmission. Data on fecal *Salmonella* shedding status, serotype, and antimicrobial resistance were collected retrospectively for all cattle admitted to the Cornell University Equine and Farm Animal Hospital between January 1, 1996 and June 1, 2007. Pulsed-field gel electrophoresis (PFGE) was performed on all available isolates. Cluster analysis was used to identify temporal clusters of cases. A total of 5,398 cattle were admitted during the study period; the prevalence of fecal *Salmonella* shedding among clinical suspects was 6.5%, while that among non-suspects tested through routine surveillance was 2.5%. Eight temporal clusters (including 57 cattle) were investigated as possible outbreaks involving nosocomial transmission, ranging in size from four to ten cases. All but one cluster were centered over the month of August or September. A total of 15 *Salmonella* serotypes were represented, with the most common being Typhimurium (33%), Newport (23%), and Agona (12%). Among the isolates available for PFGE analysis, there were 19 PFGE types represented. The majority of temporal clusters during the study period were not nosocomial in origin. However, two of the clusters were outbreaks directly resulting from nosocomial *Salmonella* transmission, based on case histories, serotype data, antimicrobial resistance patterns, and PFGE analysis. The clear seasonal pattern exhibited by these clusters underscores the need for heightened *Salmonella* vigilance during the late summer and early fall. The combination of statistical methods, routine bacteriologic data, and PFGE analysis is an effective means of conducting surveillance and outbreak investigations in a hospital setting.

Introduction

Salmonella enterica is a zoonotic pathogen that is an important cause of disease in both calves and adult cattle. Clinical signs of bovine salmonellosis may include diarrhea, fever, anorexia, dehydration, decreased milk production, abortion, and evidence of endotoxemia, although many infections remain subclinical (Divers and Peek, 2008). Hospitalized animals are likely to be at particular risk due to concurrent disease, stress associated with transport and a change in housing, and factors related to their care including surgical procedures and the administration of various drugs. Several outbreaks of salmonellosis in veterinary medical teaching hospitals have been described, primarily among horses. Such outbreaks can have a number of serious consequences, including zoonotic transmission, morbidity and mortality among patients, and economic costs associated with enhanced hygiene efforts (Tillotson *et al.*, 1997; Schott *et al.*, 2001; Ernst *et al.*, 2004). A number of these outbreaks have been severe enough to warrant temporary hospital closure (Tillotson *et al.*, 1997; Schott *et al.*, 2001; Ward *et al.*, 2005), leading to the additional losses of financial revenue and teaching caseload. The establishment of *Salmonella* transmission as nosocomial in origin requires a combination of historical, clinical, and laboratory data. However, it may be difficult to ascertain the initial source of infection in an outbreak situation (Ward *et al.*, 2005). Controlling nosocomial outbreaks of salmonellosis requires the isolation of infected animals, the identification of subclinical fecal shedders, and vigorous disinfection and biosecurity protocols.

Several studies have been published on salmonellosis in equine hospital patients, and some of these have specifically investigated the epidemiology of nosocomial infections (Hird *et al.*, 1984; Carter *et al.*, 1986; House *et al.*, 1999; Ekiri *et al.*, 2009). Risk factors for nosocomial *Salmonella* infection among hospitalized

horses include admission because of colic (Hird *et al.*, 1984) or large colon impaction in particular (House *et al.*, 1999), abdominal surgery (Ekiri *et al.*, 2009), parenteral antibiotic administration (Hird *et al.*, 1984; House *et al.*, 1999), nasogastric intubation (Hird *et al.*, 1984), an increase in the mean daily ambient temperature (House *et al.*, 1999), and shedding of the same *Salmonella* serotype by other equine patients in the days prior to admission (House *et al.*, 1999). In contrast, little has been published on *Salmonella* infections among hospitalized cattle, and we are unaware of any reports that focus on nosocomial transmission.

In this study, we examined data for cattle admitted to the Cornell University Equine and Farm Animal Hospital in Ithaca, New York between January 1, 1996 and June 1, 2007. Our objectives were to identify and characterize temporal clusters of *Salmonella* cases over this duration and to determine which clusters were very likely to have involved nosocomial transmission, using patient histories, serotype data, antimicrobial resistance patterns, and pulsed-field gel electrophoresis (PFGE) analysis. In addition, we sought to fully describe the nosocomial clusters as a means of documenting the factors that we believe in hindsight contributed to the transmission of *Salmonella* in this hospital; we hope that such information can be used to help prevent nosocomial outbreaks in the future.

Materials and Methods

Study design

As part of a larger project to determine prevalence and risk factors for *Salmonella* infection in cattle admitted to the Cornell University Equine and Farm

Animal Hospital between 1/1/96 and 6/1/07, data were collected retrospectively using the hospital's computerized medical records database (Cummings *et al.*, 2009a). Information collected for each bovine patient included age, breed, sex, state of origin, date of admission, and diagnoses. If bacteriologic culture for *Salmonella* was performed, the date of testing, result, serotype, and antimicrobial resistance profile were also gathered from the records. Fecal *Salmonella* testing took one of two forms. The most commonly used test was the *Salmonella* surveillance culture, intended to be performed on samples obtained from all admitted cattle that did not have clinical signs consistent with salmonellosis. This test was primarily a biosecurity measure aimed at identifying subclinical shedders that posed a threat to the hospital environment. The second test was used for those cattle presenting with a combination of typical signs of salmonellosis (diarrhea, fever, dehydration) or for cattle originating from a farm with a history of either endemic or epidemic salmonellosis (based on either voluntary information provided by the referring veterinarian or recollection of the hospital clinician regarding previous admission of confirmed *Salmonella*-positive cases from that farm). These patients were tested with a specific *Salmonella* culture or a broad enteric bacteriology culture panel that included *Salmonella*, depending on the age of the patient (calf vs adult) and preference of the attending clinician. The specific *Salmonella* culture was more commonly selected for samples from adult cattle, whereas the enteric culture panel was generally used for samples from calves because *Escherichia coli* is a common bacterial pathogen within this age group.

Sample collection

For the purpose of *Salmonella* testing, clinicians and students in the Equine and Farm Animal Hospital were asked to routinely collect at least 10 g of fecal matter

from each patient on the day of admission, using an examination glove. The sample was packaged in a plastic specimen cup with a tight lid and then transported directly to Cornell's Animal Health Diagnostic Center for bacteriologic culture. Fecal samples collected after 5:00 PM were refrigerated overnight at 4° C prior to submission.

Microbiologic procedures for Salmonella detection

Personnel at the Animal Health Diagnostic Center utilized standard bacteriologic culture methods to isolate *Salmonella* from feces. *Salmonella* culture procedures for both the surveillance and clinical suspect samples included selective enrichment as an initial step. Individual fecal swabs from sample containers were added to 10 ml of Tetrathionate broth (10% w/v, Difco, Detroit, MI) containing 0.2 ml of iodine solution, and the mixture was incubated at 42°C for 18–24 hours. After incubation, the sample-broth mixture was streaked onto Brilliant Green agar with novobiocin (BGN; Becton Dickinson and Company, Franklin Lakes, NJ) and Xylose Lysine Tergitol 4 (XLT-4) selective media, and both plates were incubated at 37°C for 18–24 hours. Red colonies (lactose non-fermenting bacteria) on BGN and black colonies (H₂S-producing bacteria) on XLT-4 were inoculated into Kligler Iron Agar (KIA) slants and then incubated at 37°C for 18–24 hours. XLT-4 plates without suspected colonies were re-incubated at 37°C for an additional 18–24 hours before checking again for characteristic black colonies. Colonies on KIA slants which exhibited the biochemical properties of *Salmonella* were then serogrouped by slide agglutination using standard protocols and grouping sera. Those colonies that were positive by slide agglutination were subsequently identified as *Salmonella* using the Sensititre Automated Microbiology System's A80 panel (TREK Diagnostic Systems Inc., Cleveland, OH). Serotyping of confirmed *Salmonella* isolates was performed at

the USDA, APHIS National Veterinary Services Laboratories (NVSL) in Ames, Iowa. Antimicrobial susceptibility of *Salmonella* isolates was determined by use of the broth dilution method; minimal inhibitory concentrations (MIC) were established for each isolate against a standard bovine panel in use at the AHDC at the time of submission (Sensititre, TREK Diagnostic Systems Inc.). Depending on the panel, we selected seven to nine drugs that are relevant to *Salmonella* and that represent a broad range of antimicrobial classes, although not all have practical applications in bovine medicine. Clinical and Laboratory Standards Institute (CLSI) guidelines were used to interpret MIC values (CLSI, 2008).

In addition to the enrichment process described above, direct culture of all clinical suspect samples (but not surveillance samples) on solid media was concurrently performed, regardless of whether the animal was tested with a specific *Salmonella* culture or an enteric culture panel that included *Salmonella*. This was intended to permit a quicker diagnosis when clinicians were suspicious of *Salmonella* infection. Individual fecal swabs were directly inoculated onto TSB agar with 5% sheep blood, Levine EMB agar, and Brilliant Green agar with novobiocin.

Statistical analysis

Data were imported into SAS (version 9.1; SAS Institute Inc., Cary, NC) for analysis. The prevalence of *Salmonella* fecal shedding among tested bovine patients was calculated; cattle tested for *Salmonella* more than once were considered positive if at least one fecal culture was positive. Using PROC FASTCLUS, a cluster analysis of cases was performed to search for cattle with positive *Salmonella* results grouped by test date. Consideration as a potential outbreak cluster involving nosocomial transmission required at least three positive cattle within a 30-day period.

Pulsed-field gel electrophoresis

PFGE was performed on all study isolates that were archived in the Animal Health Diagnostic Center's culture collection, using the standard CDC PulseNet protocol for *Salmonella* subtyping (Ribot *et al.*, 2006). *XbaI* was used as the restriction enzyme. *XbaI*-digested *Salmonella enterica* serotype Braenderup (CDCH9812) DNA was used as a reference size standard (Hunter *et al.*, 2005). Electrophoresis was performed for 21 h using the CHEF Mapper apparatus (Bio-Rad Laboratories, Hercules, CA). Pattern images were captured with a Bio-Rad Gel Doc and Quantity One 1-D Analysis software (Bio-Rad Laboratories). PFGE patterns were then analyzed and compared using the BioNumerics version 3.5 software (Applied Maths, Saint-Matins-Latem, Belgium). Similarity clustering analyses were performed with BioNumerics using the unweighted pair group method with arithmetic mean and the Dice correlation coefficient with a tolerance of 1.5%. PFGE patterns differing by one or more bands were considered different.

Classification of Salmonella infections as nosocomial

The decision to categorize a *Salmonella* infection as nosocomial was conditional on the following information: recovery of an isolate with a serotype, antimicrobial resistance pattern, and PFGE type which matched those of the index strain, development of diarrhea or fever at least 48-72 hours after admission, and, if applicable, having positive *Salmonella* culture results following an initial negative culture.

Results

During the study period, 5,398 bovine patients were admitted to the Cornell University Equine and Farm Animal Hospital. Among these, 2,020 (37.4%) were tested with the *Salmonella* surveillance culture, and the prevalence of fecal *Salmonella* shedding was 2.5% (50/2,020) within this group. A total of 768 bovine patients (14.2%) were classified as clinical salmonellosis suspects and therefore were tested with either a specific *Salmonella* culture or an enteric bacteriology culture panel which included *Salmonella*; the prevalence of fecal *Salmonella* shedding within this group was 6.5% (50/768).

Cluster analysis of cattle with positive *Salmonella* results revealed 23 temporal clusters ranging in size from two to 10 cases. Eight of these clusters included three or more positive animals within a 30-day time frame (Table 5.1), and these were investigated as possible outbreaks involving nosocomial transmission. The eight clusters of interest ranged in size from four to ten cases, with the median being seven cases per cluster. With the exception of one cluster, all were centered over the month of August or September. A total of 57 animals were included in these clusters; of these, 24 (42.1%) were male or female calves, 32 (56.1%) were cows, and one (1.8%) was a mature bull. Thirty (52.6%) were clinical salmonellosis suspects presenting with a combination of typical signs (diarrhea, fever, dehydration) or originating from a farm with a history of *Salmonella*, while 27 (47.4%) were non-suspect animals detected through routine surveillance. There were 15 *Salmonella* serotypes represented, with the most common being Typhimurium, including the Copenhagen variant (33%), Newport (23%), and Agona (12%). Among the 57 animals involved in the eight clusters under investigation, 42 isolates were available for PFGE analysis. A total of 19 PFGE types were represented (Figure 5.1), with the number of isolates

Table 5.1: Summary of the eight case clusters under investigation

Cluster no.	Date of admission	Date of sampling	Animal	Herd	Serotype	Antimicrobial resistance*	PFGE type
1	9/6/1996	9/12/96	Cow	A	Typhimurium (Copenhagen)	Amp, Fis, Tet	7
	9/19/1996	9/20/96	Calf	A	Typhimurium (Copenhagen)	Amp, Fis, Tet	6
	9/19/1996	9/20/96	Calf	A	Typhimurium (Copenhagen)	Amp, Fis, Tet	6
	9/19/1996	9/20/96	Calf	A	Typhimurium (Copenhagen)	NA	6
	11/18/1996	11/19/96	Cow	B	Muenster	---	18
	12/16/96	12/16/96	Calf	C	Typhimurium (Copenhagen)	Amp, Tet	NA
2	8/20/99	8/20/99	Cow	D	Typhimurium (Copenhagen)	Amp, Ffn	8
	9/27/1999	9/28/99	Calf	E	Typhimurium	Amp, Ffn, Gen, Sxt, Tet	NA
	10/2/1999	10/5/99	Calf	E	Typhimurium	Amp, Ffn, Gen, Sxt, Tet	NA
	10/2/1999	10/5/99	Calf	E	Typhimurium	Amp, Ffn, Gen, Sxt, Tet	NA
3	9/6/2001	9/7/01	Cow	F	Typhimurium	Amp, Ffn, Oxy, Sdm, Spe	NA
	9/8/2001	9/10/01	Cow	F	Typhimurium	Amp, Ffn, Oxy, Sdm, Spe	1
	9/15/2001	9/16/01	Cow	F	Typhimurium	Amp, Ffn, Oxy, Sdm, Spe	1
	9/19/2001	9/19/01	Cow	F	Unknown	NA	NA
	9/27/2001	9/28/01	Cow	G	Typhimurium	Amp, Ffn, Oxy, Sdm, Spe	1
	10/15/01	10/15/01	Cow	F	Typhimurium	Amp, Ffn, Oxy, Sdm, Spe	1
	11/6/01	11/6/01	Calf	H	Typhimurium (Copenhagen)	Amp, Ffn, Oxy, Sdm, Spe	NA
	11/6/01	11/6/01	Calf	H	Typhimurium (Copenhagen)	Amp, Ffn, Oxy, Sdm, Spe	NA
	11/6/01	11/6/01	Calf	H	Typhimurium (Copenhagen)	Amp, Ffn, Oxy, Sdm, Spe	NA

Table 5.1 (Continued)

Cluster no.	Date of admission	Date of sampling	Animal	Herd	Serotype	Antimicrobial resistance*	PFGE type
4	6/27/2002	6/29/02	Calf	I	Tennessee	Ffn, Spe	9
	7/23/2002	7/30/02	Cow	F	Kentucky	Ffn	5
	8/2/2002	8/6/02	Cow	J	Oranienburg	Ffn, Spe	NA
	8/2/2002	8/8/02	Cow	K	Typhimurium (Copenhagen)	Amp, Ffn, Oxy, Sdm, Spe, Sxt	6
	8/2/2002	8/12/02	Cow	L	Newport	Amp, Ffn, Oxy, Sdm, Tio	12
	8/7/2002	8/12/02	Cow	J	Newport	Amp, Ffn, Oxy, Sdm, Tio	12
	8/10/2002	8/20/02	Calf	M	Newport	Amp, Ffn, Oxy, Sdm, Tio	12
	9/6/2002	9/17/02	Cow	J	Newport	Amp, Ffn, Oxy, Sdm, Tio	12
	9/20/2002	9/25/02	Cow	J	Oranienburg	Ffn	14
	10/9/2002	10/9/02	Cow	J	Oranienburg	Ffn, Spe	15
5	7/5/2003	7/6/03	Cow	N	Tennessee	Ffn, Spe	10
	7/14/2003	7/15/03	Cow	O	Bardo	Amp, Ffn, Oxy, Sdm, Tio	NA
	7/30/2003	8/2/03	Calf	P	Agona	Amp, Ffn, Oxy, Sdm, Spe, Sxt, Tio	19
	9/14/2003	9/15/03	Cow	Q	Enteritidis	NA	NA
	9/16/03	9/16/03	Calf	R	Infantis	Amp, Ffn, Oxy, Sdm, Spe, Sxt, Tio	11
	9/18/2003	9/19/03	Cow	S	Newport	Amp, Ffn, Oxy, Sdm, Spe, Tio	13

Table 5.1 (Continued)

Cluster no.	Date of admission	Date of sampling	Animal	Herd	Serotype	Antimicrobial resistance*	PFGE type
5 (cont.)	9/29/2003	10/1/03	Cow	G	Newport	Amp, Ffn, Oxy, Sdm, Tio	12
6	10/27/2004	10/28/04	Calf	T	Newport	NA	NA
	1/10/2005	1/11/05	Cow	U	Newport	Amp, Ffn, Oxy, Sdm, Spe, Tio	12
	1/10/2005	1/12/05	Cow	U	Newport	NA	12
	1/11/2005	1/19/05	Cow	V	Newport	NA	12
	12/31/2004	1/24/05	Bull	W	Newport	Amp, Ffn, Oxy, Spe, Tio	12
	1/11/2005	1/24/05	Calf	X	Newport	Amp, Ffn, Oxy, Sdm, Spe, Tio	12
	1/17/2005	1/24/05	Cow	Y	Newport	Amp, Ffn, Oxy, Sdm, Spe, Tio	12
7	8/6/2005	8/13/05	Calf	Z	Agona	Amp, Ffn, Oxy, Sdm, Spe, Sxt, Tio	19
	8/6/2005	8/14/05	Calf	Z	Agona	Amp, Ffn, Oxy, Sdm, Spe, Sxt, Tio	19
	8/16/2005	8/17/05	Calf	AA	Agona	Amp, Ffn, Oxy, Sdm, Spe, Sxt, Tio	19
	8/26/2005	8/26/05	Cow	BB	Kentucky	Sdm, Spe	3
	9/28/2005	9/29/05	Cow	CC	Mbandaka	Spe	NA
	10/14/2005	10/15/05	Cow	DD	Johannesburg	Sdm, Spe	NA
	12/2/2005	12/4/05	Calf	EE	Agona	Amp, Ffn, Oxy, Sdm, Spe, Sxt, Tio	19
	11/25/2005	12/12/05	Calf	FF	Agona	Amp, Ffn, Oxy, Sdm, Spe, Sxt, Tio	19

Table 5.1 (Continued)

Cluster no.	Date of admission	Date of sampling	Animal	Herd	Serotype	Antimicrobial resistance*	PFGE type
7 (cont.)	11/25/2005	12/23/05	Calf	FF	Agona	Amp, Ffn, Oxy, Sdm, Spe, Sxt, Tio	19
8	8/13/2006	8/13/06	Cow	GG	Typhimurium	Sdm, Spe	2
	8/31/2006	8/31/06	Cow	F	Brandenburg	Sdm, Spe	17
	9/1/06	9/1/06	Calf	HH	Dublin	Amp, Ffn, Oxy, Sdm, Spe, Tio	4
	9/8/06	9/8/06	Calf	HH	Dublin	Amp, Ffn, Oxy, Sdm, Spe, Tio	4
	9/21/06	9/21/06	Cow	II	Reading	Amp, Ffn, Oxy, Sdm, Spe, Tio	16

* Abbreviations: Amp=Ampicillin, Ffn=Florfenicol, Fis=Sulfisoxazole, Gen=Gentamicin, Oxy=Oxytetracycline, Sdm=Sulfadimethoxine, Spe=Spektinomycin, Sxt=Trimethoprim/Sulfamethoxazole, Tet=Tetracycline, Tio=Ceftiofur, NA=Not available

corresponding to each type ranging from one to 11.

