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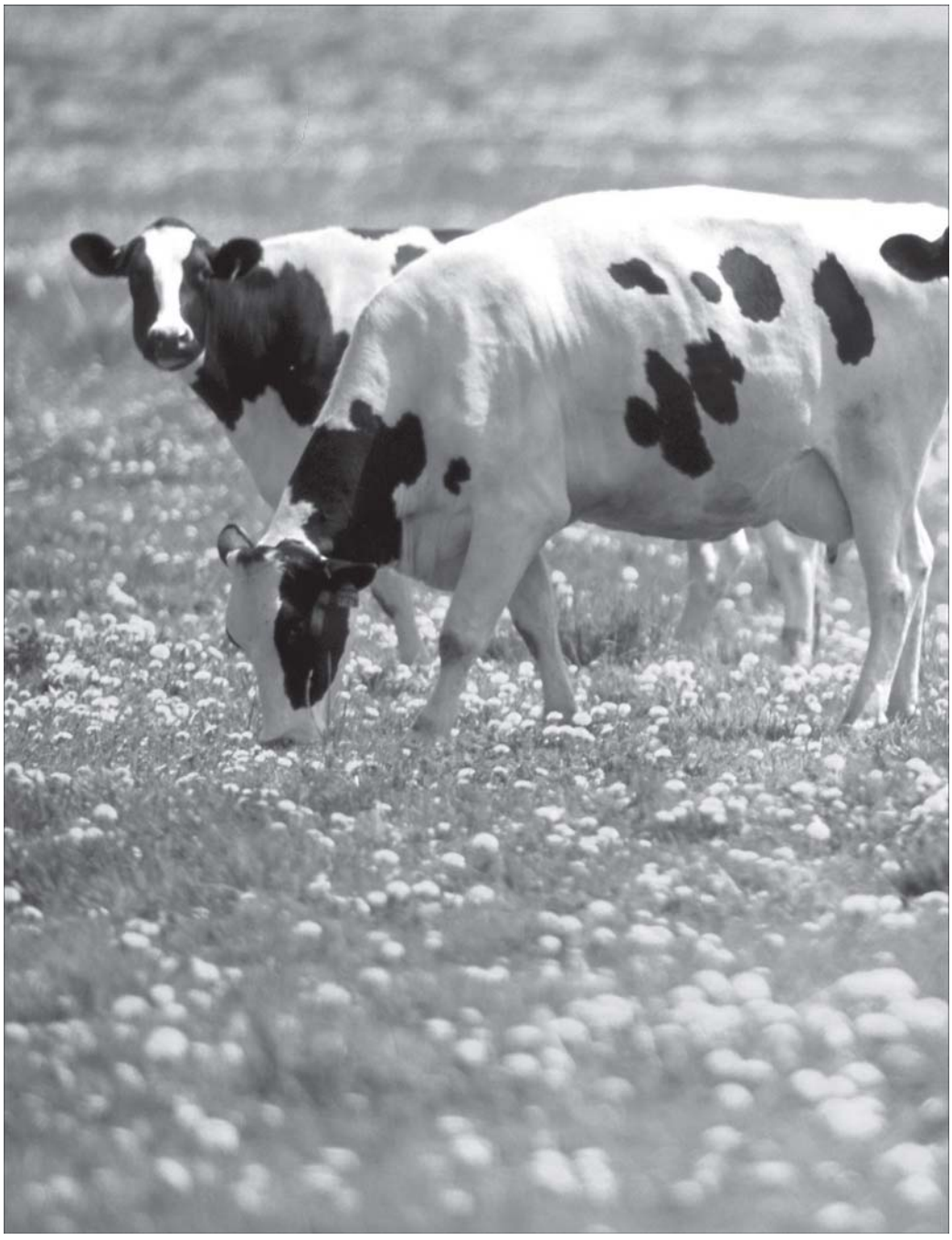
National
Animal Health
Monitoring
System

March 2011

Dairy 2007

Salmonella, Listeria, and *Campylobacter* on U.S. Dairy Operations, 1996–2007



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Selected Highlights

This report provides an in-depth look at the prevalence of food safety pathogens on U.S. dairy operations from 1996 to 2007, as identified from three National Animal Health Monitoring System studies: Dairy 1996, Dairy 2002, and Dairy 2007. Estimates in this report from bulk-tank milk testing are reported as population estimates. Estimates based on fecal culture represent a convenience sample and are not population estimates.

Here are a few highlights from the report:

In 2007, the percentage of operations on which a milk filter tested positive for *Salmonella* (24.7 percent) was more than double the percentage of operations on which a bulk-tank milk sample tested positive (10.8 percent). Likewise, the percentage of operations on which a milk filter tested positive for any *Listeria* (28.3 percent) was more than three times the percentage of operations on which a bulk-tank milk sample tested positive for any *Listeria* (9.0 percent). Milk filters were not tested in 2002 or 1996.

The percentage of operations on which bulk-tank milk tested positive for *Salmonella* by RT-PCR was similar in 2002 and 2007 (11.9 and 10.8 percent, respectively). In addition, the percentage of operations on which bulk-tank milk tested positive for *Listeria monocytogenes* was similar in 2002 and 2007 (3.8 and 3.7 percent, respectively). Bulk-tank milk was not tested in 1996.