Clusters 1, 2, 3, and 4 occurred during the late summer and early fall of 1996, 1999, 2001, and 2002 respectively (Table 5.1). Cluster 1 contained three calves and one cow from the same herd that was experiencing an outbreak of salmonellosis (herd A), and these animals presented in September of 1996. All four were positive for the Copenhagen variant of Typhimurium. Antimicrobial susceptibilities for three of the isolates (from the cow and two of the calves) were determined, and the MIC was measured against a panel of seven antimicrobial agents: ampicillin, ceftiofur, enrofloxacin, gentamicin, sulfisoxazole, tetracycline, and trimethoprim/sulfadiazine.

The MIC value for each drug was identical in each of the calf isolates, indicating *in vitro* resistance to three of the seven antibiotics. The cow isolate displayed the same overall resistance pattern, although the actual MIC value for ceftiofur was higher. The three calf isolates all shared the same PFGE type (type 6, Figure 5.1), while the cow isolate differed from this pattern by one band (type 7). Two other animals admitted later in the fall also tested positive for *Salmonella* and were included in this temporal cluster, but their infections were presumably not nosocomial because both had presented to the hospital with a chief complaint of diarrhea and were positive on the initial admission sample.

Similarly, cluster 2 contained three calves from a herd that was having an outbreak of salmonellosis (herd E), and these animals presented in late September and early October of 1999. All were positive for Typhimurium. Antimicrobial susceptibilities for the three isolates were determined, and the MIC was measured against a similar panel of seven drugs (florfenicol replaced sulfisoxazole, and trimethoprim/sulfamethoxazole substituted for trimethoprim/sulfadiazine). The MIC value for each drug was identical in all three isolates, indicating *in vitro* resistance to five of the seven antibiotics. None of the three calf isolates were available for PFGE typing. The only other animal in this temporal cluster was a cow admitted in August of that year for mastitis, but this cow was found via routine surveillance to be shedding a different serotype with a unique antimicrobial resistance profile.

Cluster 3 was comprised of nine cases, including five cows from a farm with a history of endemic salmonellosis (herd F). These five were all admitted for surgical repair of a left displaced abomasum (LDA) between September and October, 2001. Four of these animals tested positive for Typhimurium; the fifth was reported as being positive for *Salmonella* serogroup B (the same serogroup as Typhimurium) but was not serotyped. Antimicrobial susceptibilities for four of the isolates were determined,

and the MIC was measured against a panel of nine drugs: ampicillin, ceftiofur, enrofloxacin, florfenicol, gentamicin, oxytetracycline, spectinomycin, sulfadimethoxine, and trimethoprim/sulfamethoxazole. The MIC value for each antimicrobial agent was identical in all four isolates, indicating *in vitro* resistance to five of the nine drugs. Three of the four isolates were available for PFGE analysis, and these were found to share the same PFGE type (type 1) as well. Also included in this temporal cluster were three calves from a herd that was having an outbreak of salmonellosis (herd H). These animals presented in November of 2001, and all were positive for the Copenhagen variant of Typhimurium. Antimicrobial susceptibilities for all three of the isolates were determined, and the MIC was established for the same nine antimicrobial agents; the MIC value for each was the same in all three isolates, again indicating *in vitro* resistance to five of the nine drugs. None of the three isolates were available for PFGE typing. The final animal in this cluster was a cow admitted in late September of that year for repair of an LDA. Routine surveillance testing revealed that this cow was shedding Typhimurium, and the antimicrobial resistance profile and PFGE type (type 1) of this isolate matched those of the other Typhimurium isolates obtained between early September and mid-October of 2001. This was likely a nosocomial infection, but the animal did not develop clinical signs while hospitalized and was discharged shortly after surgery.

Cluster 4 contained a total of 10 cases, including five cows from a different farm with a history of endemic salmonellosis (herd J). These animals were all admitted for surgical repair of a LDA between August and October, 2002. Two of these animals tested positive for Newport, while the other three were found to be shedding Oranienburg. Antimicrobial susceptibilities for both Newport isolates were determined using the same nine-drug panel. The MIC for each antimicrobial agent was the same in both, indicating *in vitro* resistance to five of the nine antibiotics; these

Disc (0 at 1.50%) (Tot 1.5% - 1.5%) (+0.0% S+0.0%) [0.0%-100.0%]
 PFGE-XbaI PFGE-XbaI

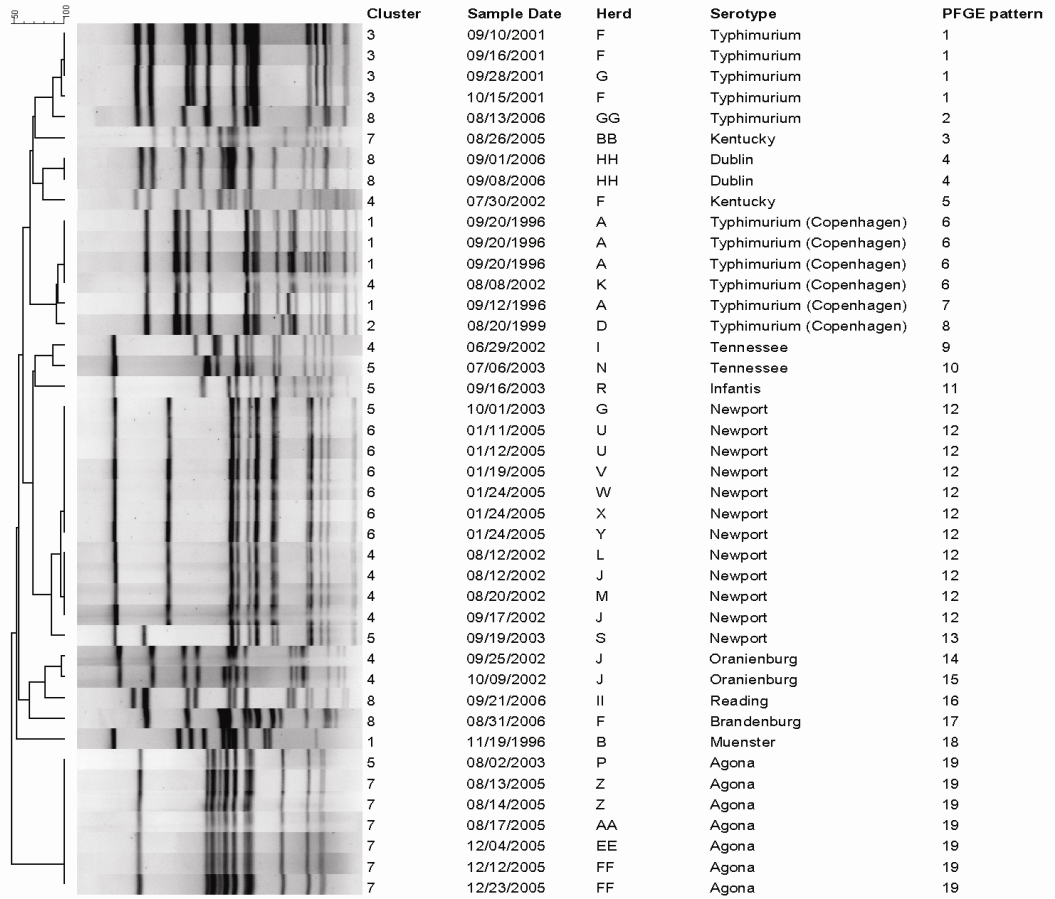


Figure 5.1: *XbaI* PFGE patterns for 42 isolates representing the eight case clusters under investigation (15 isolates were not available for PFGE analysis)

two Newport isolates also shared the same PFGE type (type 12). This temporal cluster included an additional five animals admitted late in the summer of that year, all from separate herds. Three of these tested positive for serotypes and PFGE types that were unique to those individual animals. However, the other two were found to be shedding Newport; the antibiotic resistance profiles and PFGE types (type 12) of these isolates matched those of the two Newport isolates from the other animals in this cluster, suggesting nosocomial transmission. Notably, both animals had remained in the hospital for an extended period of time. The first was a cow presenting in the beginning of August with a history of lethargy and decreased appetite. This cow developed diarrhea during the latter part of her hospital stay (15 days) and was subsequently found to be shedding Newport. The second animal was a calf presenting in early August with lameness of non-infectious etiology. Near the midpoint of this calf's hospital stay (19 days), a *Salmonella* culture came back positive for Newport, although this animal never developed clinical signs while hospitalized. Both of these patients were discharged later in the month.

Clusters 5 and 8 occurred during the late summer and early fall of 2003 and 2006, respectively (Table 5.1). These differed from the previous clusters in that they did not consist primarily of multiple animals from herds that were experiencing either endemic or epidemic salmonellosis. Cluster 5 included two calves and five cows, all from different herds. Two animals had presented to the hospital with diarrhea and were positive on the initial admission sample, and the other five were discovered to be shedding *Salmonella* through routine surveillance. All were admitted between July and October, 2003. Six serotypes were represented in this cluster, with Newport being the only one isolated from more than a single animal. The first cow shedding Newport had been admitted in mid-September for decreased appetite and a possible LDA, while the second cow presented with a chief complaint of diarrhea at the end of that month.

However, the two isolates had unique antimicrobial resistance profiles and PFGE types.

Cluster 8 included two calves and three cows admitted during August and September, 2006. The calves came from a herd that had experienced a recent rise in calf illness and mortality, with typical signs including fever, lethargy, and anorexia, eventually followed by dyspnea and diarrhea. Adult cattle in this herd were apparently unaffected. Both calves tested positive for Dublin and were ultimately euthanized. Antimicrobial susceptibility testing was performed on both isolates using the same panel, and both had matching MIC values which indicated *in vitro* resistance to six of the nine antibiotics; these two Dublin isolates also shared the same PFGE type (type 4). The three cows in this temporal cluster each came from different herds. They were not clinical suspects on admission, but all were found to be shedding *Salmonella* via routine surveillance testing. Serotypes and PFGE types were unique to these three animals.

In contrast to the temporal clusters previously described, clusters 6 and 7 appeared to be caused by nosocomial transmission. Cluster 6 included a total of seven animals, six of which were admitted in January, 2005 (Table 5.1). Around the first of the year, a herd owner from a neighboring state had called to discuss an outbreak of profuse diarrhea among his adult animals (herd U), and the consulting clinician advised a veterinary visit from one of the local ambulatory practices. One week later, however, two cows from this farm arrived unannounced at the hospital, late in the evening on January 10. They presented with fever, dehydration, and severe diarrhea containing blood and mucus. These animals were housed overnight in the main bovine ward of the hospital and were not transferred to the isolation ward until the following morning. *Salmonella* cultures on both patients were positive for *S.* Newport. Antimicrobial susceptibility testing was performed on one of the isolates,

and the MIC values indicated *in vitro* resistance to six of the nine antibiotics. Both of these isolates shared the same PFGE type (type 12). Of these two presumptive index cases, one cow died the day after admission while the other recovered and was discharged. Over the remainder of the month, four other animals presenting with various chief complaints all tested positive for Newport at least one week past their admission date; these four patients had also been tested at admission and were found to have negative *Salmonella* culture results at that time. All four Newport isolates had the same PFGE type (type 12) as the first two. Antimicrobial susceptibility testing was performed on three of these isolates, and the resistance profiles matched that of the original Newport isolate (except that one isolate had a lower MIC value for sulfadimethoxine). A calf with pneumonia developed diarrhea and was kept in the hospital until clinical recovery and five negative *Salmonella* cultures were achieved. A bull admitted for abomasal dysfunction had transient mild diarrhea and fever but also recovered clinically before being discharged. However, two cows that tested positive for Newport (one presented for RDA, the other for fatty liver syndrome) developed diarrhea and were ultimately euthanized. Thus, the case fatality rate within this cluster was 50% (3/6), including the index animal which died. The only other animal included in this group via temporal cluster analysis was a calf admitted in late October, 2004, but this case appears to have had no relationship with the nosocomial outbreak based on the earlier date of hospitalization.

Cluster 7 included a total of nine animals admitted between August and December, 2005 (Table 5.1). Two calves with atresia coli from the same herd were admitted in early August (herd Z), and both developed post-surgical diarrhea. *Salmonella* cultures on both patients were positive for Agona. Antimicrobial susceptibility testing was performed on these isolates, and the MIC values indicated *in vitro* resistance to seven of the nine antimicrobial agents. Both of the isolates were

available for PFGE analysis, and these were found to share the same PFGE type (type 19). The two calves made uneventful recoveries and were discharged. Over the next few months, four additional calves presenting for various reasons all tested positive for Agona (three of which had also been tested at admission and were initially found to be *Salmonella*-negative), and all four isolates shared the same PFGE type (type 19) as the first two. Antimicrobial susceptibility testing was also performed on all four of these isolates, and the resistance profiles matched those of the original two Agona isolates. Two newborn calves from the same herd were presented for weakness; one developed a fever and diarrhea while hospitalized, and the other became febrile only. Their *Salmonella*-positive culture samples were obtained over two weeks beyond their admission date. Both recovered clinically and were subsequently found to be *Salmonella* culture negative before being discharged. Another calf admitted for umbilical hernia also developed diarrhea but recovered prior to discharge. The fourth calf presented for dyspnea, but there was no clinical evidence of *Salmonella* infection during hospitalization; this animal was also discharged following successful treatment for an upper airway obstructive disorder. Unlike the previous nosocomial cluster, there was no mortality in this outbreak. The two calves with atresia coli were believed to be the index cases, shedding *Salmonella* into the environment after corrective surgery. There were also three cows linked with the six calves by temporal cluster analysis, but each had a unique serotype.

Discussion

Cluster analysis is a subjective process, dependent on the criteria used to define the clusters at the outset. In searching for clusters that may have involved nosocomial

transmission, it was decided that a cluster had to include at least three positive cattle within a 30-day period in order to warrant further investigation. Eight of the 23 temporal clusters were selected based upon this guideline. *Salmonella* infections were regarded as nosocomial if the isolate matched the index strain by serotype, antimicrobial resistance pattern, and PFGE type. Clinical signs of salmonellosis, if present, had to have developed at least 48-72 hours after admission; in almost all cases, a positive *Salmonella* culture result followed an initial negative culture. In particular, PFGE subtyping has been shown to be a highly effective means of analyzing veterinary hospital outbreaks (Amavisit *et al.*, 2001; Schott *et al.*, 2001; Ward *et al.*, 2005; Dunowska *et al.*, 2007) because it facilitates an assessment of epidemiologic relatedness. This study illustrates the utility of combining statistical methods, routine bacteriologic data, and molecular subtyping when conducting surveillance and outbreak investigations.

Bacteriologic culture of feces does not have perfect sensitivity for detecting the presence of *Salmonella*, and we recognize that some cattle that were shedding *Salmonella* organisms were presumably missed by culturing. It is also possible that we could have misclassified a *Salmonella* infection acquired prior to admission as being nosocomial. However, our classification of cases as nosocomial in origin was supported in all instances by the aforementioned laboratory data used for isolate characterization, in addition to patient historical information.

The majority of temporal clusters during this study period were not nosocomial outbreaks. Clusters 1 through 4 were related to the admission of a number of animals from the same herd that was experiencing either endemic or epidemic salmonellosis. Herd outbreaks of salmonellosis are not unusual in the northeastern United States. For example, a recent field study of over 800 dairy herds in this region identified salmonellosis in 11% of herds (93/831) monitored over a one-year duration; of these,

57% (53/93) had multiple cases (Cummings *et al.*, 2009b). Clusters 1 through 4, in addition to clusters 5 and 8, all occurred in the late summer and early fall. Using data from the same time frame to investigate all *Salmonella*-positive cattle admitted to our hospital during the study period, the authors found previously that there was a significant difference in the seasonal distribution of clinical suspect cattle with positive culture results, with the highest proportion in the fall and the lowest in the spring (Cummings *et al.*, 2009a). It has been reported that fecal *Salmonella* shedding among dairy cattle on farms is more common in the summer and fall (Fossler *et al.*, 2005), and other studies have found increased shedding during the summer months on dairy farms and at livestock markets (Kabagambe *et al.*, 2000; Wells *et al.*, 2001; Pangloli *et al.*, 2008). Two studies on hospitalized horses had similar results; one found that the number of *Salmonella* cases reached a peak in September, based on data gathered over a period of 11 years (Carter *et al.*, 1986), and the other found the prevalence of shedding over a one-year period to be highest between late summer and early fall (Smith *et al.*, 1978). This seasonal association is presumably related to temperature and/or moisture conditions that prevail in the summer and fall months, but whether these conditions are impacting the bacteria or the host species is unclear. *Salmonella*'s ability to thrive in warm, moist environments may increase the odds of host exposure and infection, or perhaps heat stress in cattle leads to suppressed immunity. The seasonal pattern of these clusters underscores the need for heightened awareness during the late summer and early fall. The potential for both nosocomial and zoonotic transmission will likely be greater during this time of year. Although none of the aforementioned temporal clusters originated solely from nosocomial transmission, it is very probable that three of the involved animals (one cow in cluster 3 and a cow and calf in cluster 4) did acquire their *Salmonella* infection in the hospital,

based on the timeline of positive culture status and the serotype, antimicrobial resistance, and PFGE data.

Clusters 6 and 7, on the other hand, appeared to directly result from nosocomial *Salmonella* transmission. In both instances, we believe that we have identified specific bovine patients as the original source of infection. The admission of two cows with profuse diarrhea into the main bovine ward of the hospital (cluster 6) probably occurred because of their unexpected arrival after normal business hours. It is likely that significant contamination of the hospital environment occurred prior to the transfer of these patients to the isolation ward the following morning. In addition, three of the other animals in this outbreak cluster overlapped temporally with the admission of the index cases; the bull had already been in the hospital for 10 days, and the calf with pneumonia and cow with fatty liver syndrome were admitted the next day. This suggests that hospital personnel (clinicians, students, technicians, and animal care attendants) and shared equipment may have played a role in spreading *Salmonella* among these patients.

The presumptive index cases in cluster 7 were two calves that presented for atresia coli. These animals obviously did not have diarrhea on admission and were not considered clinical suspects at that time. Both developed diarrhea post-surgically, leading to contamination of the calf housing area of the bovine ward. Four animals were believed to have become infected via nosocomial transmission in the subsequent few months, and all were calves housed in close proximity. Thus, it is suspected that the cleaning and disinfection of this area of the hospital was not adequate to completely eliminate the source of infection. Persistence of *Salmonella* in the environment for months to even years has been documented in a number of outbreaks (Hartmann *et al.*, 1996; Tillotson *et al.*, 1997; Amavisit *et al.*, 2001; Schott *et al.*, 2001; Ward *et al.*, 2005; Dunowska *et al.*, 2007), suggesting that this is an important

cause of nosocomial transmission. Areas of the hospital environment that are most likely to harbor *Salmonella* include floor drains, stall walls and floors, corners and cracks within stalls, and rubber mats such as those used in anesthetic recovery rooms (Tillotson *et al.*, 1997; Ewart *et al.*, 2001; Schott *et al.*, 2001; Alinovi *et al.*, 2003; Ward *et al.*, 2005). The ability of a single *Salmonella* clone to persist and cause nosocomial infections in a veterinary hospital for a period of up to several years (Amavisit *et al.*, 2001; Dunowska *et al.*, 2007) is truly an alarming prospect, and it emphasizes the significant danger presented by fecal contamination in this setting. Interestingly, the PFGE subtypes responsible for the two nosocomial outbreaks of 2005 (types 12 and 19) had been identified among bovine patients from different herds during the clusters of 2002 and 2003. However, we have found PFGE types 12 and 19 to be relatively common among New York dairy cattle. Combined with the historical information surrounding the 2005 nosocomial outbreaks, these data suggest separate introductions of these PFGE types into our hospital over time.

The issues that played a role in facilitating these two nosocomial outbreaks are common to large animal hospitals in general, and similar factors have been implicated in multiple *Salmonella* outbreaks among horses (Hartmann *et al.*, 1996; Tillotson *et al.*, 1997; Schott *et al.*, 2001; Ward *et al.*, 2005). Other factors have also been hypothesized to have contributed to the spread of *Salmonella* infection during equine outbreaks, and these should be considered as well when dealing with infections among hospitalized cattle. High-pressure power sprayers may be less effective at removing bacterial contamination than scrubbing by hand, and they may in fact promote the aerosolization of infectious particles (Hartmann *et al.*, 1996; Tillotson *et al.*, 1997). This could conceivably lead to inadvertent exposure of patients in neighboring stalls. Rodents located in a teaching hospital during an outbreak have been found to be

infected with the same *Salmonella* serotype (Tillotson *et al.*, 1997); thus, they may serve as vectors of infection, perhaps by contaminating the feed supply.

Antimicrobial resistance was widespread among the cluster isolates; susceptibility testing was performed on 51 of the isolates, and 47 of them (92.2%) displayed *in vitro* resistance to at least two antimicrobial agents. Although studies of fecal *Salmonella* shedding among clinically healthy cattle have shown antimicrobial resistance to be uncommon (Wells *et al.*, 2001; Blau *et al.*, 2005; Ray *et al.*, 2007), multidrug resistance was found to be highly prevalent among isolates from cattle with clinical salmonellosis in the northeastern U.S. (Cummings *et al.*, 2009b), particularly among those serotypes that were predominant in the present study (Typhimurium, Newport, and Agona). An association between clinical disease and multidrug resistance would suggest that antimicrobial therapeutic options are likely to be limited when treating cattle with salmonellosis, and it has clear public health implications as well.

The zoonotic potential of *Salmonella* adds a crucial element to the outbreak clusters reported here, regardless of whether or not they involved nosocomial transmission. While we are unaware of any human cases associated with these clusters, the risk of zoonotic transmission should never be overlooked. Clinicians and hospital administrators are obligated to educate students and clients regarding protective hygiene practices such as washing well after handling animals, thoroughly disinfecting boots and equipment, and not taking boots and coveralls home. Particular care should be taken to prevent exposure of high-risk individuals such as children, the elderly, and those with immunosuppressive health conditions.

A number of measures have been taken in our hospital to reduce both nosocomial and zoonotic transmission of salmonellosis, including biosecurity training sessions for hospital personnel, restriction of human traffic, disinfectant foot mats

(The Coburn Company, Inc., Whitewater, WI) with Trifectant (Vetoquinol USA, Buena, NJ) in front of every stall and at the entrance to each hospital ward, hand disinfection stations on the walls throughout the wards and at all hand-washing sinks, and disposable gowns and booties for working with neonatal calves, all clinical suspect cattle, and cattle from herds with a history of *Salmonella*. There is a general decrease in the use of shared equipment between patients; for example, separate thermometers are used for each individual patient and are stored outside the stall. Other types of equipment, such as feed buckets, oral specula, stomach tubes, and balling guns, are disinfected or sterilized after each patient use. All non-cleanable surfaces and equipment, such as wooden feed carts, have been removed or replaced with something having a non-porous surface. Any animal that presents as a salmonellosis suspect is admitted directly to the isolation ward, and those cattle that are found to be asymptomatic shedders through surveillance testing are promptly transferred to isolation. Floors throughout the hospital are disinfected with Trifectant or a similar product at least twice daily, using an industrial walk-behind scrubber (Advance Convertamatic; Nilfisk-Advance, Inc., Plymouth, MN). Upon discharge of each bovine patient, the stall is thoroughly cleaned and disinfected with Trifectant. If a given patient was not a clinical suspect but was found to be shedding *Salmonella* during its stay, that animal's stall is not cleared for subsequent use until *Salmonella* culture of the stall environment (floor, walls, and feed and water containers) is determined to be negative. In addition, a designated member of the hospital's infection control committee collects at least 15 samples monthly for environmental *Salmonella* surveillance culture, focusing on floors and drains throughout the hospital, stall walls and floors, and equipment. Finally, the use of PFGE is increasingly being combined with other forms of isolate characterization (serotype and antimicrobial resistance pattern) in order to monitor for persistence of particular *Salmonella* strains

within the hospital environment and to more quickly recognize potential hospital outbreaks.

REFERENCES

- Alinovi CA, Ward MP, Couetil LL, *et al.* Detection of *Salmonella* organisms and assessment of a protocol for removal of contamination in horse stalls at a veterinary teaching hospital. *J Am Vet Med Assoc* 2003; 223: 1640-1644.
- Amavisit P, Markham PF, Lightfoot D, *et al.* Molecular epidemiology of *Salmonella* Heidelberg in an equine hospital. *Vet Microbiol* 2001; 80: 85-98.
- Blau DM, McCluskey BJ, Ladely SR, *et al.* *Salmonella* in dairy operations in the United States: prevalence and antimicrobial drug susceptibility. *J Food Prot* 2005; 68:696-702.
- Carter JD, Hird DW, Farver TB, *et al.* Salmonellosis in hospitalized horses: Seasonality and case fatality rates. *J Am Vet Med Assoc* 1986; 188: 163-167.
- Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard - Third Edition*. CLSI document M31-A3. Wayne, PA: Clinical and Laboratory Standards Institute. 2008.
- Cummings KJ, Divers TJ, McDonough PL, *et al.* Fecal shedding of *Salmonella* spp among cattle admitted to a veterinary medical teaching hospital. *J Am Vet Med Assoc* 2009a; 234: 1578-1585.
- Cummings KJ, Warnick LD, Alexander KA, *et al.* The incidence of salmonellosis among dairy herds in the northeastern United States. *J Dairy Sci* 2009b; 92:3766-3774.
- Divers TJ and Peek SF. *Rebhun's diseases of dairy cattle*. St. Louis: Saunders Elsevier, 2008.
- Dunowska M, Morley PS, Traub-Dargatz JL, *et al.* Comparison of *Salmonella enterica* serotype Infantis isolates from a veterinary teaching hospital. *J Appl Microbiol* 2007; 102: 1527-1536.
- Ekiri AB, MacKay RJ, Gaskin JM, *et al.* Epidemiologic analysis of nosocomial *Salmonella* infections in hospitalized horses. *J Am Vet Med Assoc* 2009; 234: 108-119.

Ernst NS, Hernandez JA, MacKay RJ, *et al.* Risk factors associated with fecal *Salmonella* shedding among hospitalized horses with signs of gastrointestinal tract disease. J Am Vet Med Assoc 2004; 225: 275-281.

Ewart SL, Schott HC, Robison RL, *et al.* Identification of sources of *Salmonella* organisms in a veterinary teaching hospital and evaluation of the effects of disinfectants on detection of *Salmonella* organisms on surface materials. J Am Vet Med Assoc 2001; 218: 1145-1151.

Fossler CP, Wells SJ, Kaneene JB, *et al.* Cattle and environmental sample-level factors associated with the presence of *Salmonella* in a multi-state study of conventional and organic dairy farms. Prev Vet Med 2005; 67:39-53.

Hartmann FA, Callan RJ, McGuirk SM, *et al.* Control of an outbreak of salmonellosis caused by drug-resistant *Salmonella* Anatum in horses at a veterinary hospital and measures to prevent future infections. J Am Vet Med Assoc 1996; 209: 629-631.

Hird DW, Pappaioanou M, and Smith BP. Case-control study of risk factors associated with isolation of *Salmonella* Saintpaul in hospitalized horses. Am J Epidemiol 1984; 120: 852-864.

House JK, Mainar-Jaime RC, Smith BP, *et al.* Risk factors for nosocomial *Salmonella* infection among hospitalized horses. J Am Vet Med Assoc 1999; 214: 1511-1516.

Hunter SB, Vauterin P, Lambert-Fair MA, *et al.* Establishment of a universal size standard strain for use with the PulseNet standardized pulsed-field gel electrophoresis protocols: Converting the national databases to the new size standard. J Clin Microbiol 2005; 43: 1045-1050.

Kabagambe EK, Wells SJ, Garber LP, *et al.* Risk factors for fecal shedding of *Salmonella* in 91 US dairy herds in 1996. Prev Vet Med 2000; 43:177-194.

Pangloli P, Dje Y, Ahmed O, *et al.* Seasonal incidence and molecular characterization of *Salmonella* from dairy cows, calves, and farm environment. Foodborne Pathog Dis 2008; 5: 87-96.

Ray KA, Warnick LD, Mitchell RM, *et al.* Prevalence of antimicrobial resistance among *Salmonella* on midwest and northeast USA dairy farms. Prev Vet Med 2007; 79:204-223.

Ribot EM, Fair MA, Gautom R, *et al.* Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. *Foodborne Pathog Dis* 2006; 3: 59-67.

Schott HC, Ewart SL, Walker RD, *et al.* An outbreak of salmonellosis among horses at a veterinary teaching hospital. *J Am Vet Med Assoc* 2001; 218: 1152-9.

Smith BP, Reina-Guerra M, and Hardy AJ. Prevalence and epizootiology of equine salmonellosis. *J Am Vet Med Assoc* 1978; 172: 353-356.

Tillotson K, Savage CJ, Salman MD, *et al.* Outbreak of *Salmonella* Infantis infection in a large animal veterinary teaching hospital. *J Am Vet Med Assoc* 1997; 211: 1554-1557.

Ward MP, Brady TH, Couetil LL, *et al.* Investigation and control of an outbreak of salmonellosis caused by multidrug-resistant *Salmonella* Typhimurium in a population of hospitalized horses. *Vet Microbiol* 2005; 107: 233-240.

Wells SJ, Fedorka-Cray PJ, Dargatz DA, *et al.* Fecal shedding of *Salmonella* spp. by dairy cows on farm and at cull cow markets. *J Food Prot* 2001; 64:3-11.

CHAPTER 6: The effect of clinical outbreaks of salmonellosis on the prevalence of fecal *Salmonella* shedding among dairy cattle in New York*

*Cummings KJ, Warnick LD, Elton M, Gröhn YT, McDonough PL, Siler JD. The effect of clinical outbreaks of salmonellosis on the prevalence of fecal *Salmonella* shedding among dairy cattle in New York. *Foodborne Pathogens and Disease* (In press).

Abstract

The objective of this study was to determine if the within-herd prevalence of fecal *Salmonella* shedding is higher in dairy herds with clinical outbreaks of disease, as compared to herds with subclinical infections only. Data were collected prospectively from dairy herds throughout New York that had at least 150 lactating cows and that received clinical service from participating veterinarians. Following enrollment, *Salmonella* surveillance consisted of both environmental screening and disease monitoring within the herd. Herds positive by either environmental or fecal culture were sampled during three visits to estimate the within-herd prevalence of *Salmonella*. We characterized isolates by serovar and antimicrobial resistance pattern. Among 57 enrolled herds, 44 (77%) yielded *Salmonella*-positive samples during the study period; 27 (61%) of the positive herds had *Salmonella* isolated from environmental samples only, and 17 (39%) had one or more laboratory-confirmed clinical cases. The within-herd prevalence of fecal *Salmonella* shedding ranged from 0 to 53%. *Salmonella* Cerro was the predominant serovar, accounting for 56% of all isolates. Antimicrobial resistance ranged from zero to nine drugs, and 14 (32%) of the positive farms generated multidrug-resistant isolates. Herds with laboratory-confirmed clinical cases had a higher prevalence of fecal *Salmonella* shedding than herds which only generated positive environmental samples, as estimated by a Poisson regression model (prevalence ratio, 2.7; $P = 0.01$). An association between dairy herd outbreaks of salmonellosis and a higher prevalence of asymptomatic shedding should help guide strategies for reducing the public health threat of *Salmonella*, as the ability to recognize high-risk herds by clinical laboratory submissions presents an obvious opportunity to maximize food safety at the pre-harvest level. This is in contrast with

other foodborne zoonotic pathogens, such as *Campylobacter jejuni* and *E. coli* O157:H7, which occur widely in adult cattle without accompanying clinical disease.

Introduction

Salmonella enterica is an important zoonotic pathogen, causing an estimated 1.4 million illnesses, 16,000 hospitalizations, and between 400 – 600 deaths annually in the U.S. alone (Mead *et al.*, 1999; Voetsch *et al.*, 2004). Though primarily a cause of self-limiting acute enteritis (diarrhea, abdominal pain, and fever, with a typical duration of four to seven days), *Salmonella* can produce invasive infections that lead to sepsis and death. Young children, the elderly, and those with compromised immune systems are especially susceptible to severe disease. The prevalence of multidrug resistance among *Salmonella* strains has increased over the past two decades (Glynn *et al.*, 1998; Dunne *et al.*, 2000; Gupta *et al.*, 2003; Davis *et al.*, 2007), making treatment failures more common among those with serious disease. In addition, infections with resistant strains of *Salmonella* tend to be more severe and lead to higher rates of hospitalization than those caused by susceptible strains (Helms *et al.*, 2002; Helms *et al.*, 2004; Varma *et al.*, 2005a; Varma *et al.*, 2005b).

People generally acquire salmonellosis through foodborne exposure, although direct contact with infected animals is another possible route (Mead *et al.*, 1999; L Plym and Wierup, 2006). Preliminary CDC FoodNet data from 2008 show that *Salmonella* accounted for 40% of all laboratory-confirmed cases of foodborne infections, based on surveillance in 10 states (Centers for Disease Control and Prevention (CDC), 2009b). Dairy cattle are considered an important source of several *Salmonella* serovars that are a threat to human health, including multidrug-resistant

Newport and Typhimurium (Gupta *et al.*, 2003; Dechet *et al.*, 2006; Varma *et al.*, 2006; Karon *et al.*, 2007). Fecal contamination of beef carcasses at the time of slaughter is thought to represent the predominant source of transmission. According to the 1996 USDA National Animal Health Monitoring System (NAHMS) Dairy report, 14.9% of culled dairy cows were shedding *Salmonella* at livestock markets, and 66.0% of markets had at least one cow shedding *Salmonella* (Wells *et al.*, 2001). Contamination of crops, either by manure used as fertilizer or by irrigation water that has been contaminated by manure run-off, is another key source of transmission (Islam *et al.*, 2004; Sivapalasingam *et al.*, 2004; Centers for Disease Control and Prevention (CDC), 2008a). Those who work or otherwise interact with livestock are also at risk of infection via direct exposure when cattle are shedding *Salmonella*.

Introduction of *Salmonella* onto a dairy farm can occur through a variety of routes, including purchased cattle, contaminated feed or water, wild animals such as rodents and birds, human traffic, and insects (Bender, 1994; Evans and Davies, 1996; Sanchez *et al.*, 2002; Nielsen *et al.*, 2007). Clinical signs of bovine salmonellosis may include diarrhea, fever, anorexia, dehydration, decreased milk production, abortion, and evidence of endotoxemia, although many infections remain asymptomatic (Divers and Peek, 2008). Infected cattle can shed the organism for variable periods and intermittently following either clinically apparent or subclinical infections. Widespread environmental contamination can result from *Salmonella* shedding, and the organism can survive for prolonged periods in suitable conditions outside a host (Wray and Wray, 2000; You *et al.*, 2006). Fecal *Salmonella* shedding can also augment the risk of within-herd transmission and inadvertent spread to other herds. In addition to impacting the health and productivity of dairy cattle, these factors lead to an increased risk of zoonotic transmission. Although the prevalence of fecal *Salmonella* shedding among asymptomatic dairy cattle has been estimated in a number

of studies (Wells *et al.*, 2001; Huston *et al.*, 2002; Fossler *et al.*, 2004; Blau *et al.*, 2005), the relationship between clinical outbreaks of salmonellosis and fecal shedding is not well understood.

Our hypothesis was that the within-herd prevalence of fecal *Salmonella* shedding is higher in herds with clinical outbreaks of disease, as compared to herds with subclinical infections only. Thus, the objective of this study was to determine the effect of clinical disease (salmonellosis) on the prevalence of asymptomatic fecal *Salmonella* shedding within dairy herds. The identification of such a link would provide a clear point of intervention to mitigate public health risk; herds posing the greatest danger to human health could therefore be recognized by clinical laboratory submissions, potentially reducing the need for surveillance among herds without clinical disease. In addition, we described the serovars and antimicrobial resistance patterns of the isolates in order to enhance our understanding of the epidemiology of *Salmonella* on dairy farms.

Materials and Methods

Study design

Data for this study were collected prospectively from a convenience sample of dairy herds throughout New York that had at least 150 lactating cows and that received clinical service from participating veterinarians. Following enrollment, *Salmonella* surveillance consisted of both environmental screening and disease monitoring within the herd, for a period of at least 12 months. Environmental surveillance involved the repeated collection of samples from four locations per herd

for *Salmonella* culture (cow housing, calf housing, manure storage area, and sick pen); the targeted interval between sample collections was monthly. In addition, veterinarians submitted fecal samples from suspected clinical cases for *Salmonella* culture. The diagnostic criteria provided to the veterinarians included diarrhea with blood, mucus, or a foul odor, fever of at least 103°F, depression, and decreased appetite, as well as sudden death in the absence of specific clinical signs or death following a course of diarrhea. In order to encourage the submission of samples from every clinical suspect animal, all shipping and laboratory costs were covered by the study. A positive culture result arising by either surveillance method prompted a series of three herd visits (at four to eight week intervals) for cattle sampling by project personnel, with the goal of estimating the within-herd prevalence of *Salmonella*. The number of animals sampled at each visit ranged from 50 – 70, depending on herd size (< 500 lactating cows: 50 animals sampled, 500 – 1000 lactating cows: 60, and > 1000 lactating cows: 70). A subset of each sample was comprised of pre-weaned calves, i.e. a total of 10, 15, and 20 calves made up the samples of 50, 60, and 70 animals, respectively. A conscious effort was made to sample cattle from each pen on the farm, and animals within a given pen were sampled systematically to the extent possible. No attempt was made to collect samples from the same cattle during the subsequent herd visits, though some animals may have been sampled again by chance.

Sample collection and processing

Environmental samples were collected using sterile 4x4 inch gauze pads saturated in double-strength skim milk, which had been placed beforehand into a sterile flip-top container. For each of the four sampling locations per farm, four

different gauze pads were used to collect samples and were subsequently pooled into a single flip-top container. Locations sampled in the cow housing area included four sites on the floor within high-traffic sections of the barn. Calf housing samples consisted of either four swabs of the floor in group housing areas or four swabs of the bedding in individual hutches or pens. Manure storage areas were sampled by sticking an instrument deep into the lagoon or slurry pit and then swabbing it. Sick pen samples consisted of either four swabs of the floor in group pens or four swabs of the bedding in individual sick pens. All environmental samples were maintained at 4°C until processing; samples were shipped to the research laboratory for bacteriologic culture.