The percentage of operations positive for *Salmonella* via fecal culture increased from 1996 to 2007. In 1996, 20.0 percent of operations had any *Salmonella*-positive cows compared with 30.9 percent of operations in 2002 and 39.7 percent in 2007. In 1996 and 2007, the percentage of cows positive for *Salmonella* was 5.4 and 13.8 percent, respectively.

During the Dairy 1996, 2002, and 2007 studies, a higher percentage of operations with 500 or more cows were *Salmonella* positive than operations with fewer than 100 cows.

The percentage of *Salmonella* isolates resistant to at least one antimicrobial decreased from 2002 to 2007 (17.7 and 3.4 percent, respectively). Similarly, for any specific antimicrobial to which resistance was observed, a lower percentage of isolates were resistant in 2007 than in 2002.

In the Dairy 1996, 2002, and 2007 studies, nearly all operations had at least one cow that was shedding *Campylobacter* (100, 97.9, and 92.6 percent of operations, respectively).

In 2002 and 2007, less than 5 percent of *C. jejuni* isolates were resistant to any single antimicrobial tested, with the exception of tetracycline. In 2007, 62.4 percent of *C. jejuni* isolates were resistant to tetracycline compared with 47.5 percent in 2002.

Acknowledgments

This report has been prepared from material received and analyzed by the U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS) via three national studies of health management and animal health on U.S. dairy operations conducted in 1996, 2002, and 2007.

The Dairy 1996, Dairy 2002, and Dairy 2007 studies were cooperative efforts between State and Federal animal health officials, statisticians, university researchers, and extension personnel. We want to thank the National Agricultural Statistics Service (NASS) enumerators, State and Federal veterinary medical officers (VMOs), and animal health technicians (AHTs) who visited the farms and collected the data. Their hard work and dedication to the National Animal Health Monitoring System (NAHMS) are invaluable. The roles of the producers, Area Veterinarians in Charge (AVIC), NAHMS Coordinators, VMOs, AHTs, and NASS enumerators were critical in providing quality data for all dairy reports. Recognition also goes to the personnel at the Centers for Epidemiology and Animal Health (CEAH) for their efforts in generating and distributing valuable reports from NAHMS dairy studies.

Additional biological sampling and testing were afforded by the generous contributions of collaborators for the dairy studies, including

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- University of Wisconsin, Madison; and
- Wisconsin Veterinary Diagnostic Laboratory.

All participants are to be commended, particularly the producers whose voluntary efforts made the dairy studies possible.

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Feedback

Feedback, comments, and suggestions regarding the Dairy 2007 study reports are welcome. Please forward correspondence via email to: NAHMS@aphis.usda.gov, or you may submit feedback via online survey at: http://nahms.aphis.usda.gov (Click on “FEEDBACK on NAHMS reports.”)

Table of Contents

Introduction

There are more than 250 known diseases caused by bacteria, fungi, viruses, and parasites transmitted through food to humans. Foodborne pathogens or toxins enter the body through the gastrointestinal tract where the first symptoms of illness often appear. As a result, nausea, vomiting, abdominal cramps, and diarrhea are common symptoms in many foodborne diseases. The majority of foodborne illnesses are mild and cause symptoms for only 1 to 2 days; however, some cases are more serious, resulting in severe illness or death (CDC, 2005).

While the food supply in the United States is one of the safest in the world, the Centers for Disease Control and Prevention (CDC) estimates that each year 76 million people in the United States get sick from foodborne pathogens, of which 325,000 are hospitalized and 5,000 die (Mead et al., 1999). The most commonly recognized foodborne infections caused by bacteria are due to *Campylobacter*, *Salmonella*, and *Escherichia coli (E. coli)* O157:H7 (CDC, 2005). The Foodborne Diseases Active Surveillance Network (FoodNet) of CDC's Emerging Infections Program collects data in 10 U.S. States on diseases caused by enteric pathogens transmitted commonly through food. In 2008, FoodNet reported that the incidence per 100,000 people for *Salmonella, Campylobacter*, *E. coli* O157:H7, and *Listeria* remained unchanged for the preceding 3 years (CDC, 2009). Preventing illness and death associated with foodborne pathogens remains a major public health challenge.

In addition to the effect on human health, foodborne illnesses have an economic impact. The health-related cost of foodborne illness in the United States is estimated to be approximately $152 billion annually (Scharff, 2010).

Many organisms capable of causing foodborne illness are present in the intestines of healthy animals raised for food. As a result, food can be contaminated as it is produced. For example, meat and poultry carcasses can be contaminated if they come in contact with small amounts of intestinal contents during slaughter. Similarly, fresh fruits and vegetables can be contaminated if they are washed or irrigated with water contaminated with animal manure or human sewage (Doyle and Erickson, 2008; Hanning et al., 2009). Other foods of animal origin, such as raw eggs, unpasteurized milk, and raw shellfish might also be contaminated. In general, commingling products from many individual animals—such as bulk raw milk, pooled raw eggs, or ground beef—presents an increased risk of contamination; a pathogen present in any one animal can contaminate products from multiple animals.

There are several reasons that food safety is of concern to the dairy industry. Raw milk can contain *Salmonella*, *Campylobacter*, or *Listeria,* all of which can cause human disease; however, outbreaks of disease in humans caused by milk products have primarily been due to the consumption of unpasteurized milk or cheeses made from unpasteurized milk. In addition, cull dairy cows account for about 17 percent of the

ground beef available for national consumption (Troutt and Osburn, 1997) and may be a potential source of human exposure to foodborne pathogens if the meat from these animals is contaminated with fecal material during slaughter or processing.