Fecal samples from suspected clinical cases were collected by veterinarians via rectal retrieval, with a new glove being used to collect each sample. Approximately 10 g of fecal matter was placed into a Para-Pak bottle (Meridian Bioscience Inc., Cincinnati, OH) and sealed. These samples were shipped to the Animal Health Diagnostic Center (College of Veterinary Medicine, Cornell University, Ithaca, NY) for bacteriologic culture.

Fecal samples obtained by project personnel during the three monthly visits were collected via rectal retrieval, again with a new glove being used to collect each sample. Approximately 10 g of fecal matter was placed into a Para-Pak bottle and sealed. All of these samples were transported to the research laboratory for bacteriologic culture.

Standard culture methods were used to isolate *Salmonella* from feces. Individual fecal swabs from sample bottles were enriched in 10 ml of Tetrathionate broth (Difco, Detroit, MI) containing 0.2 ml of iodine solution; the mixture was incubated at 42°C for 18–24 hours. After incubation, the sample-broth mixture was streaked onto Brilliant Green agar with novobiocin (BGN; Becton Dickinson and

Company, Franklin Lakes, NJ) and Xylose Lysine Tergitol 4 (XLT-4) selective media, and both plates were incubated at 37°C for 18–24 hours. Red colonies (lactose non-fermenting bacteria) on BGN and black colonies (H₂S-producing bacteria) on XLT-4 were inoculated into Kligler Iron Agar (KIA) slants and then incubated at 37°C for 18–24 hours. XLT-4 plates without suspected colonies were re-incubated at 37°C for an additional 18–24 hours before checking again for characteristic black colonies. Colonies on KIA slants which exhibited the biochemical properties of *Salmonella* were then serogrouped by slide agglutination using standard protocols. Those colonies that were positive by slide agglutination were then identified as *Salmonella* using the Sensititre Automated Microbiology System's A80 panel (TREK Diagnostic Systems Inc., Cleveland, OH). Confirmed *Salmonella* isolates were sent to the USDA, APHIS National Veterinary Services Laboratories (NVSL) in Ames, Iowa for serotyping using standard protocols.

Antimicrobial susceptibility testing

Antimicrobial susceptibility of *Salmonella* isolates was determined by use of the broth dilution method. Minimal inhibitory concentrations (MIC) were established for each isolate against a panel of up to 15 antimicrobial agents (Sensititre, TREK Diagnostic Systems Inc.). The panel used for *Salmonella* organisms isolated via environmental and follow-up sampling included 15 drugs (amikacin, amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim/sulfamethoxazole); the panel used for clinical isolates included 11 drugs (ampicillin, ceftiofur, chlortetracycline, enrofloxacin, florfenicol, gentamicin, neomycin, oxytetracycline, spectinomycin,

sulfadimethoxine, and trimethoprim/sulfamethoxazole). Clinical and Laboratory Standards Institute (CLSI) guidelines were used to interpret MIC values when available (CLSI, 2008). Otherwise, MIC values were interpreted using National Antimicrobial Resistance Monitoring System (NARMS) breakpoints (Centers for Disease Control and Prevention (CDC). 2009a). Isolates were classified as being resistant or susceptible to each agent; those isolates with intermediate susceptibility were categorized as being susceptible. Quality control was performed weekly using four strains of bacteria: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* 29213, *Enterococcus faecalis* 29212, and *Pseudomonas aeruginosa* 27853. The MIC ranges for quality control recommended by the CLSI were used, and results were accepted if the MIC values were within expected ranges for these bacterial strains.

Data analysis

Study herds were considered *Salmonella*-positive either if *Salmonella* was isolated from one or more environmental samples or if there was at least one laboratory-confirmed clinical case. The median within-herd prevalence of *Salmonella* shedding was estimated for herds that only yielded *Salmonella*-positive environmental samples and for herds that had one or more clinical cases confirmed by bacteriologic culture. Descriptive analysis of serovar data and level of antimicrobial resistance was performed, stratified by positive herd type. The proportion of multidrug-resistant isolates by serovar was also determined. In this study, multidrug resistance was defined as having *in vitro* resistance to five or more antimicrobial agents.

Repeated measures Poisson regression analysis was performed to study the relationship between the within-herd prevalence of *Salmonella* shedding and the dichotomous predictor variable for positive herd status (positive environmental

samples only vs. laboratory-confirmed clinical cases). The response variable was the number of cattle positive for *Salmonella* upon follow-up sampling, and the offset variable was the logarithm of the number of animals tested via this method. A random effects regression model was used to account for the clustering of the three sequential prevalence estimates per herd. Time of sampling for the three within-herd prevalence measurements was added to the model as a fixed effect, thus allowing a comparison of the change in prevalence over time for the two positive herd types. Herd size was forced into the final model because it was considered an important potential confounding variable. This Poisson regression model was of the form

$$\ln (C/N) = \alpha + \sum \beta_i x_i$$

where C was the number of cattle positive for *Salmonella* upon follow-up sampling, N was the number of cattle tested via follow-up, α was the intercept term, x_i were the model covariates, and β_i were the regression coefficients. The generalized estimating equations (GEE) method was used for this regression model.

Separate logistic regression models were utilized to determine whether herds with confirmed clinical cases were more likely to yield either serovars that are important human pathogens (Newport and Typhimurium) or multidrug-resistant (MDR) isolates, as compared to herds with positive environmental samples only. Logistic regression analysis was also used to investigate any associations between herd size and *Salmonella* status. All data analysis was performed in SAS (version 9.1; SAS Institute Inc., Cary, NC), and p-values < 0.05 were considered significant.

Results

Thirty-four veterinarians representing 11 veterinary practices participated in this study. A total of 62 dairy farms were enrolled, although five farms withdrew their involvement. Among the remaining 57 study herds, the median herd size was 875 female dairy cattle (range: 245–7,412). Forty-four herds (77.2%) yielded positive samples over the course of the study period. *Salmonella* was isolated from 22.0% (409/1,857) of environmental surveillance samples, 42.1% (120/285) of suspected clinical case samples, and 9.1% (674/7,400) of follow-up samples for estimating within-herd prevalence. Of the positive clinical cases, 101 (84.2%) were adult cows and 19 (15.8%) were calves. The prevalence of fecal *Salmonella* shedding among clinical suspects was 49.3% (101/205) for adults and 23.8% (19/80) for calves. Of the positive follow-up samples, 588 (87.2%) were from adult cows and 86 (12.8%) were from calves. The prevalence of fecal *Salmonella* shedding among non-suspect animals tested through follow-up was 10.1% (588/5,797) for adults and 5.4% (86/1,603) for calves.

Twenty-seven (61.4%) of the positive herds had *Salmonella* isolated from environmental samples only, and 17 (38.6%) had one or more laboratory-confirmed clinical cases (median: 3 confirmed clinical cases, range: 1 – 48). The within-herd prevalence of fecal *Salmonella* shedding ranged from 0 to 53%. The median prevalence in herds that only generated *Salmonella*-positive environmental samples (0.5%) was significantly lower (Wilcoxon rank sum p-value=0.01) than the median prevalence in herds that had at least one laboratory-confirmed clinical case (8.9%, Figure 6.1).

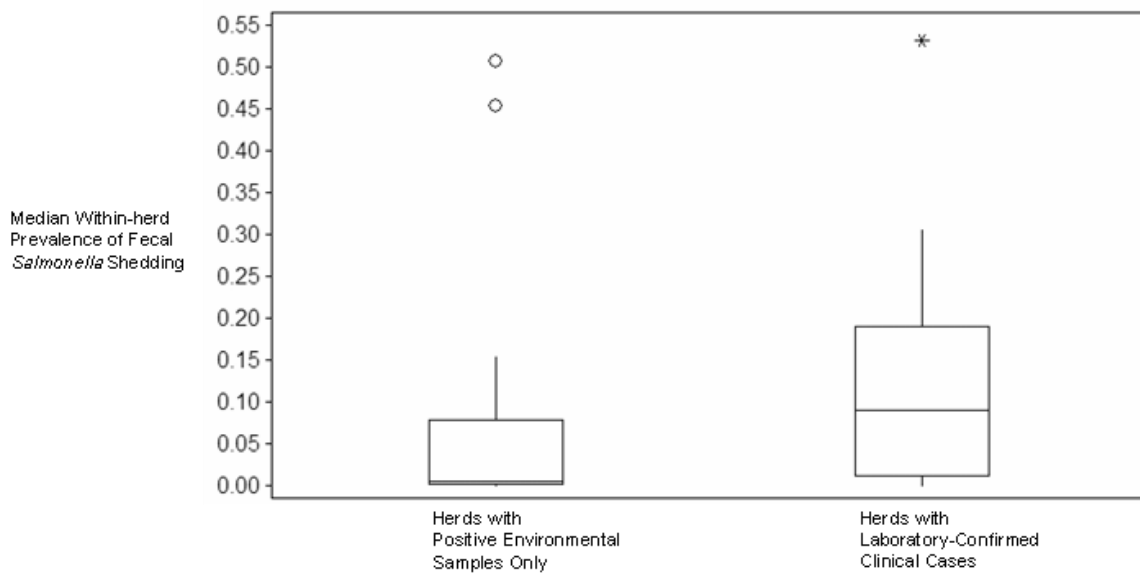


Figure 6.1: Box-and-whiskers plot illustrating the median within-herd prevalence of fecal *Salmonella* shedding among herds in both categories of *Salmonella*-positive status

Serotyping was performed on 96.7% (1,163/1,203) of the *Salmonella* isolates, yielding 31 serovars during the study period (Table 6.1). The predominant serovar among herds with only positive environmental samples was Cerro, which accounted for 57.5% (253/440) of the isolates, followed by Kentucky (14.8%, 65/440), Anatum (including the 15+ variant; 11.1%, 49/440), and Meleagridis (2.7%, 12/440). Cerro was the most common environmental serovar (44.5%, 87/182) among these herds, as well as the one most frequently isolated during follow-up sampling (64.3%, 166/258). The most common serovar among herds that had at least one laboratory-confirmed clinical case was also Cerro, accounting for 55.6% (402/723) of the isolates, followed by Kentucky (14.0%, 101/723), Typhimurium (including the Copenhagen variant; 9.4%, 68/723), and Newport (5.9%, 43/723). Among these herds, Cerro was the leading serovar in all sampling categories (environment: 40.8%, 84/206, clinical cases: 59.2%, 71/120, follow-up sampling: 62.2%, 247/397). Of the predominant serovars in

Table 6.1: *Salmonella* serovars isolated from dairy cattle and the environment of farms in New York*

<i>Serovar</i>	<i>No. of Environmental Isolates</i>	<i>No. of Clinical Case Isolates</i>	<i>No. of Isolates from Follow-up Sampling</i>	<i>Total</i>	
				<i>No.</i>	<i>%</i>
3,10:-:1,5	3	---	1	4	0.3
3,10:-:1,w	1	---	---	1	0.1
3,10:e,h:-	1	1	---	2	0.2
4,12:i:-	---	2	---	2	0.2
4,5,12:i:-	---	---	1	1	0.1
6,7:-:1,5	1	---	---	1	0.1
8,20:-:z6	1	---	---	1	0.1
Agona	4	1	---	5	0.4
Anatum	19	3	21	43	3.7
Anatum var. 15+	4	---	13	17	1.5
Cerro	171	71	413	655	56.3
Heidelberg	1	---	---	1	0.1
Infantis	7	3	---	10	0.9
Kentucky	59	9	98	166	14.3
Lexington	---	---	1	1	0.1
Mbandaka	7	---	---	7	0.6
Meleagridis	26	8	20	54	4.6
Minnesota	3	---	---	3	0.3
Montevideo	7	---	1	8	0.7
Muenster	3	2	7	12	1.0
Newport	18	9	38	65	5.6
Oranienburg	5	1	---	6	0.5
Orion var. 15+,34+	2	---	---	2	0.2
Paratyphi B var. L-tartrate+	1	---	---	1	0.1
Rubislaw	---	---	1	1	0.1
Senftenberg	---	---	1	1	0.1
Tennessee	1	---	---	1	0.1
Thompson	---	1	1	2	0.2
Typhimurium	25	6	26	57	4.9
Typhimurium var. Copenhagen	9	---	5	14	1.2
Rough isolates	---	2	---	2	0.2
Untypeable	9	1	7	17	1.5

*40 isolates excluded from this table due to lack of serovar data

this study, Cerro and Kentucky were the most widespread among farms based on follow-up cattle sampling; *S. Cerro* was isolated from 10 herds, of which five had clinical disease, while *S. Kentucky* was isolated from nine herds, of which four had clinical disease. *S. Newport* (six herds [four clinical]) and *S. Typhimurium* (five herds [four clinical]) were isolated from fewer farms but were more closely associated with clinical disease.

Antimicrobial susceptibility testing was performed on 98.3% (1,183/1,203) of the *Salmonella* isolates. Antimicrobial resistance among isolates ranged from zero (pan-susceptible) to nine drugs. Herds with only positive environmental samples generated 26/449 (5.8%) multidrug-resistant isolates and 404/449 (90.0%) that were pan-susceptible. Multidrug resistance was seen in 4.8% (9/186) of the environmental samples and 6.5% (17/263) of the follow-up samples from these herds. Among herds that had at least one laboratory-confirmed clinical case, 47/734 (6.4%) isolates were MDR and 580/734 (79.0%) were pan-susceptible. The MDR phenotype was observed in 8.1% (17/211) of the environmental samples, 6.7% (8/120) of the clinical case samples, and 5.5% (22/403) of the follow-up samples from these herds. Fourteen (31.8%) of the *Salmonella*-positive herds in our study yielded MDR isolates from cattle, the environment, or both.

There was considerable variation in antimicrobial resistance across the serovars most commonly isolated in this study. For instance, 86.2% (56/65) of the Newport isolates and 8.5% (6/71) of the Typhimurium isolates were multidrug-resistant, whereas the proportion of isolates that were MDR among the Cerro, Kentucky, Anatum, and Meleagridis serovars ranged from 0 to 1.7%.

Using a multivariable Poisson regression model to account for the effects of other factors, we found that positive herd status (positive environmental samples only vs. laboratory-confirmed clinical cases) was a significant predictor of *Salmonella*

Table 6.2: Association between within-herd *Salmonella* prevalence and positive herd status among dairy cattle in NY, when forcing herd size into a Poisson regression model

<i>Variable</i>	<i>Prevalence Ratio</i>	<i>95% Confidence Interval</i>	<i>p-value</i>
<i>Positive herd status</i>			
Laboratory-confirmed clinical cases	2.7	(1.3, 5.9)	0.01
Positive environmental samples only	1.0	---	---
<i>Herd size (female dairy cattle)</i>			
≥ 1,500	0.6	(0.2, 1.5)	0.3
1,000-1,499	0.5	(0.2, 1.7)	0.3
500-999	0.7	(0.2, 2.4)	0.6
< 500	1.0	---	---

prevalence within the herd (Table 6.2). Herds with confirmed clinical cases had a higher prevalence than herds with just positive environmental samples (prevalence ratio, 2.7; $P = 0.01$). Herd size was forced into the model but was not associated with within-herd *Salmonella* prevalence. There was not a significant interaction between positive herd status and time of sampling ($P = 0.1$). Among herds that had at least one laboratory-confirmed clinical case, however, there was a significant decrease in the median within-herd prevalence between the first sampling visit (12.9%) and the last (2.9%; Wilcoxon rank sum p -value=0.04). The median prevalence for the first and last sampling visits did not differ significantly among herds that only generated *Salmonella*-positive environmental samples.

Logistic regression analysis showed that herds with confirmed clinical cases were more likely (odds ratio, 5.6; $P = 0.03$) to yield either Newport or Typhimurium on follow-up sampling than were herds with positive environmental samples only (Table 6.3). Clinical herds also tended to be more likely (OR, 4.4; $P = 0.06$) to yield

Table 6.3: Association between herd-level isolation of *Salmonella* Newport/Typhimurium and positive herd status among dairy cattle in NY, as estimated by a logistic regression model

<i>Variable</i>	<i>Odds Ratio</i>	<i>95% Confidence Interval</i>	<i>p-value</i>
<i>Positive herd status</i>			
Laboratory-confirmed clinical cases	5.6	(1.2, 26.1)	0.03
Positive environmental samples only	1.0	---	---

Deviance = 41.9 (df = 42)

Table 6.4: Association between the presence of laboratory-confirmed clinical cases of salmonellosis and herd size among *Salmonella*-positive dairy herds in NY, as estimated by a logistic regression model

<i>Variable</i>	<i>Odds Ratio</i>	<i>95% Confidence Interval</i>	<i>p-value</i>
<i>Herd size (female dairy cattle)</i>			
≥ 1,500	7.2	(1.1, 48.0)	0.04
1,000-1,499	3.0	(0.3, 25.9)	0.3
500-999	1.2	(0.2, 9.0)	0.9
< 500	1.0	---	---

Likelihood Ratio Chi-square = 6.8 (df = 3)

multidrug-resistant isolates on follow-up sampling than herds with just positive environmental samples.

Herd size was not a significant predictor of whether or not *Salmonella* was isolated from a study herd. Among the positive herds, however, herd size was significantly associated with the presence of laboratory-confirmed clinical cases (Table 6.4). Larger herds with at least 1,500 female dairy cattle were more likely to have clinical cases than smaller herds with fewer than 500 female dairy cattle (OR,

7.2; $P = 0.04$). The odds of having clinical cases among the three smaller herd size categories (1,000-1,499; 500-999; and <500 female dairy cattle) did not differ significantly.

Discussion

A number of studies have described the prevalence of fecal *Salmonella* shedding among apparently healthy dairy cattle (Wells *et al.*, 2001; Huston *et al.*, 2002; Fossler *et al.*, 2004; Blau *et al.*, 2005), but there is little in the literature regarding the incidence of clinical disease associated with *Salmonella* infections in cattle (Cummings *et al.*, 2009). To our knowledge, no studies have investigated the relationship between clinical outbreaks of bovine salmonellosis and the within-herd prevalence of fecal *Salmonella* shedding. This study had the advantage of utilizing both environmental surveillance and disease monitoring in order to assess the occurrence of *Salmonella* within each enrolled dairy herd. These herds were located throughout New York and were characterized by a wide range of sizes and management types representative of the dairy industry in this area of the country. Another strength of this study was the longitudinal sampling approach for estimating the within-herd prevalence of *Salmonella* when herds were identified as being positive. A random effects regression model accounted for the clustering of the three sequential prevalence estimates per herd, and the addition of sampling time to the model as a fixed effect allowed us to characterize the prevalence over time for the two positive herd types.

Environmental surveillance has been shown to be a relatively effective means of monitoring for the presence of *Salmonella* on dairy farms (Warnick *et al.*, 2003).

However, it is possible that the number of clinical cases in the present study was underestimated if clinically affected cattle went undetected by herd managers or veterinarians. Furthermore, fecal culture does not have perfect sensitivity for detecting the presence of *Salmonella*, and we recognize that some positive cattle were presumably missed by culturing. Thus, the possibility exists that we could have missed *Salmonella*-positive herds altogether or misclassified herds with clinical cases as being positive by virtue of environmental samples only. Such non-differential misclassification would bias our results towards the null.

It is also conceivable that participating veterinarians had a tendency to enroll client herds that had a history of diarrhea over herds without clinical disease. However, only 17 of the 57 enrolled herds had one or more laboratory-confirmed clinical cases over the duration of the study. Moreover, it was not our intention for study herds to be representative of herds throughout New York in terms of the herd-level prevalence of *Salmonella* infection. Our goal was to have a sufficient number of study herds positive by either method (positive environmental samples only vs. laboratory-confirmed clinical cases) to allow a comparison between the two.

Of the positive study herds, 39% had at least one confirmed case of salmonellosis, while the remaining 61% had *Salmonella* isolated from the environment only. The median within-herd prevalence of asymptomatic fecal *Salmonella* shedding was significantly higher in herds that had at least one confirmed clinical case, as compared to herds that only generated positive environmental samples. Positive herd status was also a significant predictor of within-herd *Salmonella* prevalence in a Poisson regression model controlling for herd as a random effect, after adjusting for the effect of herd size. Those herds with a high prevalence of asymptomatic fecal *Salmonella* shedding presumably represent a greater threat to public health than herds with few shedders. An association between dairy herd outbreaks of salmonellosis and

higher within-herd *Salmonella* prevalence should help guide strategies for reducing this threat, as the ability to recognize high-risk herds by clinical laboratory submissions presents an obvious opportunity to maximize food safety at the pre-harvest level. This is in contrast with other foodborne zoonotic pathogens, such as *Campylobacter jejuni* and *E. coli* O157:H7, which occur widely in adult cattle without accompanying clinical disease (Dunn *et al.*, 2004; Cho *et al.*, 2006; Kwan *et al.*, 2008; Ellis-Iversen *et al.*, 2009; Huang *et al.*, 2009). The occurrence of disease outbreaks among herds with a higher within-herd prevalence of *Salmonella* shedding also provides a tangible incentive for dairy producers to enhance their biosecurity and hygiene efforts on the farm. Salmonellosis can be a costly disease for producers on account of mortality, treatment expenses, reduced milk yield, and weight loss within the herd (Peters, 1985; Huston *et al.*, 2002); the economic benefits of *Salmonella* control, coupled with the promotion of public health, should encourage the implementation of strategies for preventing the introduction and spread of this pathogen. Finally, the association between clinical disease and high-risk herds implies that steps to reduce the public health threat of *Salmonella* on dairy farms can yield improvements that are directly observed and quantified.

Among herds with at least one laboratory-confirmed clinical case, there was a significant decrease in the within-herd prevalence of fecal *Salmonella* shedding between the first and last sampling visits; such was not the case among herds with *Salmonella*-positive environmental samples only. The concentration of *Salmonella* within the manure of an infected cow ranges from 10^2 to 10^7 organisms per gram of feces (You *et al.*, 2006). It seems likely that the presence of cattle with diarrhea due to salmonellosis will lead to an initial peak in the within-herd prevalence of fecal *Salmonella* shedding; the prevalence would be expected to decline as clinical signs of salmonellosis abate in the herd. In contrast, herds with *Salmonella* contamination of

the environment but no clinical cases would presumably have consistent, repeated exposure over time. The within-herd prevalence of fecal *Salmonella* shedding is likely to persist at some level without peaking at any point.

S. Cerro was the major serovar in this study, accounting for 56% (655/1,163) of the isolates and leading all herd sampling categories. *Cerro* was the only serovar isolated from the four herds in this study with the highest within-herd prevalence of fecal *Salmonella* shedding (31%, 45%, 51%, and 53%). Two of these herds had at least one laboratory-confirmed case of salmonellosis, while the other two had *Salmonella* isolated from environmental samples only. The role of *S. Cerro* in causing clinical disease in cattle is unclear (Cummings *et al.*, in press). This serovar has been a rare isolate among people in the U.S. with laboratory-confirmed *Salmonella* infections, accounting for 0.1% of the cases in 2006 (Centers for Disease Control and Prevention (CDC), 2008b).

Whereas *S. Cerro* and *S. Kentucky* were predominant among both positive herd types, *S. Newport* and *S. Typhimurium* (including the Copenhagen variant) were common only among those herds that had at least one laboratory-confirmed clinical case. *Newport* and *Typhimurium* were previously shown to be the two leading serovars in a large study on the incidence of clinical disease due to *Salmonella* infection among dairy herds in the northeastern U.S (Cummings *et al.*, 2009). CDC FoodNet data from 2008 showed that these serovars were also two of the three most common *Salmonella* serovars isolated from people with laboratory-confirmed foodborne infection, accounting for 26% of the human cases (Centers for Disease Control and Prevention (CDC), 2009b). In contrast, the serovars most commonly isolated in studies of fecal *Salmonella* shedding among clinically healthy cattle differ from those that most frequently cause human disease. According to the 1996 NAHMS Dairy report, *S. Montevideo* (21%) was the most prevalent serovar isolated from

healthy lactating cows, and neither Newport nor Typhimurium was among the 10 most common serovars isolated (Wells *et al.*, 2001). The 2002 NAHMS Dairy study found *S. Meleagridis* (24%) to be the most prevalent serovar, while Newport and Typhimurium accounted for only 3% and 10% of all isolates, respectively (Blau *et al.*, 2005). Similarly, *S. Meleagridis* was the most common serovar isolated from the environment of dairy herds without clinical signs of salmonellosis; neither Newport nor Typhimurium was identified on these farms (Peek *et al.*, 2004). In the present study, herds experiencing clinical disease were more likely to yield either Newport or Typhimurium on follow-up sampling than were herds with *Salmonella*-positive environmental samples only. Thus, herds with clinical outbreaks of salmonellosis may present a greater threat to public health, as there is a higher probability that the cattle are shedding serovars that are also important human pathogens.

With the exception of *S. Newport*, there was a low frequency of antimicrobial resistance among isolates from any of the sampling categories in this study. Over 86% of the Newport isolates across all sample types displayed multidrug resistance, but virtually none of the isolates representing other common serovars showed a MDR phenotype. Studies of fecal *Salmonella* shedding among clinically healthy cattle across the U.S. have shown antimicrobial resistance to be uncommon (Wells *et al.*, 2001; Blau *et al.*, 2005; Ray *et al.*, 2007). However, multidrug resistance was found to be highly prevalent among isolates from cattle with clinical signs of salmonellosis in the northeastern U.S. (Cummings *et al.*, 2009), particularly among those serovars that were common in the present study such as Newport and Typhimurium. In this study, multidrug resistance was infrequent among both the broad population of cattle (follow-up sampling) as well as the cattle with salmonellosis, likely due to the distribution of serovars among the clinical isolates. Although 77.8% (7/9) of the clinical Newport isolates were MDR, *S. Newport* was the isolated serovar in only

7.5% (9/120) of the clinical isolates. The low prevalence of MDR isolates in the dairy farm environment is consistent with another study on antimicrobial resistance among *Salmonella* on dairy farms, in which investigators found that 9.7% of environmental samples yielded isolates resistant to five or more antimicrobial agents (Ray *et al.*, 2007). Despite the generally low levels of antimicrobial resistance detected in the present study, herds experiencing clinical disease tended to be more likely to yield multidrug-resistant isolates on follow-up sampling than herds with *Salmonella*-positive environmental samples only. This further reinforces the notion that herds with clinical outbreaks of salmonellosis may pose a greater risk to public health.

Among the *Salmonella*-positive herds, herd size was significantly associated with the presence of laboratory-confirmed clinical cases. The largest herds in this study were more likely to have clinical cases than herds of the three smaller size categories. Herd size was also found to be a significant predictor of the incidence of salmonellosis among dairy herds in the northeastern United States, when included in a multivariable regression model (Cummings *et al.*, 2009). Larger herds may have a greater likelihood of purchasing cattle from outside sources, with the accompanying risk of introducing *Salmonella* via a subclinical shedder that has been stressed by transport. High cattle density may also occur in larger herds and could promote *Salmonella* transmission and clinical disease; animal crowding enhances contact among cattle and may also encourage stressful group dynamics. Finally, larger herds may be characterized by management practices which somehow play a role in increasing the incidence of salmonellosis. Herd size is a risk factor that does not easily lend itself to practical intervention due to the management trends and economic constraints that prevail in the modern dairy industry. However, it is possible that certain attributes of larger herds that contribute to the incidence of salmonellosis could in fact be modified to reduce the occurrence of this disease.

Conclusions

In this study, dairy herds with laboratory-confirmed clinical cases of salmonellosis had a higher prevalence of fecal *Salmonella* shedding than herds which only generated positive environmental samples. Herds with confirmed clinical cases were also more likely to yield either *Salmonella* Newport or *S. Typhimurium* on follow-up sampling, and they had a greater tendency to generate multidrug-resistant isolates. Clinical laboratory submissions may thus aid in recognizing herds that represent an increased threat to public health. *S. Cerro* was the predominant serovar isolated from clinical cases, asymptomatic cattle, and the farm environment.

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REFERENCES

- Bender JB. Reducing the risk of *Salmonella* spread and practical control measures in dairy herds. *Bovine Pract* 1994; 28:62-65.
- Blau DM, McCluskey BJ, Ladely SR, *et al.* *Salmonella* in dairy operations in the United States: prevalence and antimicrobial drug susceptibility. *J Food Prot* 2005; 68:696-702.
- Centers for Disease Control and Prevention (CDC). Outbreak of *Salmonella* serotype Saintpaul infections associated with multiple raw produce items--United States, 2008. *MMWR Morb.Mortal.Wkly.Rep.* 2008a; 57:929-934.
- Centers for Disease Control and Prevention (CDC). *Salmonella* Surveillance: Annual Summary, 2006. U.S. Department of Health and Human Services, CDC, Atlanta, GA. 2008b.
- Centers for Disease Control and Prevention (CDC). National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS): Human Isolates Final Report, 2006. U.S. Department of Health and Human Services, CDC, Atlanta, GA. 2009a.
- Centers for Disease Control and Prevention (CDC). Preliminary FoodNet Data on the incidence of infection with pathogens transmitted commonly through food--10 States, 2008. *MMWR Morb Mortal Wkly Rep* 2009b; 58:333-337.
- Cho S, Bender JB, Diez-Gonzalez F, *et al.* Prevalence and characterization of *Escherichia coli* O157 isolates from Minnesota dairy farms and county fairs. *J Food Prot* 2006; 69:252-259.
- Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard - Third Edition*. CLSI document M31-A3. Wayne, PA: Clinical and Laboratory Standards Institute. 2008.
- Cummings KJ, Warnick LD, Alexander KA, *et al.* The incidence of salmonellosis among dairy herds in the northeastern United States. *J Dairy Sci* 2009; 92:3766-3774.
- Cummings KJ, Warnick LD, Elton M, *et al.* *Salmonella enterica* serotype Cerro among dairy cattle in New York: an emerging pathogen? *Foodborne Pathog Dis* 2010; Epub ahead of print.

- Davis MA, Besser TE, Eckmann K, *et al.* Multidrug-resistant *Salmonella* Typhimurium, Pacific Northwest, United States. *Emerg Infect Dis* 2007; 13:1583-1586.
- Dechet AM, Scallan E, Gensheimer K, *et al.* Outbreak of multidrug-resistant *Salmonella enterica* serotype Typhimurium Definitive Type 104 infection linked to commercial ground beef, northeastern United States, 2003-2004. *Clin Infect Dis* 2006; 42:747-752.
- Divers TJ and Peek SF. *Rebhun's diseases of dairy cattle*. St. Louis: Saunders Elsevier, 2008.
- Dunn JR, Keen JE, Thompson RA. Prevalence of Shiga-toxigenic *Escherichia coli* O157:H7 in adult dairy cattle. *J Am Vet Med Assoc* 2004; 224:1151-1158.
- Dunne EF, Fey PD, Kludt P, *et al.* Emergence of domestically acquired ceftriaxone-resistant *Salmonella* infections associated with AmpC beta-lactamase. *JAMA* 2000; 284:3151-3156.
- Ellis-Iversen J, Cook AJ, Smith RP, *et al.* Temporal patterns and risk factors for *Escherichia coli* O157 and *Campylobacter* spp, in young cattle. *J Food Prot* 2009; 72:490-496.
- Evans S and Davies R. Case control study of multiple-resistant *Salmonella* Typhimurium DT104 infection of cattle in Great Britain. *Vet Rec* 1996; 139:557-558.
- Fossler CP, Wells SJ, Kaneene JB, *et al.* Prevalence of *Salmonella* spp on conventional and organic dairy farms. *J Am Vet Med Assoc* 2004; 225:567-573.
- Glynn MK, Bopp C, Dewitt W, *et al.* Emergence of multidrug-resistant *Salmonella enterica* serotype Typhimurium DT104 infections in the United States. *N Engl J Med* 1998; 338:1333-1338.
- Gupta A, Fontana J, Crowe C, *et al.* Emergence of multidrug-resistant *Salmonella enterica* serotype Newport infections resistant to expanded-spectrum cephalosporins in the United States. *J Infect Dis* 2003; 188:1707-1716.
- Helms M, Simonsen J, Molbak K. Quinolone resistance is associated with increased risk of invasive illness or death during infection with *Salmonella* serotype Typhimurium. *J Infect Dis* 2004; 190:1652-1654.

- Helms M, Vastrup P, Gerner-Smidt P, *et al.* Excess mortality associated with antimicrobial drug-resistant *Salmonella* Typhimurium. *Emerg Infect Dis* 2002; 8:490-495.
- Huang JL, Xu HY, Bao GY, *et al.* Epidemiological surveillance of *Campylobacter jejuni* in chicken, dairy cattle and diarrhoea patients. *Epidemiol Infect* 2009; 137:1111-1120.
- Huston CL, Wittum TE, Love BC, *et al.* Prevalence of fecal shedding of *Salmonella* spp in dairy herds. *J Am Vet Med Assoc* 2002; 220:645-649.
- Islam M, Morgan J, Doyle MP, *et al.* Persistence of *Salmonella enterica* serovar Typhimurium on lettuce and parsley and in soils on which they were grown in fields treated with contaminated manure composts or irrigation water. *Foodborne Pathog Dis* 2004; 1:27-35.
- Karon AE, Archer JR, Sotir MJ, *et al.* Human multidrug-resistant *Salmonella* Newport infections, Wisconsin, 2003-2005. *Emerg Infect Dis* 2007; 13:1777-1780.
- Kwan PS, Birtles A, Bolton FJ, *et al.* Longitudinal study of the molecular epidemiology of *Campylobacter jejuni* in cattle on dairy farms. *Appl Environ Microbiol* 2008; 74:3626-3633.
- L Plym F and Wierup M. *Salmonella* contamination: a significant challenge to the global marketing of animal food products. *Rev Sci Tech* 2006; 25:541-554.
- Mead PS, Slutsker L, Dietz V, *et al.* Food-related illness and death in the United States. *Emerg Infect Dis* 1999; 5:607-625.
- Nielsen LR, Warnick LD, Greiner M. Risk factors for changing test classification in the Danish surveillance program for *Salmonella* in dairy herds. *J Dairy Sci* 2007; 90:2815-2825.
- Peek SE, Hartmann FA, Thomas CB, *et al.* Isolation of *Salmonella* spp from the environment of dairies without any history of clinical salmonellosis. *J Am Vet Med Assoc* 2004; 225:574-577.
- Peters AR. An estimation of the economic impact of an outbreak of *Salmonella* Dublin in a calf rearing unit. *Vet Rec* 1985; 117:667-668.

Ray KA, Warnick LD, Mitchell RM, *et al.* Prevalence of antimicrobial resistance among *Salmonella* on midwest and northeast USA dairy farms. *Prev Vet Med* 2007; 79:204-223.

Sanchez S, Hofacre CL, Lee MD, *et al.* Animal sources of salmonellosis in humans. *J Am Vet Med Assoc* 2002; 221:492-497.

Sivapalasingam S, Friedman CR, Cohen L, *et al.* Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *J Food Prot* 2004; 67:2342-2353.

Varma JK, Greene KD, Ovitt J, *et al.* Hospitalization and antimicrobial resistance in *Salmonella* outbreaks, 1984-2002. *Emerg Infect Dis* 2005a; 11:943-946.

Varma JK, Marcus R, Stenzel SA, *et al.* Highly resistant *Salmonella* Newport-MDRampC transmitted through the domestic US food supply: a FoodNet case-control study of sporadic *Salmonella* Newport infections, 2002-2003. *J Infect Dis* 2006; 194:222-230.

Varma JK, Molbak K, Barrett TJ, *et al.* Antimicrobial-resistant nontyphoidal *Salmonella* is associated with excess bloodstream infections and hospitalizations. *J Infect Dis* 2005b; 191:554-561.

Voetsch AC, Van Gilder TJ, Angulo FJ, *et al.* FoodNet estimate of the burden of illness caused by nontyphoidal *Salmonella* infections in the United States. *Clin Infect Dis* 2004; 38 Suppl 3:S127-34.

Warnick LD, Kaneene JB, Ruegg PL, *et al.* Evaluation of herd sampling for *Salmonella* isolation on midwest and northeast US dairy farms. *Prev Vet Med* 2003; 60:195-206.

Wells SJ, Fedorka-Cray PJ, Dargatz DA, *et al.* Fecal shedding of *Salmonella* spp. by dairy cows on farm and at cull cow markets. *J Food Prot* 2001; 64:3-11.

Wray C and Wray A. *Salmonella* in domestic animals. Oxford ; New York: CABI Pub., 2000.

You Y, Rankin SC, Aceto HW, *et al.* Survival of *Salmonella enterica* serovar Newport in manure and manure-amended soils. *Appl Environ Microbiol* 2006; 72:5777-5783.

CHAPTER 7: *Salmonella enterica* serotype Cerro among dairy cattle in New York: an emerging pathogen?*

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Abstract

The focus of this study was *Salmonella enterica* serotype Cerro, a potentially emerging pathogen of cattle. Our objectives were to document the within-herd prevalence of *S. Cerro* among a sample of New York dairy herds, to describe the antimicrobial resistance patterns and pulsed-field gel electrophoresis (PFGE) types of the isolates, and to elucidate the status of this serotype as a bovine pathogen. Data were collected prospectively from dairy herds throughout NY that had at least 150 lactating cows and that received clinical service from participating veterinarians. Following enrollment, *Salmonella* surveillance consisted of both environmental screening and disease monitoring within the herd. Herds positive by either environmental or fecal culture were sampled during three visits to estimate the within-herd prevalence of *Salmonella*. Among 57 enrolled herds, 44 (77%) yielded *Salmonella*-positive samples during the study period. Of these, 20 herds (46%) were positive for *S. Cerro*. Upon follow-up sampling for estimation of prevalence, Cerro was identified in 10 of the 20 herds; the median within-herd Cerro prevalence was 17%, with a maximum of 53%. Antimicrobial resistance ranged from zero to nine drugs, and eight (40%) of the Cerro-positive farms generated drug-resistant isolates. Eight *Xba*I PFGE types were represented among 116 isolates tested, although 89% of these isolates shared the predominant type. Among herds with clinical cases, cattle that had signs consistent with salmonellosis were more likely to test positive for Cerro than apparently healthy cattle, as estimated by a logistic regression model which controlled for herd as a random effect (OR, 3.9). There is little in the literature concerning *S. Cerro*, and published reports suggest a lack of disease association in cattle. However, in our region there has been an apparent increase in the prevalence of this serotype among cattle with salmonellosis. Other *Salmonella* serotypes important

to bovine health have emerged to become leading causes of human foodborne disease, and close monitoring of Cerro is warranted.