This report compares the prevalence and antimicrobial resistance of *Salmonella, Campylobacter*, and *Listeria* on U.S. dairy operations as reported in the NAHMS Dairy 1996, Dairy 2002, and Dairy 2007 studies. These pathogens were selected because data relating to them could be compared across study years; only results that could be compared with Dairy 2007 results were included. For example, results from the composite fecal sample testing for *Salmonella* conducted during Dairy 2007 are not reported here because composite fecal *Salmonella* samples were not collected and tested during the Dairy 1996 and Dairy 2002 studies.

Further information on NAHMS studies and reports is available online at:
http://nahms.aphis.usda.gov

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NAHMS Dairy Studies

The National Animal Health Monitoring System (NAHMS) is a nonregulatory division of the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS). NAHMS is designed to help meet the Nation's animal-health information needs and has collected data on dairy health and management practices through four previous studies.

The NAHMS 1991-92 National Dairy Heifer Evaluation Project (NDHEP) provided the dairy industry's first national information on the health and management of dairy heifers in the United States. Just months after the study's first results were released in 1993, cases of acute bovine viral diarrhea (BVD) surfaced in the United States following a 1993 outbreak in Canada. NDHEP information on producer vaccination and biosecurity practices helped officials address the risk of disease spread and target educational efforts on vaccination protocols. An outbreak of human illness was reported in 1993 in the Pacific Northwest, this time related to *Escherichia coli* 0157:H7. NDHEP data on the bacteria's prevalence in dairy cattle helped officials define public risks as well as research needs. This baseline picture of the industry also helped identify additional research and educational efforts in various production areas, such as feed management and weaning age.

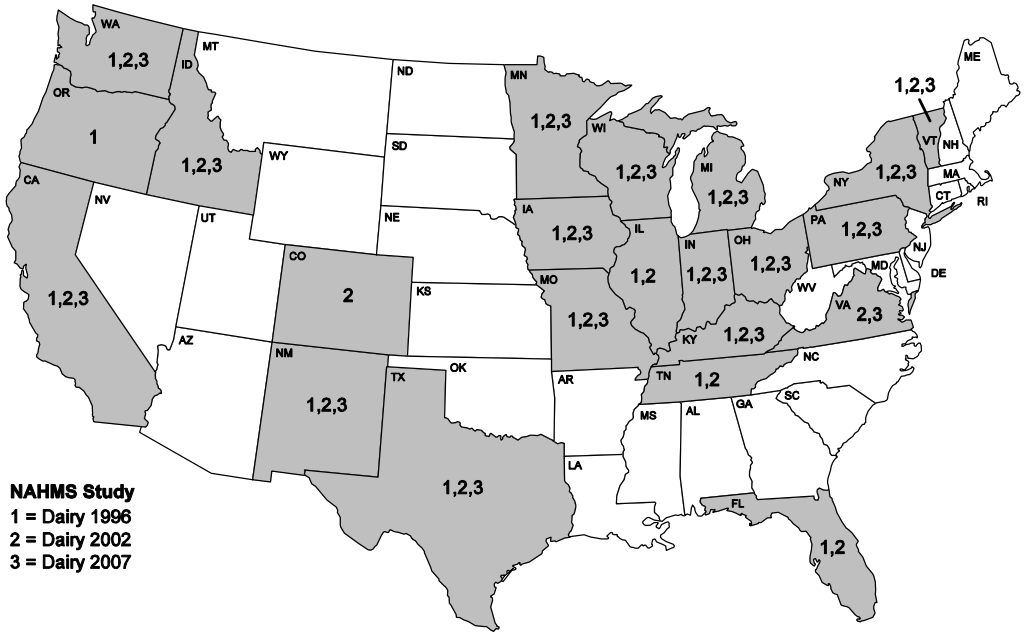
Information from the NAHMS Dairy 1996 study helped the U.S. dairy industry identify educational needs and prioritize research efforts on such timely topics as antibiotic usage and Johne's disease, as well as digital dermatitis, bovine leukosis virus, and potential foodborne pathogens, including *E. coli*, *Salmonella*, and *Campylobacter.*

A major focus of the Dairy 2002 study was to describe management strategies that prevent and reduce Johne's disease and to determine management factors associated with *Mycoplasma* and *Listeria* in bulk-tank milk. Additionally, levels of participation in quality assurance programs, the incidence of digital dermatitis, a profile of animal waste handling systems used on U.S. dairy operations, and industry changes since the NDHEP in 1991 and Dairy 1996 were examined.

One of the objectives of the Dairy 2007 study was calf health, including colostrum management and passive transfer of immunity. Additional study topics included an evaluation of cow comfort and the analysis of hygiene and hock scores. Additionally, diseases of concern such as BVD, Johne's disease, and contagious mastitis were evaluated. The Dairy 2007 study also took and in-depth look at reproductive practices.

An objective for all three studies, Dairy 1996, Dairy 2002, and Dairy 2007, was to determine the prevalence of specific food safety pathogens and to describe antimicrobial resistance patterns on U.S. dairy operations.

States Participating in NAHMS 1996, 2002, and 2007 Dairy Studies



Study Objectives and Related Outputs

1. Describe trends in dairy cattle health and management practices
- Part II: Changes in the U.S. Dairy Cattle Industry, 1991–2007, March 2008
- Part V: Changes in Dairy Cattle Health and Management Practices in the United States, 1996–2007, July 2009

2. Evaluate management factors related to cow comfort and removal rates
- Facility Characteristics and Cow Comfort on U.S. Dairy Operations, 2007, Interpretive Report, October 2010

3. Describe dairy calf health and nutrition from birth to weaning and evaluate heifer disease prevention practices
- Part I: Reference of Dairy Cattle Health and Management Practices in the United States, 2007, October 2007
- Off-Site Heifer Raising on U.S. Dairy Operations, 2007, info sheet, November 2007
- Colostrum Feeding and Management on U.S. Dairy Operations, 1991–2007, info sheet, March 2008
- Part IV: Reference of Dairy Cattle Health and Management Practices in the United States, 2007, February 2009
- Calving Intervention on U.S. Dairy Operations, 2007, info sheet, February 2009
- Heifer Calf Health and Management Practices on U.S. Dairy Operations, 2007, Interpretive Report, February 2010
- Passive Transfer in Dairy Heifer Calves, 1991–2007, info sheet, March 2010

4. Estimate the prevalence of herds infected with bovine viral diarrhea virus (BVDV)
- Bovine Viral Diarrhea (BVD) Management Practices and Detection in Bulk Tank Milk in the United States, 2007, info sheet, October 2008

5. Describe current milking procedures and estimate the prevalence of contagious mastitis pathogens
- Part III: Reference of Dairy Cattle Health and Management Practices in the United States, 2007, September 2008
- Milking Procedures on U.S. Dairy Operations, 2007, info sheet, October 2008
- Prevalence of Contagious Mastitis Pathogens on U.S. Dairy Operations, 2007, info sheet, October 2008

6. Estimate the herd-level prevalence and associated costs of *Mycobacterium avium* subspecies *paratuberculosis*
- Johne's Disease on U.S. Dairies, 1991–2007, info sheet, April 2008

7. Describe current biosecurity practices and determine producer motivation for implementing or not implementing biosecurity practices
- Part I: Reference of Dairy Cattle Health and Management Practices in the United States, 2007, October 2007
- Part III: Reference of Dairy Cattle Health and Management Practices in the United States, 2007, September 2008
- Biosecurity Practices on U.S. Dairy operations, 1991–2007, Interpretive Report, May 2010

8. Determine the prevalence of specific food-safety pathogens and describe antimicrobial resistance patterns

- Antibiotic Use on U.S. Dairy Operations, 2002 and 2007, info sheet, October 2008
- Prevalence of *Salmonella* and *Listeria* in Bulk Tank Milk and In-line Filters on U.S. Dairies, 2007, info sheet, July 2009
- *Salmonella* and *Campylobacter* on U.S. Dairy Operations, 2002–07, info sheet, July 2009
- ***Salmonella, Listeria,* and *Campylobacter* on U.S. Dairy Operations, 2007, Interpretive Report, March 2011**
- Prevalence of *Coxiella burnetii* on U.S. Dairy Operations, 2007, technical brief, March 2011
- Prevalence of *Clostridium difficile* on U.S. Dairy Operations, 2007, technical brief, April 2011

Additional information sheets

- Dairy Cattle Identification Practices in the United States, 2007, info sheet, November 2007
- Bovine Leukosis Virus (BLV) on U.S. Dairy Operations, 2007, info sheet, October 2008
- Reproduction Practices on U.S. Dairy Operations, 2007, info sheet, February 2009
- Injection Practices on U.S. Dairy Operations, 2007, info sheet, February 2009
- Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolation from Bulk Tank Milk in the United States, 2007,technical brief, March 2011

Terms Used in This Report

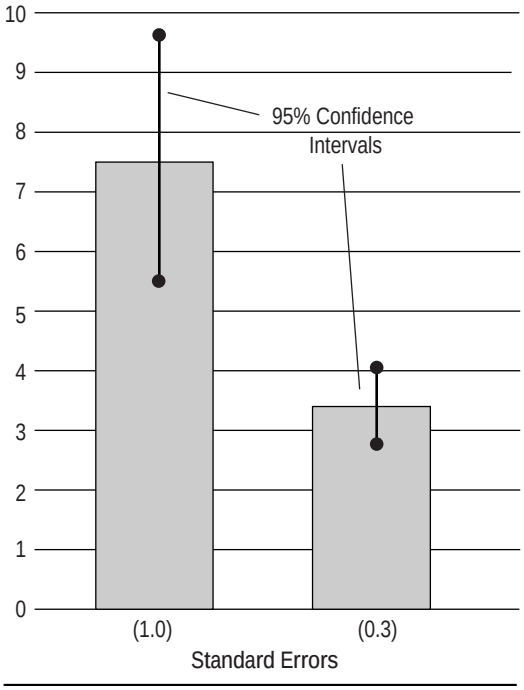
Herd size: Herd size is based on January 1 dairy cow inventory for each study year. Small herds are those with fewer than 100 head; medium herds are those with 100 to 499 head; and large herds are those with 500 or more head.

Population estimates: The estimates in this report for bulk-tank milk and milk filter sampling make inference to all of the operations with dairy cows in the target population. Data from the operations responding to the survey are weighted to reflect their probability of selection during sampling and to account for survey nonresponse.