Introduction

Salmonella enterica is a zoonotic pathogen that poses a considerable threat to public health, resulting in approximately 1.4 million illnesses, 16,000 hospitalizations, and between 400 – 600 deaths annually in the U.S. alone (Mead *et al.*, 1999; Voetsch *et al.*, 2004). People generally become infected with *Salmonella* through foodborne exposure or direct contact with infected animals (Mead *et al.*, 1999; L Plym and Wierup, 2006). Human infections may be asymptomatic or result in varying degrees of clinical disease (Jones *et al.*, 2008), ranging from self-limiting acute enteritis to sepsis and death. The prevalence of multidrug resistance among *Salmonella* strains has increased over the past two decades (Glynn *et al.*, 1998; Dunne *et al.*, 2000; Gupta *et al.*, 2003; Davis *et al.*, 2007), causing an increase in treatment failures and hospitalization rates (Helms *et al.*, 2002; Helms *et al.*, 2004; Varma *et al.*, 2005a; Varma *et al.*, 2005b). Over 2,500 *Salmonella* serotypes have been identified to date, but relatively few are responsible for a large proportion of clinical infections (Jones *et al.*, 2008). Preliminary CDC FoodNet data from 2008 show that ten *Salmonella* serotypes comprised 73% of the laboratory-confirmed cases of disease, based on surveillance in 10 states (Centers for Disease Control and Prevention (CDC), 2009b).

Salmonella is also an important cause of clinical illness in both calves and adult dairy cattle. Salmonellosis can be a costly disease for producers on account of treatment expenses, mortality, reduced milk yield, and weight loss within the herd (Peters, 1985; Huston *et al.*, 2002b). Clinical signs of salmonellosis in cattle may

include diarrhea, fever, anorexia, dehydration, decreased milk production, abortion, and evidence of endotoxemia, although many infections remain subclinical (Divers and Peek, 2008). As in people, however, relatively few serotypes are recognized as causing the majority of clinical disease. For example, a recent field study on the incidence of salmonellosis among 831 dairy herds in the northeastern United States found that just seven serotypes accounted for 87% of the cases: *S. Newport*, Typhimurium (including the Copenhagen variant), *Infantis*, 4,5,12:i:-, *Agona*, *Muenster*, and *Kentucky* (Cummings *et al.*, 2009). In contrast, the serotypes most commonly isolated from clinically healthy cattle differ from those that most frequently cause disease. According to the three most recent National Animal Health Monitoring System (NAHMS) Dairy reports (1996, 2002, and 2007), *S. Meleagridis*, *Montevideo*, and *Mbandaka* were consistently among the most prevalent serotypes isolated from healthy lactating cows (Centers for Epidemiology and Animal Health, 2009). Clearly there is a great diversity in *Salmonella* serotypes shed by dairy cattle, both in terms of serotype numbers and their pathogenicity.

The focus of this study was *Salmonella enterica* serotype Cerro, an infrequent human pathogen (CDC, 2008) whose role in causing clinical disease in cattle is unclear (Huston *et al.*, 2002a; Peek *et al.*, 2004; Van Kessel *et al.*, 2007). Our objectives were to document the within-herd prevalence of *S. Cerro* among a sample of dairy herds throughout New York, to describe the antimicrobial resistance patterns and pulsed-field gel electrophoresis (PFGE) types of the Cerro isolates, and to further clarify the status of this serotype as a bovine pathogen.

Materials and Methods

Study design

As part of a larger project to evaluate the prevalence of fecal *Salmonella* shedding among dairy herds with clinical vs. subclinical infections (Cummings *et al.*, 2010), data were collected prospectively from a convenience sample of herds throughout New York that had at least 150 lactating cows and that received clinical service from participating veterinarians. Following enrollment, *Salmonella* surveillance consisted of both environmental screening and disease monitoring within the herd, for a period of at least 12 months. Environmental surveillance involved the repeated collection of samples from four locations per herd for *Salmonella* culture (cow housing, calf housing, manure storage area, and sick pen); the targeted interval between sample collections was monthly. In addition, veterinarians submitted fecal samples from suspected clinical cases for *Salmonella* culture. The diagnostic criteria provided to the veterinarians included diarrhea with blood, mucus, or a foul odor, fever of at least 103°F, depression, and decreased appetite, as well as sudden death in the absence of specific clinical signs or death following a course of diarrhea. In order to encourage the submission of samples from every clinical suspect animal, all shipping and laboratory costs were covered by the study. A positive culture result arising by either surveillance method prompted a series of three herd visits (at four to eight week intervals) for cattle sampling by project personnel, with the goal of estimating the within-herd prevalence of *Salmonella*. The number of animals sampled at each visit ranged from 50 – 70, depending on herd size (< 500 lactating cows: 50 animals sampled, 500 – 1000 lactating cows: 60, and > 1000 lactating cows: 70). A subset of each sample was comprised of pre-weaned calves, i.e. a total of 10, 15, and

20 calves made up the samples of 50, 60, and 70 animals, respectively. A conscious effort was made to sample cattle from each pen on the farm, and animals within a given pen were sampled systematically (every n th animal) to the extent possible. No attempt was made to collect samples from the same cattle during the subsequent herd visits, though some animals may have been sampled again by chance.

Sample collection and processing

Environmental samples were collected using sterile 4x4 inch gauze pads saturated in double-strength skim milk, which had been placed beforehand into a sterile flip-top container. For each of the four sampling locations per farm, four different gauze pads were used to collect samples and were subsequently pooled into a single flip-top container. Locations sampled in the cow housing area included four sites on the floor within high-traffic sections of the barn. Calf housing samples consisted of either four swabs of the floor in group housing areas or four swabs of the bedding in individual hutches or pens. Manure storage areas were sampled by sticking an instrument deep into the lagoon or slurry pit and then swabbing it. Sick pen samples consisted of either four swabs of the floor in group pens or four swabs of the bedding in individual sick pens. All environmental samples were maintained at 4°C until processing; samples were shipped to the research laboratory for bacteriologic culture.

Fecal samples from suspected clinical cases were collected by veterinarians via rectal retrieval, with a new glove being used to collect each sample. Approximately 10 g of fecal matter was placed into a Para-Pak bottle (Meridian Bioscience Inc., Cincinnati, OH) and sealed. These samples were shipped to the Animal Health

Diagnostic Center (College of Veterinary Medicine, Cornell University, Ithaca, NY) for bacteriologic culture.

Fecal samples obtained by project personnel during the three monthly visits were collected via rectal retrieval, again with a new glove being used to collect each sample. Approximately 10 g of fecal matter was placed into a Para-Pak bottle and sealed. All of these samples were transported to the research laboratory for bacteriologic culture.

Standard culture methods were used to isolate *Salmonella* from fecal samples. Fecal swab specimens from each sample container were enriched in 10 ml of Tetrathionate broth (Difco, Detroit, MI) containing 0.2 ml of iodine solution; the mixture was incubated at 42°C for 18–24 hours. After incubation, the sample-broth mixture was streaked onto Brilliant Green agar with novobiocin (BGN; Becton Dickinson and Company, Franklin Lakes, NJ) and Xylose Lysine Tergitol 4 (XLT-4) selective media, and both plates were incubated at 37°C for 18–24 hours. Red colonies (lactose non-fermenting bacteria) on BGN and black colonies (H₂S-producing bacteria) on XLT-4 were inoculated into Kligler Iron Agar (KIA) slants and then incubated at 37°C for 18–24 hours. XLT-4 plates without suspected colonies were re-incubated at 37°C for an additional 18–24 hours before checking again for characteristic black colonies. Colonies on KIA slants which exhibited the biochemical properties of *Salmonella* were then serogrouped by slide agglutination using standard protocols. Those colonies that were positive by slide agglutination were then identified as *Salmonella* using the Sensititre Automated Microbiology System's A80 panel (TREK Diagnostic Systems Inc., Cleveland, OH). Confirmed *Salmonella* isolates were sent to the USDA, APHIS National Veterinary Services Laboratories (NVSL) in Ames, Iowa for serotyping using standard protocols.

Antimicrobial susceptibility testing

Antimicrobial susceptibility of *Salmonella* isolates was determined by use of the broth dilution method. Minimal inhibitory concentrations (MIC) were established for each isolate against a panel of up to 15 antimicrobial agents (Sensititre, TREK Diagnostic Systems Inc.). The panel used for *Salmonella* organisms isolated via environmental and follow-up sampling included 15 drugs (amikacin, amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim/sulfamethoxazole); the panel used for clinical isolates included 11 drugs (ampicillin, ceftiofur, chlortetracycline, enrofloxacin, florfenicol, gentamicin, neomycin, oxytetracycline, spectinomycin, sulfadimethoxine, and trimethoprim/sulfamethoxazole). Clinical and Laboratory Standards Institute (CLSI) guidelines were used to interpret MIC values when available (CLSI, 2008). Otherwise, MIC values were interpreted using National Antimicrobial Resistance Monitoring System (NARMS) breakpoints (CDC, 2009a). Isolates were classified as being resistant or susceptible to each agent; those isolates with intermediate susceptibility were categorized as being susceptible. Quality control was performed weekly using four strains of bacteria: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* 29213, *Enterococcus faecalis* 29212, and *Pseudomonas aeruginosa* 27853. The MIC ranges for quality control recommended by the CLSI were used, and results were accepted if the MIC values were within expected ranges for these bacterial strains.

Pulsed-field gel electrophoresis

PFGE was performed on a representative sample of study isolates, using the standard CDC PulseNet protocol for *Salmonella* subtyping (Ribot *et al.*, 2006). Our goal was to select one isolate per farm per sample date per source (clinical suspect animal, asymptomatic animal tested via follow-up sampling, or environment) per antimicrobial resistance pattern; when there were a number of isolates with the same resistance profile from the same farm/date/source, a random number generator was utilized to select one. *XbaI* was used as the restriction enzyme. *XbaI*-digested *Salmonella enterica* serotype Braenderup (CDCH9812) DNA was used as a reference size standard (Hunter *et al.*, 2005). Electrophoresis was performed for 21 h using the CHEF Mapper apparatus (Bio-Rad Laboratories, Hercules, CA). Pattern images were captured with a Bio-Rad Gel Doc and Quantity One 1-D Analysis software (Bio-Rad Laboratories). PFGE patterns were then analyzed and compared using the BioNumerics version 4.5 software (Applied Maths, Saint-Matins-Latem, Belgium). Similarity clustering analyses were performed in BioNumerics based on the Dice correlation coefficient with a tolerance of 1.5%, using the unweighted pair group method with arithmetic mean (UPGMA) algorithm. PFGE patterns differing by one or more bands were considered different.

Data analysis

Study herds were considered positive for *Salmonella* Cerro either if this serotype was isolated from one or more environmental samples or if there was at least one laboratory-confirmed clinical case. The distribution of Cerro isolates was characterized by herd and by sample type. Descriptive analysis of antimicrobial

resistance and PFGE data was performed. Finally, statistical methods including chi-squared testing and logistic regression analysis were used to assess the importance of *S. Cerro* as a pathogen in cattle, both at the herd and animal level. All data analysis was performed in SAS (version 9.1; SAS Institute Inc., Cary, NC), and p-values < 0.05 were considered significant.

Results

Thirty-four veterinarians representing 11 veterinary practices participated in this study. A total of 62 dairy farms were enrolled, although five farms withdrew their involvement. Among the remaining 57 study herds, the median herd size was 875 female dairy cattle (range: 245–7,412). Forty-four herds (77.2%) yielded *Salmonella*-positive samples over the course of the study period. Of these, 20 herds (45.5%) in 13 counties across New York were positive for *S. Cerro* (Table 7.1). All 20 of these herds yielded Cerro isolates from the environment, and six also had one or more clinical cases of salmonellosis (as identified by the herd veterinarian) that were positive for this serotype. Upon follow-up sampling for estimation of within-herd prevalence, Cerro was identified in 10 of the 20 herds; five had clinical disease attributed to salmonellosis, and five had positive environmental samples but no clinical cases. The within-herd Cerro prevalence ranged from 3.3% to 53.1%, with a median of 17.4%. This median within-herd prevalence among Cerro-positive herds was significantly greater (Wilcoxon rank sum p-value = 0.007) than the median within-herd prevalence of fecal *Salmonella* shedding among herds that were positive for other serotypes (4.1%). The estimated prevalence in three of the Cerro-positive herds was approximately 50% (45%, 51%, and 53%).

Table 7.1: Summary of results from 20 New York dairy herds that were positive for *Salmonella enterica* serotype Cerro

<i>Herd</i>	<i>Cerro-positive environmental samples</i>	<i>Cerro-positive clinical cases</i>	<i>Within-herd Cerro prevalence</i>
1	+	+	53.1%
2	+	+	30.6%
3	+	+	20.0%
4	+	+	14.7%
5	+	+	7.8%
6	+	+	---
7	+	-	51.3%
8	+	-	45.3%
9	+	-	9.2%
10	+	-	4.0%
11	+	-	3.3%
12	+	-	---
13	+	-	---
14	+	-	---
15	+	-	---
16	+	-	---
17	+	-	---
18	+	-	---
19	+	-	---
20	+	-	---

A total of 31 *Salmonella* serotypes were isolated during the study period. Cerro was the predominant serotype among herds with confirmed clinical cases as well as herds with positive environmental samples only, accounting for 56.3% (655/1,163) of all isolates. Cerro was also the most commonly isolated serotype in the three individual sampling categories (environment: 44.1%, 171/388, clinical cases: 59.2%, 71/120, follow-up sampling: 63.1%, 413/655). Of the six herds with Cerro-positive clinical cases, three yielded Cerro as the only serotype from environmental, clinical suspect, and follow-up sampling. Two herds also generated *S. Kentucky* from these sample types. In the final herd, both *S. Cerro* and *S. Montevideo* were isolated

from the environment, and follow-up samples yielded primarily *S. Cerro* (33 out of 38 positive cattle) but also three *S. Newport* isolates and a single isolate each of *S. Typhimurium* (Copenhagen) and *S. Thompson*; however, *Cerro* was the only serotype isolated from clinical cases.

Antimicrobial resistance among *Salmonella Cerro* isolates ranged from zero (pan-susceptible) to nine drugs. Resistance to at least one antimicrobial agent was found in 5.5% (36/651) of the isolates on which susceptibility testing was performed, whereas 94.5% were pan-susceptible. Eight (40.0%) of the *Cerro*-positive herds in our study generated drug-resistant isolates from cattle, the environment, or both. The antimicrobial drugs to which isolates were most commonly resistant included sulfadimethoxine (15 isolates), sulfisoxazole (12), tetracycline (11), ampicillin (9), ceftiofur (8), and amoxicillin/clavulanic acid (8). Chi-squared testing revealed that drug resistance was significantly more common among isolates from clinically ill cattle (21.2%, 14/66) than among either environmental isolates (4.1%, 7/171; Fisher's exact p-value < 0.0001) or isolates from healthy cattle (3.6%, 15/414; Fisher's exact p-value < 0.0001).

Among 116 isolates selected for PFGE typing, eight PFGE types were differentiated (Figure 7.1). A total of 103 isolates shared the predominant PFGE type, whereas the number of isolates corresponding to each of the other seven types ranged from one to four. The main PFGE type was identified on 17 farms. This pattern was shared by 84.2% (32/38) of the clinical case isolates, 97.0% (32/33) of the asymptomatic cattle isolates, and 86.7% (39/45) of the environmental isolates. The other seven PFGE types differed by just one or two bands from the predominant type. Twelve of the 13 isolates with these sporadic PFGE patterns were pan-susceptible; one was resistant only to sulfadimethoxine.

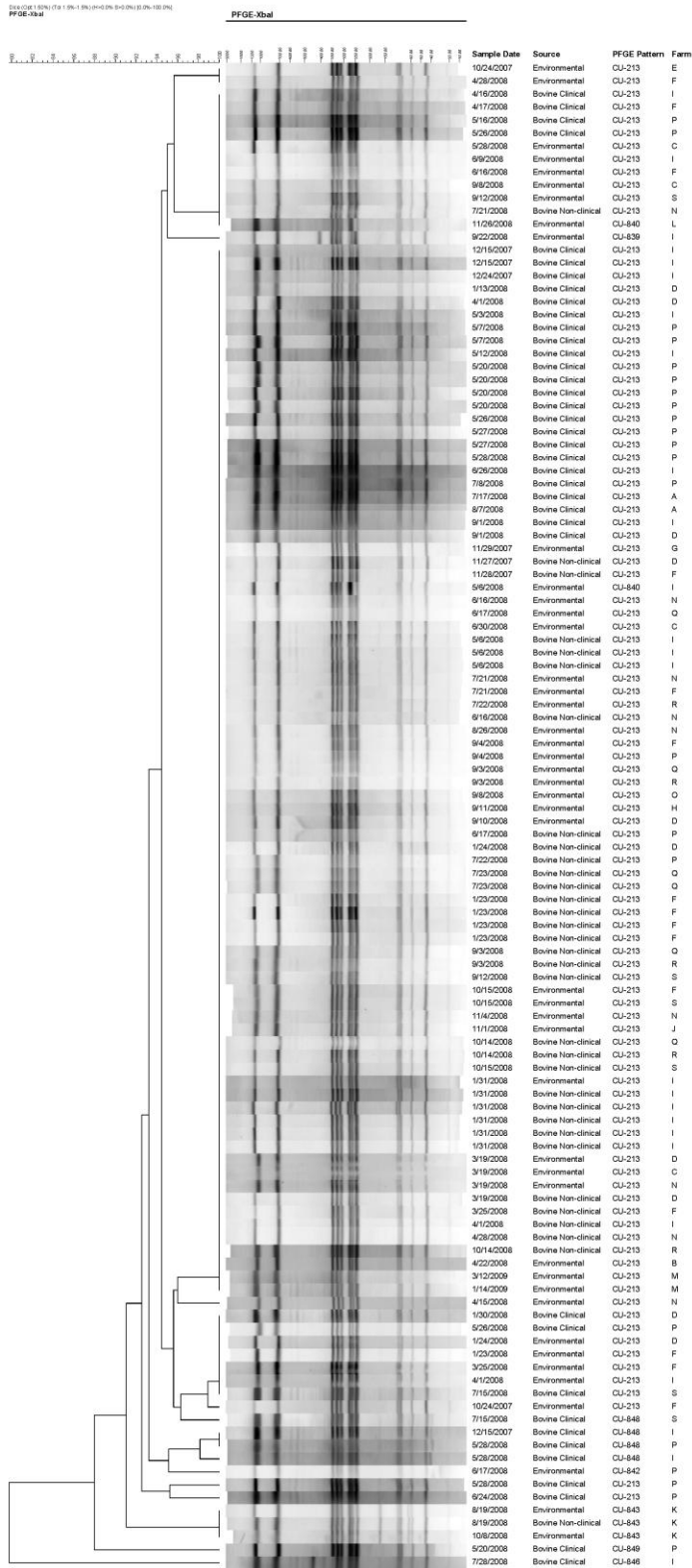


Figure 7.1: *Xba*I pulsed-field gel electrophoresis (PFGE) patterns for 116 *Salmonella enterica* serotype Cerro isolates selected for typing

The median within-herd Cerro prevalence among herds that had clinical cases (20.0%) was greater than that among herds that only had positive environmental samples (9.2%), but this difference was not statistically significant. Among herds with clinical cases, chi-squared testing showed that the prevalence of fecal Cerro shedding among clinical suspect cattle (56.6%, 69/122) was significantly higher (p-value < 0.0001) than that among apparently healthy cattle tested via follow-up sampling (25.6%, 249/974). Clinical suspect cattle were also more likely to test positive for Cerro than apparently healthy cattle as estimated by a logistic regression model which controlled for herd as a random effect (OR, 3.9; p-value = 0.05).