Precision of population estimates: Population estimates in this report are provided with a measure of precision called the standard error. A 95-percent confidence interval can be created with bounds equal to the estimate plus or minus two standard errors. If the only error is sampling error, the confidence intervals created in this manner will contain the true population mean 95 out of 100 times. In the example to the right, an estimate of 7.5 with a standard error of 1.0 results in limits of 5.5 to 9.5 (two times the standard error above and below the estimate). The second estimate of 3.4 shows a standard error of 0.3 and results in limits of 2.8 and 4.0. Alternatively, the 90-percent confidence interval would be created by multiplying the standard error by 1.65 instead of 2. Most estimates in this report are rounded to the nearest tenth. If rounded to 0, the standard error was reported (0.0). References to population estimates being higher or lower than other estimates are based on the 95-percent confidence intervals not overlapping. The estimates in this report without standard errors are not considered populatin estimates.

Regions:
West: California, Colorado, Idaho, New Mexico, Oregon, Texas, Washington
East: Florida, Illinois, Indiana, Iowa, Kentucky, Michigan, Minnesota, Missouri, Ohio, Pennsylvania, New York, Tennessee, Vermont, Virginia, Wisconsin

Examples of a 95% Confidence Interval



Section I: Population Estimates

A. *Salmonella* Detection in Bulk-tank Milk and Milk Filters

1. Background

Salmonellae are gram-negative bacteria that can cause gastrointestinal infection in animals and humans. *Salmonella* causes an estimated 1.4 million human illnesses and over 500 deaths annually in the United States (Mead et al., 1999). Clinical signs of salmonellosis in humans include diarrhea, fever, and abdominal cramps 12 to 72 hours after infection. Clinical signs usually last 4 to 7 days, and most people recover without treatment (CDC, 2008a). In the elderly, infants, and immunocompromised individuals, *Salmonella* infection may spread from the intestines to the bloodstream and cause more severe, sometimes life-threatening, infections. Economic losses associated with human *Salmonella* infections have attracted increasing attention in a number of countries. Salmonellosis is estimated to cost the United States $14.6 billion annually (Scharff, 2010).

In dairy cows, *Salmonella* infection can result in mortality of adult cows, higher treatment costs, increased cull rates, higher labor costs, and lower milk production. Calf mortality and morbidity also add to the total cost of disease. Clinical signs of salmonellosis in adult cattle include depression, dehydration, diarrhea, fever (106–108°F), anorexia, vaginal discharge, abortion, and decreased milk production. The effects of infection can range from no clinical signs to endotoxemia and death. Calves with clinical *Salmonella* infections can present with diarrhea, fever, lethargy, and an inability to rise. Infected calves can also become septic and die (Smith, 2002). Evidence indicates that calves are more likely to experience mortality than cows (Cummings et al., 2009b), and preweaned calves are more likely to be affected by clinical salmonellosis compared with other cattle (Cummings et al., 2009a). Cattle can shed *Salmonella* in their feces without showing clinical signs.

Dairy operations represent a potential source of *Salmonella* infection for humans. *Salmonella* species can colonize the gastrointestinal tracts of cattle and other animals. Humans can become infected with *Salmonella* through fecal contamination of food products or water. Several outbreaks of salmonellosis have been linked to beef and dairy products (CDC, 2003, 2006a, 2006b; Van Duynhoven et al., 2009). Another source of human infection, primarily affecting farm families, employees, and visitors, is direct contact with ill animals (Holmberg et al., 1984; Troutt and Osburn, 1997). Cull dairy cows contribute about 17 percent of the ground beef available for national consumption (Troutt and Osburn, 1997) and can be a potential source of human exposure to *Salmonella* when the meat is contaminated with fecal material during slaughter. Pasteurization is very effective against *Salmonella* organisms, and foodborne outbreaks associated with this pathogen in pasteurized milk or dairy products are very rare.

Testing for *Salmonella* in milk is not a routine practice by milk producers. Bacteriological analysis of raw milk is typically limited to tests for bacteria (i.e., standard plate count and coliform count) or for specific mastitis-causing bacteria (Jayarao et al., 2001). *Salmonella* serotyping allows for monitoring changes in the

causative organisms. A change in a herd's serotype profile could indicate a new source of infection. Antimicrobial susceptibility testing is important for determining effective therapy and for guiding prudent antibiotic use.

Salmonella contamination in bulk-tank milk is believed to result from fecal contamination attributable to poor hygiene during the milking process rather than from intramammary infection with *Salmonella*, which is rare (Van Kessel et al., 2004; Jayarao et al., 2006). Standard hygiene practices during milking reduce but do not eliminate the risk of milk contamination. Pasteurization decreases the number of pathogenic organisms, decreases transmission of pathogens, and improves the safety of milk more than other measures, including certification of raw milk (Potter et al., 1984). Interstate sale of raw milk is banned in the United States by the Food and Drug Administration, but intrastate sales are allowable on a State-by-State basis, depending upon each State's regulation. Consumption of raw bulk-tank milk is a common practice among farm families (Jayarao et al., 2006). Among the nonfarming population, a growing number of consumers claim that raw milk is healthier, and they choose raw milk over pasteurized milk (Bren, 2004; Jayarao et al., 2006). Pasteurizing raw milk is an important public health tool for preventing foodborne disease. Because of pasteurization, contamination of dairy products currently accounts for a small percentage of foodborne illness in the United States. However, it is clear that consuming raw milk and products made with raw milk present a risk of foodborne illness to humans.

2. Sampling and testing overview

Bulk-tank milk samples were collected and tested for the presence of *Salmonella* during the Dairy 2002 and Dairy 2007 studies. Bulk-tank milk was not tested for *Salmonella* in Dairy 1996. In 2002 and 2007, one bulk-tank milk sample was collected per operation using aseptic techniques. In addition, a milk filter was collected from each operation in 2007.

For Dairy 2002, both culture and real-time polymerase chain reaction (RT-PCR) were used to detect *Salmonella* in bulk-tank milk samples, while only PCR was used in Dairy 2007. Culture was performed on PCR-positive samples from Dairy 2007 so that serotyping could be done. In 2002, culture results for bulk-tank milk were available from 852 dairy operations, and RT-PCR results were available from 838 operations. In the Dairy 2007 study, test results from bulk-tank milk or milk filters were available from 538 dairy operations: 517 from bulk-tank milk and milk filters, 19 from bulk-tank milk only, and 2 from milk filters only.

For more information on sampling and diagnostic testing methods, see Section III, p 48.

3. Prevalence

In 2007, the percentage of operations on which a milk filter tested positive for *Salmonella* (24.7 percent) was more than double the percentage of operations on which a bulk-tank milk sample tested positive (10.8 percent).

a. Percentage of operations on which a bulk-tank milk and/or a milk-filter sample tested positive for *Salmonella* in Dairy 2007, by testing method

Testing Method					
Bulk-tank Milk RT-PCR		Milk Filter RT-PCR		Bulk-tank Milk or Milk Filter RT-PCR	
Percent	Std. Error	Percent	Std. Error	Percent	Std. Error
10.8	(1.8)	24.7	(2.4)	28.1	(2.6)

The percentage of operations on which bulk-tank milk tested positive for *Salmonella* by RT-PCR was similar in 2002 and 2007 (11.9 and 10.8 percent of operations, respectively). The percentage of operations on which a bulk-tank milk sample tested positive for *Salmonella* by RT-PCR was similar across herd sizes in 2002 and 2007.

b. Percentage of operations on which a bulk-tank milk sample tested positive for *Salmonella* by RT-PCR, by herd size

	Herd Size (Number of Cows)							
	Small (Fewer than 100)		Medium (100–499)		Large (500 or More)		All Operations	
Study	Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error
Dairy 2002	12.4	(2.2)	10.2	(2.1)	13.9	(3.1)	11.9	(1.7)
Dairy 2007	8.1	(2.3)	16.2	(3.2)	19.6	(4.6)	10.8	(1.8)

In 2002 and 2007, there was no regional difference in the percentage of operations on which a bulk-tank milk sample tested positive for *Salmonella* by RT-PCR.

c. Percentage of operations on which a bulk-tank milk sample tested positive for *Salmonella* by RT-PCR, by region

	Region			
	East		West	
Study	**Percent**	**Std. Error**	**Percent**	**Std. Error**
Dairy 2002	11.9	(1.8)	11.5	(3.8)
Dairy 2007	10.7	(2.0)	12.7	(3.1)

4. Serotypes

Eight *Salmonella* serotypes were found in bulk-tank milk in 2002. *S.* Montevideo was found in bulk-tank milk on seven operations in 2002. In 2007, 14 and 22 *Salmonella* serotypes were found in bulk-tank milk and milk filters, respectively. *S.* Cerro was identified in the highest number of both sample types.

a. Number of operations on which the following *Salmonella* serotypes were identified, by sample type used for identification

	Sample Type		
	Dairy 2002	Dairy 2007	
	Bulk-tank Milk	Bulk-tank Milk	Milk Filters
Serotype	Number Operations (852 Sampled)	Number Operations (536 Sampled)	Number Operations (519 Sampled)
Cerro	3	8	27
Kentucky	0	5	16
Muenster	2	5	10
Newport	4	1	9
Anatum*	1	4	8
Montevideo	7	2	7
Meleagridis	2	1	6
Mbandaka	0	3	5
Typhimurium*	0	1	4
Dublin	1	2	3
Senftenberg	0	1	2
Give*	0	0	2
Untypable	3	0	2
Agona	0	1	1
Infantis	0	1	1
Schwarzengrund	0	1	1
Derby	0	0	1
Muenchen	0	0	1
Reading	0	0	1
Saintpaul	0	0	1
Soerenga	0	0	1
Thompson	0	0	1

*Includes variant species (e.g., Typhimurium var. 5-, formerly Typhimurium var. Copenhagen).

B. *Listeria* Detection in Bulk-tank Milk

1. Background

Listeria species are gram-positive bacteria that can cause serious infections in humans. *Listeria monocytogenes* is the most important *Listeria* species in terms of public health risk and frequency of appearance in foodstuffs.

L. monocytogenes is widespread in the environment. The main source of infection for ruminants is spoiled silage, but cattle may also ingest the organism by fecal-oral transmission. Adult cattle observed with clinical disease (listeriosis) most often have encephalitis, a nervous system disorder. Signs of disease in cattle include facial paralysis, depression, circling, and abortion.

Although the occurrence of human listeriosis is generally infrequent, it often leads to serious illness. Listeriosis in humans can be accompanied by fever, muscle aches, nausea, and diarrhea. If infection spreads to the nervous system, symptoms such as headache, stiff neck, loss of balance, or convulsions can occur. Infections during pregnancy can lead to miscarriage or stillbirth. Pregnant women, the elderly, and those with immunosuppression are most susceptible to listeriosis. In the United States, the annual cost of illness in humans due to *L. monocytogenes* is estimated at $8.8 billion (Scharff, 2010). Estimates indicate that approximately 2,500 listeriosis cases in humans occur each year in the United States, with nearly all cases attributed to a food source (Mead et al., 1999). Approximately 92 percent of individuals with illness caused by *L. monocytogenes* listeriosis are hospitalized (20 percent of these cases are fatal), making *L. monocytogenes* responsible for the highest hospitalization rate among foodborne pathogens (Mead et al., 1999).