Discussion

There is very little in the literature concerning *Salmonella* Cerro in cattle or other species. This study had the advantage of utilizing both environmental surveillance and disease monitoring in order to assess the occurrence of *S. Cerro* within each enrolled dairy herd. These herds were located throughout New York and were characterized by a wide range of sizes and management types representative of the dairy industry in this area of the country. Another strength of this study was the longitudinal sampling approach for estimating the within-herd prevalence of *S. Cerro* when herds were identified as being positive. To our knowledge, no field studies have identified this serotype on so many intensively sampled dairy farms, thus permitting a thorough description of its within-herd prevalence, clinical implications, and isolate characteristics.

Culture of environmental samples has been shown to be a relatively effective means of monitoring for the presence of *Salmonella* on dairy farms (Warnick *et al.*,

2003). However, identifying clinical cases based on detection of suspect animals by herd owners or veterinarians may have underestimated the number of cases in the present study. Furthermore, fecal culture does not have perfect sensitivity for detecting the presence of *Salmonella*, and we recognize that some positive cattle were presumably missed by culturing. On the other hand, it is also plausible that some animals with a positive *Salmonella* culture result and compatible clinical signs were actually symptomatic because of another primary disease process.

Salmonella was identified in 77% of the study herds, and nearly half of the positive herds (20/44) yielded *S. Cerro*. In a study of a single herd in Pennsylvania experiencing an outbreak of *S. Cerro*, investigators found that this serotype was also isolated from other dairy herds in the same geographic area before and during their study period (Van Kessel *et al.*, 2007). Introduction of *Salmonella* onto a dairy farm can occur through a variety of routes, including purchased cattle, contaminated feed or water, wild animals such as rodents and birds, human traffic, and insects (Bender, 1994; Evans and Davies, 1996; Anderson *et al.*, 2001; Sanchez *et al.*, 2002; Murinda *et al.*, 2004; Nielsen *et al.*, 2007). More information on the biosecurity procedures, feed sources, and cattle importation practices of the Cerro-positive herds would be required to ascertain the likely means of introduction and potentially establish an epidemiologic linkage.

The median within-herd Cerro prevalence was 17%, and in some herds the estimated prevalence approximated 50%. Of the 10 herds from which *S. Cerro* was identified on follow-up sampling, five had experienced clinical disease compatible with a diagnosis of salmonellosis. The role of *S. Cerro* in causing clinical illness among dairy cattle is unclear, although published reports suggest a previous lack of disease association in cattle. This serotype persisted in the Pennsylvania dairy herd for nearly two years at a high within-herd prevalence (reaching a peak of 88%) in the

absence of clinical signs of salmonellosis (Van Kessel *et al.*, 2007). *S. Cerro* was also isolated from four dairy herds in Ohio for up to 18 months in the absence of clinical signs; however, three of these herds had experienced clinical outbreaks (diarrhea, abortion, and evidence of endotoxemia) prior to the study period, and *S. Cerro* was isolated from some of the affected cattle in each instance (Huston *et al.*, 2002a). *S. Cerro* was isolated from the environment of one Wisconsin dairy farm without a history of salmonellosis, but no cattle were sampled in that study (Peek *et al.*, 2004). The NAHMS Dairy 2007 study, based on a single sampling visit to 121 farms in 17 states, found *S. Cerro* to be the most common serotype isolated from healthy lactating cows (Centers for Epidemiology and Animal Health, 2009).

Although many asymptomatic cattle shed *S. Cerro* in their feces, there has been an apparent increase in the prevalence of this serotype among cattle with clinical signs of salmonellosis. *Cerro* was the main serotype isolated from clinical cases in this study (59%), and the prevalence of fecal *Cerro* shedding among clinical suspect cattle was significantly higher than that among apparently healthy cattle tested via follow-up sampling. In herds with *Cerro*-positive clinical cases, *Cerro* was the predominant or only *Salmonella* serotype isolated from the farm. In contrast, a recent comprehensive study on the incidence of salmonellosis among dairy herds in New York and other northeastern states, based on data collected between 2004 and 2005, found *S. Cerro* to be a very rare isolate (Cummings *et al.*, 2009). In that study, *Cerro* accounted for 0.2% of the cases, whereas Newport and Typhimurium accounted for 41% and 19%, respectively. *S. Cerro* has also been isolated from clinically affected chickens, turkeys, pigs, and horses in recent years (CDC, 2008; CDC, 2007).

S. Cerro has been a rare isolate among people in the U.S. with laboratory-confirmed *Salmonella* infections, accounting for 0.1% of the cases in both 2005 (CDC, 2007) and 2006 (CDC, 2008). Reports of human disease due to this serotype are

sparse. An outbreak of *S. Cerro* involving 29 known patients occurred in New Mexico in 1985, traced to contaminated beef jerky (CDC, 1985). This serotype has also been incriminated as a sporadic cause of infant diarrhea (Fule and Kaundinya, 1986), pyemia (Bhore *et al.*, 1980), and osteomyelitis (Le, 1982). Finally, a rise in *S. Cerro* isolations was documented in Italy between 1997 and 1999, although these were primarily from healthy food handlers and samples of urban sewage plant effluent (Mammina *et al.*, 2000). Future surveillance for confirmed cases of salmonellosis will determine if *S. Cerro* is seen increasingly among people. Other serotypes important to bovine health (Newport and Typhimurium) have emerged across the United States and become leading causes of human foodborne disease (Glynn *et al.*, 1998; Gupta *et al.*, 2003; CDC, 2009b), and close monitoring of Cerro is warranted.

There was a low frequency of antimicrobial resistance among Cerro isolates in this study, with 95% being pan-susceptible. However, drug-resistant isolates were widespread among farms. We found drug resistance to be significantly more common among isolates from clinically ill cattle than among isolates from other sources. This is in agreement with the apparent correlation between antimicrobial resistance and the presence of clinical disease in dairy cattle. Studies of fecal *Salmonella* shedding among healthy cattle across the U.S. have shown antimicrobial resistance to be uncommon (Wells *et al.*, 2001; Blau *et al.*, 2005; Ray *et al.*, 2007), but multidrug resistance was found to be highly prevalent among isolates from cattle with clinical signs of salmonellosis in the northeastern U.S (Cummings *et al.*, 2009). A similar phenomenon has also been observed in people, as infections with resistant *Salmonella* strains tend to be more severe and lead to higher rates of hospitalization than those caused by susceptible strains (Helms *et al.*, 2002; Helms *et al.*, 2004; Varma *et al.*, 2005a; Varma *et al.*, 2005b).

Regardless of their source, the isolates in this study displayed tremendous homogeneity with respect to PFGE types. Of the 116 isolates that were typed, 89% had an identical PFGE pattern. This particular pattern was found to be stable and conservative across herds. It remains possible that there was a disparity in virulence between isolates obtained from clinical cases as opposed to those obtained from asymptomatic cattle; however, a more discriminatory typing method or combination of methods may be needed to differentiate these isolates. Alternatively, it is conceivable that host factors impacting immune status were responsible for the discrepancy in clinical outcomes observed in this study. Such factors could include stage of lactation, concurrent illness, and plane of nutrition. The minor differences in banding patterns exhibited by the 13 isolates with sporadic PFGE types apparently did not reflect an acquisition of antimicrobial resistance genes, as 12 of these isolates were pan-susceptible.

The isolation of a predominant PFGE type from cattle throughout New York could indicate the rapid spread of a single clone on account of being a successful phenotype. PFGE homogeneity was also noted among bovine Newport–MDRAmpC isolates from Massachusetts when this serotype was discovered to be an emergent threat in the United States (Gupta *et al.*, 2003). Evidence for widespread dissemination of a single Cerro strain over a short duration of time should further prompt concern over the potential danger to animal and human health. It is noteworthy that the New York State Department of Health (NYSDOH) has isolated *S.* Cerro from two human patients since 2003 (in August, 2007 and August, 2008), and both isolates had a PFGE banding pattern that was identical to that of the predominant PFGE type seen among cattle in this study (unpublished data).

Conclusions

Salmonella Cerro appears to be an emerging pathogen of dairy cattle. In contrast to previously published reports, our results suggest that this serotype may be associated with extensive clinical outbreaks in dairy herds. The apparent increase in the prevalence of *S. Cerro* among cattle with salmonellosis, coupled with evidence for rapid clonal spread, may have important implications for public health.

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REFERENCES

Anderson RJ, House JK, Smith BP, *et al.* Epidemiologic and biological characteristics of salmonellosis in three dairy herds. *J Am Vet Med Assoc* 2001; 219:310-322.

Bender JB. Reducing the risk of *Salmonella* spread and practical control measures in dairy herds. *Bovine Pract* 1994; 28:62-65.

Bhore AV, Phadke SA, Joshi BN. *Salmonella* Cerro causing pyaemia in man--report of a case. *Indian J Pathol Microbiol* 1980; 23:309-311.

Blau DM, McCluskey BJ, Ladely SR, *et al.* *Salmonella* in dairy operations in the United States: prevalence and antimicrobial drug susceptibility. *J Food Prot* 2005; 68:696-702.

Centers for Disease Control and Prevention (CDC). Salmonellosis associated with carne seca--New Mexico. *MMWR Morb Mortal Wkly Rep* 1985; 34:645-646.

Centers for Disease Control and Prevention (CDC). *Salmonella* Surveillance: Annual Summary, 2005. U.S. Department of Health and Human Services, CDC, Atlanta, GA. 2007.

Centers for Disease Control and Prevention (CDC). *Salmonella* Surveillance: Annual Summary, 2006. U.S. Department of Health and Human Services, CDC, Atlanta, GA. 2008.

Centers for Disease Control and Prevention (CDC). National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS): Human Isolates Final Report, 2006. U.S. Department of Health and Human Services, CDC, Atlanta, GA. 2009a.

Centers for Disease Control and Prevention (CDC). Preliminary FoodNet Data on the incidence of infection with pathogens transmitted commonly through food--10 States, 2008. *MMWR Morb Mortal Wkly Rep* 2009b; 58:333-337.

Centers for Epidemiology and Animal Health. National Animal Health Monitoring System (NAHMS) Dairy 2007: *Salmonella* and *Campylobacter* on U.S. Dairy Operations, 1996-2007. United States Department of Agriculture, Fort Collins, CO. 2009.

Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from*

Animals; Approved Standard - Third Edition. CLSI document M31-A3. Wayne, PA: Clinical and Laboratory Standards Institute. 2008.

Cummings KJ, Warnick LD, Alexander KA, *et al*. The incidence of salmonellosis among dairy herds in the northeastern United States. *J Dairy Sci* 2009; 92:3766-3774.

Cummings KJ, Warnick LD, Elton M, *et al*. The effect of clinical outbreaks of salmonellosis on the prevalence of fecal *Salmonella* shedding among dairy cattle in New York. *Foodborne Pathog Dis* 2010; Epub ahead of print.

Davis MA, Besser TE, Eckmann K, *et al*. Multidrug-resistant *Salmonella* Typhimurium, Pacific Northwest, United States. *Emerg Infect Dis* 2007; 13:1583-1586.

Divers TJ and Peek SF. *Rebhun's diseases of dairy cattle*. St. Louis: Saunders Elsevier, 2008.

Dunne EF, Fey PD, Kludt P, *et al*. Emergence of domestically acquired ceftriaxone-resistant *Salmonella* infections associated with AmpC beta-lactamase. *JAMA* 2000; 284:3151-3156.

Evans S and Davies R. Case control study of multiple-resistant *Salmonella* Typhimurium DT104 infection of cattle in Great Britain. *Vet Rec* 1996; 139:557-558.

Fule RP and Kaundinya DV. *S. Cerro* (18:z4,z23-), a rare serotype isolation from infants with diarrhoea in Ambajogai. *Indian J Pathol Microbiol* 1986; 29:205-207.

Glynn MK, Bopp C, Dewitt W, *et al*. Emergence of multidrug-resistant *Salmonella enterica* serotype Typhimurium DT104 infections in the United States. *N Engl J Med* 1998; 338:1333-1338.

Gupta A, Fontana J, Crowe C, *et al*. Emergence of multidrug-resistant *Salmonella enterica* serotype Newport infections resistant to expanded-spectrum cephalosporins in the United States. *J Infect Dis* 2003; 188:1707-1716.

Helms M, Simonsen J, Molbak K. Quinolone resistance is associated with increased risk of invasive illness or death during infection with *Salmonella* serotype Typhimurium. *J Infect Dis* 2004; 190:1652-1654.

Helms M, Vastrup P, Gerner-Smidt P, *et al.* Excess mortality associated with antimicrobial drug-resistant *Salmonella* Typhimurium. *Emerg Infect Dis* 2002; 8:490-495.

Hunter SB, Vauterin P, Lambert-Fair MA, *et al.* Establishment of a universal size standard strain for use with the PulseNet standardized pulsed-field gel electrophoresis protocols: converting the national databases to the new size standard. *J Clin Microbiol* 2005; 43:1045-1050.

Huston CL, Wittum TE, Love BC. Persistent fecal *Salmonella* shedding in five dairy herds. *J Am Vet Med Assoc* 2002a; 220:650-655.

Huston CL, Wittum TE, Love BC, *et al.* Prevalence of fecal shedding of *Salmonella* spp in dairy herds. *J Am Vet Med Assoc* 2002b; 220:645-649.

Jones TF, Ingram LA, Cieslak PR, *et al.* Salmonellosis outcomes differ substantially by serotype. *J Infect Dis* 2008; 198:109-114.

L Plym F and Wierup M. *Salmonella* contamination: a significant challenge to the global marketing of animal food products. *Rev Sci Tech* 2006; 25:541-554.

Le CT. *Salmonella* vertebral osteomyelitis: a case report with literature review. *Am J Dis Child* 1982; 136:722-724.

Mamina C, Cannova L, CarfiPavia S, *et al.* Endemic presence of *Salmonella enterica* serotype Cerro in southern Italy. *Euro Surveill* 2000; 5:84-86.

Mead PS, Slutsker L, Dietz V, *et al.* Food-related illness and death in the United States. *Emerg Infect Dis* 1999; 5:607-625.

Murinda SE, Nguyen LT, Nam HM, *et al.* Detection of sorbitol-negative and sorbitol-positive Shiga toxin-producing *Escherichia coli*, *Listeria monocytogenes*, *Campylobacter jejuni*, and *Salmonella* spp. in dairy farm environmental samples. *Foodborne Pathog Dis* 2004; 1:97-104.

Nielsen LR, Warnick LD, Greiner M. Risk factors for changing test classification in the Danish surveillance program for *Salmonella* in dairy herds. *J Dairy Sci* 2007; 90:2815-2825.

Peek SE, Hartmann FA, Thomas CB, *et al.* Isolation of *Salmonella* spp from the environment of dairies without any history of clinical salmonellosis. J Am Vet Med Assoc 2004; 225:574-577.

Peters AR. An estimation of the economic impact of an outbreak of *Salmonella* Dublin in a calf rearing unit. Vet Rec 1985; 117:667-668.

Ray KA, Warnick LD, Mitchell RM, *et al.* Prevalence of antimicrobial resistance among *Salmonella* on midwest and northeast USA dairy farms. Prev Vet Med 2007; 79:204-223.

Ribot EM, Fair MA, Gautom R, *et al.* Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. Foodborne Pathog Dis 2006; 3:59-67.

Sanchez S, Hofacre CL, Lee MD, *et al.* Animal sources of salmonellosis in humans. J Am Vet Med Assoc 2002; 221:492-497.

Van Kessel JS, Karns JS, Wolfgang DR, *et al.* Longitudinal study of a clonal, subclinical outbreak of *Salmonella enterica* subsp. *enterica* serovar Cerro in a U.S. dairy herd. Foodborne Pathog Dis 2007; 4:449-461.

Varma JK, Greene KD, Ovitt J, *et al.* Hospitalization and antimicrobial resistance in *Salmonella* outbreaks, 1984-2002. Emerg Infect Dis 2005a; 11:943-946.

Varma JK, Molbak K, Barrett TJ, *et al.* Antimicrobial-resistant nontyphoidal *Salmonella* is associated with excess bloodstream infections and hospitalizations. J Infect Dis 2005b; 191:554-561.

Voetsch AC, Van Gilder TJ, Angulo FJ, *et al.* FoodNet estimate of the burden of illness caused by nontyphoidal *Salmonella* infections in the United States. Clin Infect Dis 2004; 38 Suppl 3:S127-34.

Warnick LD, Kaneene JB, Ruegg PL, *et al.* Evaluation of herd sampling for *Salmonella* isolation on midwest and northeast US dairy farms. Prev Vet Med 2003; 60:195-206.

Wells SJ, Fedorka-Cray PJ, Dargatz DA, *et al.* Fecal shedding of *Salmonella* spp. by dairy cows on farm and at cull cow markets. J Food Prot 2001; 64:3-11.

CHAPTER 8: Summary

The epidemiologic approach to *Salmonella*

The field of epidemiology offers a variety of tools for addressing important research questions concerning the health implications of *Salmonella* among both cattle and people. Baseline surveillance data are used for assessing levels of *Salmonella* throughout the food production chain, including the pre-harvest stage. Such data are essential for monitoring broad *Salmonella* patterns as well as subtype-specific trends, evaluating the success of interventions, and detecting the emergence of pathogenic serovars. Surveillance is likewise crucial for monitoring trends in antimicrobial resistance and discovering novel resistance phenotypes. These types of descriptive epidemiologic data may also be used for constructing infectious disease models and performing microbiologic risk assessments. Analytic epidemiology, via careful study design and statistical analysis, is necessary for investigating various risk factors at the population level, e.g. risk factors for positive *Salmonella* status, infection with multidrug-resistant strains, or extended duration of fecal *Salmonella* shedding among cattle. Human studies may seek to find significant associations with salmonellosis in general, or they may focus on risk factors for infection with certain *Salmonella* subtypes or resistance patterns. In the process, these analytic methods may also shed new light on *Salmonella* reservoirs and transmission routes. The addition of molecular subtyping to the epidemiologist's repertoire allows human pathogens to be microbiologically linked to animals and animal products. Thus, molecular epidemiology greatly facilitates outbreak investigations and source attribution efforts. Molecular subtyping combined with traditional epidemiologic methods can be used to confirm the existence of an outbreak and to subsequently ascertain the outbreak source. Similarly, molecular techniques are vital for attempting to estimate the proportion of human salmonellosis cases attributable to specific food items. These

efforts, in turn, are used for shaping public health policy and prioritizing both intervention strategies and research dollars.

Dissertation scope and conclusions

Several previous studies have described the prevalence of fecal *Salmonella* shedding among asymptomatic dairy cattle, using either cross-sectional or longitudinal designs (Wells et al. 2001, Huston et al. 2002b, Fossler et al. 2004, Blau et al. 2005). However, there is very little in the literature regarding fecal shedding among cattle with clinical disease due to *Salmonella* infection (salmonellosis). This dissertation has addressed this gap, both in the field and in a hospital setting, and has emphasized the relevance to public health. The research included herein has shed light on a number of facets of *Salmonella* epidemiology in dairy cattle, including (1) incidence of salmonellosis in the northeastern United States, along with the serovars and antimicrobial resistance patterns of isolates shed by cattle with clinical illness; (2) prevalence and risk factors for fecal *Salmonella* shedding among cattle presenting to a teaching hospital; (3) duration of *Salmonella* shedding following clinical disease in cattle; (4) prevalence of *Salmonella* shedding among dairy herds with clinical vs. subclinical infections; and (5) emergence of a previously sporadic pathogen (*Salmonella* Cerro) as a cause of widespread clinical disease in New York. This work supports the view that cattle with salmonellosis present a greater threat to public health than cattle that are asymptotically shedding *Salmonella* organisms.

The herd-level incidence rate for salmonellosis was approximately nine positive herds per 100 herd-years. However, fewer than 20% of the positive study herds accounted for over 70% of the clinical *Salmonella* cases. Clustering of disease

among dairy herds suggests that a relatively small number of herds may represent a substantial proportion of the public health risk attributed to bovine salmonellosis. It seems likely that the most efficient approach to controlling *Salmonella* at the farm level would be to focus efforts on addressing biosecurity and hygiene practices among the relatively few herds with high frequency of disease, as well as preventing pathogen spread from such herds to those that remain uninfected. The animal-level incidence rate for pre-weaned female calves was 8.1 cases per 1,000 animal-years, as compared to 1.8 cases per 1,000 animal-years for adult cows. As mortality was found to be higher among calves with salmonellosis, dairy herd outbreaks that involve this age group are an especially important economic concern for producers. Calves are further removed from the food chain than cows but may serve as a source of *Salmonella* persistence in the dairy farm environment. In addition, *Salmonella* isolates from calves were more likely to be multidrug-resistant (MDR) than isolates from adult cattle.

Clinically affected cattle often shed serovars that are also important human pathogens, as *Salmonella* Newport and *Salmonella* Typhimurium were the predominant serovars in the incidence and hospital studies. These serovars together accounted for 60% of the isolates in the study of incidence of salmonellosis in the northeastern United States, and they comprised 64% of the teaching hospital isolates obtained from clinical suspect cattle. Preliminary CDC FoodNet data from 2009 show that Newport and Typhimurium were two of the three most common *Salmonella* serovars isolated from people with laboratory-confirmed salmonellosis, accounting for over 28% of the cases (Centers for Disease Control and Prevention (CDC). 2010). Furthermore, the use of multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) has demonstrated overlap of *Salmonella* subtypes between humans and cattle (Alcaine et al. 2006, Soyer et al. 2010). In contrast, the serovars

most commonly isolated from clinically healthy cattle (e.g. *Salmonella* Cerro, Kentucky, Mbandaka, Meleagridis, and Montevideo) differ from those that most frequently cause human or bovine disease. Furthermore, multidrug resistance was found to be highly prevalent among isolates from cattle in the incidence study (over 68% of isolates were resistant to five or more antimicrobial agents), yet studies of fecal *Salmonella* shedding among clinically healthy cattle have shown antimicrobial resistance to be uncommon (Wells et al. 2001,Blau et al. 2005,Ray et al. 2007). The rise in multidrug resistance among *Salmonella* strains isolated from people over the past two decades (Glynn et al. 1998,Dunne et al. 2000,Gupta et al. 2003,Davis et al. 2007) has made treatment failures more common among those with serious disease. Moreover, infections with resistant strains of *Salmonella* tend to be more severe and lead to higher rates of hospitalization than those caused by susceptible strains (Helms et al. 2002,Helms et al. 2004,Varma et al. 2005b,Varma et al. 2005a).

Additional evidence for the increased risk posed by cattle with salmonellosis was found in the field study comparing dairy herds with clinical vs. subclinical infections. In this study, herds with laboratory-confirmed clinical cases of salmonellosis had a higher prevalence of fecal *Salmonella* shedding than herds which only generated positive environmental samples, and they were more likely to yield either Newport or Typhimurium on follow-up sampling. Dairy herds with a high prevalence of asymptomatic fecal *Salmonella* shedding presumably represent a greater threat to public health than herds with few shedders. An association between herd outbreaks of salmonellosis and higher within-herd *Salmonella* prevalence should help guide strategies for reducing this threat, as the ability to recognize high-risk herds by clinical laboratory submissions presents an obvious opportunity to improve food safety at the pre-harvest level. This is in contrast with other important foodborne zoonotic pathogens, such as *Campylobacter jejuni* and *E. coli* O157:H7, which occur

widely in adult cattle without accompanying clinical disease (Dunn et al. 2004, Cho et al. 2006, Kwan et al. 2008, Ellis-Iversen et al. 2009, Huang et al. 2009).

The median duration of fecal *Salmonella* shedding following clinical disease was found to be 50 days, which is well beyond the typical period of clinical signs in cattle with salmonellosis. The duration of fecal *Salmonella* shedding exceeded a year in two animals. Based on the daily output of *Salmonella* organisms from infected cattle, a typical adult cow with salmonellosis would thus be expected to excrete between 4×10^8 and 4×10^{13} organisms during its shedding period. This undoubtedly leads to extensive environmental contamination and an increased risk of within-herd transmission and spread to other herds, thereby enhancing the risk of zoonotic transmission via direct contact. Fecal contamination of beef carcasses and subsequent foodborne exposure would also be more likely when *Salmonella*-positive cattle are sent to the slaughterhouse following clinical disease.

Seasonality of fecal *Salmonella* shedding among cattle was explored in the hospital studies. Clinical suspect animals that were admitted in the fall were more likely to be shedding *Salmonella* than were patients admitted during other times of the year. Cluster analysis revealed eight temporal clusters of bovine *Salmonella* cases between 1996 and 2007, and all but one were centered over the month of August or September. It was previously reported that fecal *Salmonella* shedding among dairy cattle on farms is more common in the summer and fall (Fossler et al. 2005), and other studies found increased shedding during the summer months on dairy farms and at livestock markets (Wells et al. 2001, Kabagambe et al. 2000, Pangloli et al. 2008). This seasonal association is presumably related to temperature and/or moisture conditions that prevail in the summer and fall months, but it is unclear whether these conditions are impacting the bacteria or the host species. *Salmonella*'s ability to thrive in warm, moist environments may increase the odds of host exposure and infection, or perhaps

heat stress in cattle leads to suppressed immunity. The seasonal pattern of *Salmonella* shedding among cattle suggests that the potential for zoonotic transmission will be greater during the late summer and early fall. A seasonal increase in human salmonellosis as a result of bovine-associated subtypes would partially account for the peak incidence of human cases during the summer months.

Rapid and widespread emergence of *Salmonella* serovars may occur over time, and this phenomenon was illustrated in these studies. There was a clear temporal demarcation for the two most common serovars isolated in the hospital studies; over 90% of the Typhimurium isolates were obtained from cattle admitted between 1996 and late 2001, whereas all of the Newport isolates were obtained from cattle admitted between 2002 and 2007. This temporal shift seems to coincide with the historical incidence of *Salmonella* infections in people. During the early 1990s, MDR *Salmonella* Typhimurium definitive phage type 104 emerged across the United States (Glynn et al. 1998). By 1998, approximately 30% of all foodborne *Salmonella* infections in this country were caused by *S. Typhimurium* (Centers for Disease Control and Prevention (CDC). 1999). In 2000, however, the CDC noted a sharp increase in the incidence of infections attributable to *Salmonella* Newport, primarily as a result of MDR strains (Gupta et al. 2003). *S. Newport* has continued to increase in importance as a pathogen for humans, while *S. Typhimurium* appears to be less prevalent. According to CDC FoodNet data from 2006, the annual incidence of foodborne infections caused by *S. Newport* in the United States had increased by 42% over the average annual incidence for 1996 through 1998, whereas the incidence of infections caused by *S. Typhimurium* had decreased by 41% over the same period (Centers for Disease Control and Prevention (CDC). 2007). Recently, an apparent increase in the prevalence of *Salmonella* Cerro was noted among cattle with salmonellosis in New York. Nearly 60% of the clinical cases were positive for this

serovar, whereas the incidence study, based on data collected just a few years earlier, found *S. Cerro* to be a very rare isolate from cattle with salmonellosis (accounting for just 0.2% of the cases). The emergence of this serovar as a cause of widespread clinical disease in this region is striking, as the few published reports on *S. Cerro* suggest a lack of disease association in cattle (Huston et al. 2002a, Peek et al. 2004, Van Kessel et al. 2007, Centers for Epidemiology and Animal Health. 2009). The *Cerro* isolates from New York displayed tremendous homogeneity with respect to pulsed-field gel electrophoresis (PFGE) types, which could indicate the rapid spread of a single clone on account of being a successful phenotype. Other *Salmonella* serovars important to bovine health have emerged to become leading causes of human foodborne disease, and close monitoring of *Cerro* is warranted.

Future directions

A number of key questions have surfaced from this dissertation and should help guide future research. What is the nature of the association between increased herd size and fecal *Salmonella* shedding? In the incidence study, herds of the largest size category (≥ 400 female dairy cattle) were found to have a significantly higher incidence of salmonellosis than herds of the three smaller size categories. In the field study comparing dairy herds with clinical vs. subclinical infections, large herd size was significantly associated with the presence of laboratory-confirmed clinical cases among the *Salmonella*-positive herds. Increased likelihood of purchasing cattle from outside sources, high cattle density, or particular management practices of larger herds may play a role in augmenting the incidence of salmonellosis. Herd size itself is a risk factor that does not easily lend itself to practical intervention due to the management

trends and economic constraints that prevail in the modern dairy industry. However, it is possible that certain attributes of larger herds that contribute to the incidence of salmonellosis, if ascertained, could in fact be modified to reduce the occurrence of this disease.

Once an animal has become infected with *Salmonella*, the duration and magnitude of fecal shedding are fundamental determinants of public health risk. However, factors associated with prolonged shedding remain unknown. Are there various herd- and animal-level factors that are predictors of the duration of fecal *Salmonella* shedding among cattle? Serovar was not found to be significantly associated with *Salmonella* shedding duration, and it may be that host (immune status) and environmental factors (herd management and hygiene practices) play a more prominent role in determining the length of shedding. Alternatively, other pathogen factors such as dose of inoculum may have a significant effect on shedding duration. Additional work is needed to determine whether certain herd-level covariates, such as housing type and manure management system, are significantly associated with length of *Salmonella* shedding among cattle. A large sample of herds with diverse production methods would be required for such a study. It would also be valuable to examine specific animal-level factors, including stage of lactation and concurrent disease, as potential predictors of *Salmonella* shedding duration.

Evidence for changing trends in serovar prominence among dairy cattle was found throughout this research. What factors contribute to the periodic emergence of different *Salmonella* strains in cattle or other species, and what is the effect on human foodborne disease? Will *S. Cerro* become an important human pathogen? DNA sequencing or microarray analysis would be valuable tools for comparing Cerro isolates obtained from cattle before and after the emergence of this serovar in New York. Likewise, classification of the Cerro isolates from the current study via a typing

method more discriminatory than PFGE, or a combination of methods, might reveal a disparity in virulence between isolates obtained from clinical cases as opposed to those obtained from asymptomatic cattle. Such comparisons could shed light on the pathogen characteristics that allow particular strains to take hold and emerge as a cause of disease in animal populations. Future surveillance for confirmed cases of salmonellosis by CDC and state health departments will determine if *S. Cerro* is seen increasingly among people.

Other important research questions, though not derived directly from the work included herein, should be considered as priorities for future investigation. For example, are certain characteristics of cattle arriving at a slaughterhouse associated with increased odds of shedding *Salmonella* organisms? These might include physical traits evident at live inspection, reasons for culling, or farm-level attributes such as prior *Salmonella* history, herd size, or driving distance from the slaughterhouse. Cattle more likely to be shedding *Salmonella* could be slaughtered last or in a separate facility to minimize the contamination of carcasses. Similarly, what interventions during the immediate pre-harvest period can reduce fecal *Salmonella* shedding at the slaughterhouse? The administration of bacteriophage or probiotic agents could serve to safely and effectively decrease the quantity of organisms shed by *Salmonella*-positive cattle. Finally, what is the relative importance of clonal spread versus selective pressure due to antimicrobial use as means of promoting the dissemination of multidrug-resistant *Salmonella* within the dairy farm environment? Clonal spread of MDR *Salmonella* could occur via purchased cattle or other routine animal movements (e.g. off-site heifer raising), contaminated feed, or unregulated human traffic. However, data from recent NAHMS Dairy studies indicate that biosecurity measures such as maintenance of a closed herd, animal quarantine, and employee training are often not practiced. If lapses in biosecurity were shown to be significantly associated

with the dissemination of MDR strains, management strategies could be readily implemented to mitigate the spread of antimicrobial resistance among dairy farms.

In summary, this dissertation has elucidated several aspects of the epidemiology of clinical *Salmonella* infections in dairy cattle. It can be inferred that dairy cattle with salmonellosis present a greater threat to public health than cattle that are asymptotically shedding *Salmonella* organisms. Thus, efforts to reduce this threat may be facilitated by clinical disease surveillance in dairy cattle populations.

REFERENCES

- Alcaine SD, Soyer Y, Warnick LD, *et al.* Multilocus sequence typing supports the hypothesis that cow- and human-associated *Salmonella* isolates represent distinct and overlapping populations. *Appl Environ Microbiol* 2006; 72:7575-85.
- Blau DM, McCluskey BJ, Ladely SR, *et al.* *Salmonella* in dairy operations in the United States: prevalence and antimicrobial drug susceptibility. *J Food Prot* 2005; 68:696-702.
- Centers for Disease Control and Prevention (CDC). Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food - 10 states, 2009. *MMWR Morb Mortal Wkly Rep* 2010; 59:418-22.
- Centers for Disease Control and Prevention (CDC). Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food--10 states, 2006. *MMWR Morb Mortal Wkly Rep* 2007; 56:336-9.
- Centers for Disease Control and Prevention (CDC). Incidence of foodborne illnesses: preliminary data from the Foodborne Diseases Active Surveillance Network (FoodNet)--United States, 1998. *MMWR Morb Mortal Wkly Rep* 1999; 48:189-94.
- Centers for Epidemiology and Animal Health. National Animal Health Monitoring System (NAHMS) Dairy 2007: *Salmonella* and *Campylobacter* on U.S. Dairy Operations, 1996-2007. United States Department of Agriculture, Fort Collins, CO. 2009.
- Cho S, Bender JB, Diez-Gonzalez F, *et al.* Prevalence and characterization of *Escherichia coli* O157 isolates from Minnesota dairy farms and county fairs. *J Food Prot* 2006; 69:252-259.
- Davis MA, Besser TE, Eckmann K, *et al.* Multidrug-resistant *Salmonella* Typhimurium, Pacific Northwest, United States. *Emerg Infect Dis* 2007; 13:1583-1586.
- Dunn JR, Keen JE, Thompson RA. Prevalence of Shiga-toxigenic *Escherichia coli* O157:H7 in adult dairy cattle. *J Am Vet Med Assoc* 2004; 224:1151-1158.
- Dunne EF, Fey PD, Kludt P, *et al.* Emergence of domestically acquired ceftriaxone-resistant *Salmonella* infections associated with AmpC beta-lactamase. *JAMA* 2000; 284:3151-3156.

- Ellis-Iversen J, Cook AJ, Smith RP, *et al.* Temporal patterns and risk factors for *Escherichia coli* O157 and *Campylobacter* spp, in young cattle. *J Food Prot* 2009; 72:490-496.
- Fossler CP, Wells SJ, Kaneene JB, *et al.* Prevalence of *Salmonella* spp on conventional and organic dairy farms. *J Am Vet Med Assoc* 2004; 225:567-573.
- Fossler CP, Wells SJ, Kaneene JB, *et al.* Cattle and environmental sample-level factors associated with the presence of *Salmonella* in a multi-state study of conventional and organic dairy farms. *Prev Vet Med* 2005; 67:39-53.
- Glynn MK, Bopp C, Dewitt W, *et al.* Emergence of multidrug-resistant *Salmonella enterica* serotype Typhimurium DT104 infections in the United States. *N Engl J Med* 1998; 338:1333-1338.
- Gupta A, Fontana J, Crowe C, *et al.* Emergence of multidrug-resistant *Salmonella enterica* serotype Newport infections resistant to expanded-spectrum cephalosporins in the United States. *J Infect Dis* 2003; 188:1707-1716.
- Helms M, Simonsen J, Molbak K. Quinolone resistance is associated with increased risk of invasive illness or death during infection with *Salmonella* serotype Typhimurium. *J Infect Dis* 2004; 190:1652-1654.
- Helms M, Vastrup P, Gerner-Smidt P, *et al.* Excess mortality associated with antimicrobial drug-resistant *Salmonella* Typhimurium. *Emerg Infect Dis* 2002; 8:490-495.
- Huang JL, Xu HY, Bao GY, *et al.* Epidemiological surveillance of *Campylobacter jejuni* in chicken, dairy cattle and diarrhoea patients. *Epidemiol Infect* 2009; 137:1111-1120.
- Huston CL, Wittum TE, and Love BC. Persistent fecal *Salmonella* shedding in five dairy herds. *J Am Vet Med Assoc* 2002a; 220:650-5.
- Huston CL, Wittum TE, Love BC, *et al.* Prevalence of fecal shedding of *Salmonella* spp in dairy herds. *J Am Vet Med Assoc* 2002b; 220:645-9.
- Kabagambe EK, Wells SJ, Garber LP, *et al.* Risk factors for fecal shedding of *Salmonella* in 91 US dairy herds in 1996. *Prev Vet Med* 2000; 43:177-194.

- Kwan PS, Birtles A, Bolton FJ, *et al.* Longitudinal study of the molecular epidemiology of *Campylobacter jejuni* in cattle on dairy farms. *Appl Environ Microbiol* 2008; 74:3626-3633.
- Pangloli P, Dje Y, Ahmed O, *et al.* Seasonal incidence and molecular characterization of *Salmonella* from dairy cows, calves, and farm environment. *Foodborne Pathog Dis* 2008; 5: 87-96.
- Peek SE, Hartmann FA, Thomas CB, *et al.* Isolation of *Salmonella* spp from the environment of dairies without any history of clinical salmonellosis. *J Am Vet Med Assoc* 2004; 225:574-577.
- Ray KA, Warnick LD, Mitchell RM, *et al.* Prevalence of antimicrobial resistance among *Salmonella* on midwest and northeast USA dairy farms. *Prev Vet Med* 2007; 79:204-223.
- Soyer Y, Alcaine SD, Schoonmaker-Bopp DJ, *et al.* Pulsed-field gel electrophoresis diversity of human and bovine clinical *Salmonella* isolates. *Foodborne Pathog Dis* 2010; Epub ahead of print.
- Van Kessel JS, Karns JS, Wolfgang DR, *et al.* Longitudinal study of a clonal, subclinical outbreak of *Salmonella enterica* subsp. *enterica* serovar Cerro in a U.S. dairy herd. *Foodborne Pathog Dis* 2007; 4:449-461.
- Varma JK, Greene KD, Ovitt J, *et al.* Hospitalization and antimicrobial resistance in *Salmonella* outbreaks, 1984-2002. *Emerg Infect Dis* 2005a; 11:943-946.
- Varma JK, Molbak K, Barrett TJ, *et al.* Antimicrobial-resistant nontyphoidal *Salmonella* is associated with excess bloodstream infections and hospitalizations. *J Infect Dis* 2005b; 191:554-561.
- Wells SJ, Fedorka-Cray PJ, Dargatz DA, *et al.* Fecal shedding of *Salmonella* spp. by dairy cows on farm and at cull cow markets. *J Food Prot* 2001; 64:3-11.