It is not possible to remove all *Listeria* organisms from the environment. *L. monocytogenes* is found in soil and water, which can lead to contamination of fruits, vegetables, and other foods typically eaten raw. *Listeria* is killed by pasteurization and cooking but is relatively cold tolerant. *L. monocytogenes* survives refrigeration temperatures and can grow under these conditions, an unusual characteristic among foodborne pathogens (Walker et al., 1990). With regard to milk and dairy products, listeriosis is most often associated with products made from unpasteurized milk. Because of its ability to grow under refrigeration, contamination of cold cuts or other ready-to-eat foods after processing is a concern and has been associated with human illness.

Pasteurizing raw milk is an important public health tool for foodborne disease prevention. Because of pasteurization, contamination of dairy products currently accounts for a small percentage of foodborne illness in the United States. However, it is clear that consuming raw milk and products made with raw milk present a risk of foodborne illness to humans.

2. Sampling and testing overview

Bulk-tank milk samples were collected and tested for the presence of *Listeria* as part of the Dairy 2002 and Dairy 2007 studies. Bulk-tank milk was not tested for *Listeria* in Dairy 1996. In 2002 and 2007, one bulk-tank milk sample was collected per participating operation using aseptic techniques. In addition, in 2007 a milk filter was collected from each operation.

Culture methods were used to identify *Listeria* in bulk-tank milk samples in 2002 and 2007. In 2002, PCR was used as a component of the process to confirm isolates as *Listeria*. Results for bulk-tank milk testing for *Listeria* were available from 851 operations for Dairy 2002 and from 538 operations for Dairy 2007. In 2007, bulk-tank milk or milk-filter results were available from 538 dairy operations: 517 from bulk-tank milk and milk filters, 19 from bulk-tank milk only, and 2 from milk filters only.

For more information on sampling and diagnostic testing methods, see Section III, p 48.

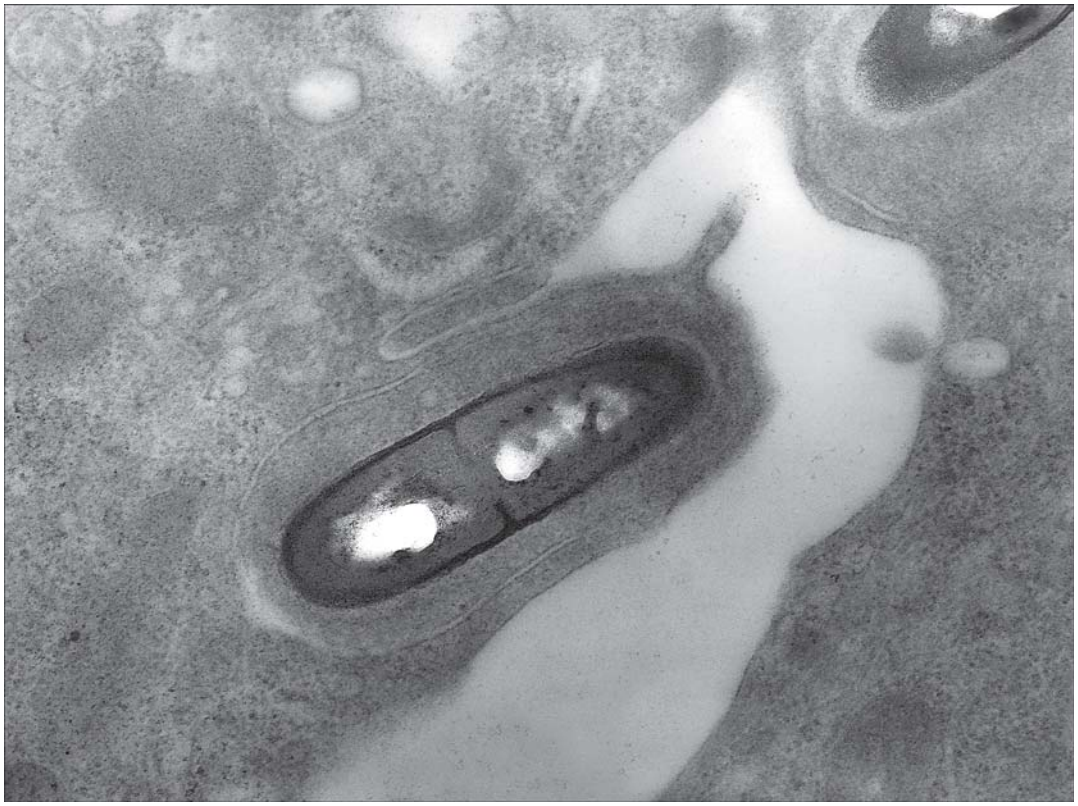


Photo of *Listeria* courtesy of CDC.

3. Prevalence

The percentage of operations on which a bulk-tank milk sample tested positive for *L. monocytogenes* was similar in 2002 and 2007 (3.8 and 3.7 percent, respectively). In Dairy 2007, the percentage of operations on which a milk filter tested positive for any *Listeria* species (28.3 percent) was more than three times the percentage of operations on which a bulk-tank milk sample tested positive for any *Listeria* species (9.0 percent).

a. Percentage of operations on which a bulk-tank milk and/or a milk filter sample tested positive for *Listeria*, by sample type

	Dairy 2002		Dairy 2007			
	L. mono-cytogenes		Any *Listeria*		*L. mono-cytogenes*	
Sample Type	Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error
Bulk-tank milk	3.8	(0.7)	9.0	(1.9)	3.7	(1.2)
Milk filter	NA		28.3	(2.9)	5.1	(1.2)
Bulk-tank milk or milk filter	NA		32.1	(2.9)	7.1	(1.5)

The percentage of operations on which a bulk-tank milk sample tested positive for *L. monocytogenes* was similar across herd sizes in 2002 and 2007.

b. Percentage of operations on which a bulk-tank milk sample tested positive for *L. monocytogenes*, by herd size

	Herd Size (Number of Cows)							
	Small (Fewer than 100)		Medium (100–499)		Large (500 or More)		All Operations	
Study	Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error	Pct.	Std. Error
Dairy 2002	3.0	(0.9)	5.4	(1.2)	7.8	(2.4)	3.8	(0.7)
Dairy 2007	2.3	(1.4)	7.5	(2.5)	4.0	(1.7)	3.7	(1.2)

There were no regional differences in 2002 and 2007 in the percentage of operations on which a bulk-tank milk sample tested positive for *L. monocytogenes*.

c. Percentage of operations on which a bulk-tank milk sample tested positive for *L. monocytogenes*, by region

	Region			
	East		West	
Study	Percent	Std. Error	Percent	Std. Error
Dairy 2002	3.9	(0.8)	2.9	(1.4)
Dairy 2007	3.3	(1.2)	8.3	(4.2)

Section II: Pathogen Detection in Feces

A. *Salmonella*

1. Sampling and testing overview

NAHMS has examined *Salmonella* occurrence using individual fecal samples from dairy cows in three separate studies: Dairy 1996, Dairy 2002, and Dairy 2007. Typically, NAHMS studies generate population-based estimates, and appropriate sample sizes are used to arrive at such estimates. Field resources, laboratory capacity, and the expense of culturing samples make it difficult to provide a national estimate of *Salmonella* prevalence based on fecal culturing of individual animals. Therefore, for the *Salmonella* estimates in this section, all three NAHMS dairy studies used a sample of approximately 100 operations, which is not an optimal sample size for providing national estimates of prevalence. Despite this limitation, the NAHMS studies provide valuable information on *Salmonella* occurrence and antimicrobial susceptibility patterns on U.S. dairies and represent the only national examination of *Salmonella* on dairy operations in the United States. Other research studies have examined *Salmonella* occurrence in dairy cattle but have been limited to specific regions of the United States.

At the time of sampling, records were kept as to whether each cow was sick, healthy (from the milking string), scheduled for culling (within 7 days of leaving the operation), or dry (dry cows were sampled only in Dairy 1996 and Dairy 2002). Dry cows were grouped with healthy cows in the following estimates. Dairy 1996 compared the prevalence of *Salmonella* in milk cows on-farm to that of cows on-farm that were scheduled for culling within the next 7 days and to cull cows at markets.

Dairy 2007 evaluated strategies for detecting *Salmonella* using fecal samples from individual cows, fecal samples pooled from five cows, and composite fecal samples from the dairy environment. To allow for comparison across the three studies, results presented in this report are primarily limited to healthy cows. An operation was classified as infected if one or more fecal samples were culture positive for *Salmonella*. The following table presents an overview of the sampling procedures used for the three dairy studies.

For more information on sampling and diagnostic testing methods, see Section III, p 48.

***Salmonella* fecal sampling methods, by study**				
Study	**Number of Operations Sampled***	**Sampling Period**	**Number of Samples per Operation**	**Notes**
Dairy 1996	91 dairy operations/ 19 States	Feb. 26 to July 10, 1996	40 or 50, depending on herd size, plus all cows scheduled for culling	All samples were taken rectally from individual cows. There were no specific targets as to the number of sick, dry, or milking string cows, other than the sample was to be representative of the cows on hand on the day of the visit. Cow type was noted at the time of collection. **Dairies with 30–99 cows**: Operations were visited once. Up to 40 fecal samples were collected, which included samples from all cows scheduled for culling present on the day of the visit. **Dairies with 100 or more cows:** Operations were visited three times. During one visit, 50 cows (from milking string, dry, or sick) were sampled along with up to 20 cows scheduled for culling in the next 7 days. On 2 other visits, up to 20 samples were taken from cows scheduled for culling in the next 7 days.
	97 cull cow markets/ 20 States	Feb. 26 to July 10, 1996	25	Twenty-five fresh fecal samples per market—either by rectal retrieval from individual cows or from pen floors if restraining facilities were not available.
Dairy 2002	97 dairy operations/ 21 States	Mar. 27 to Sept. 25, 2002	40	The goal was to collect 40 individual fecal samples during a single visit, all via rectal retrieval. If the herd had fewer than 40 cows, all cows were sampled. There were no specific targets for number of sick, dry, or milking string cows to be sampled, but cow type was noted at the time of collection.
Dairy 2007	121 dairy operations/ 17 States	Feb. 28 to Aug. 30, 2007	35	The goal was to collect 35 individual fecal samples during a single visit, all via rectal retrieval. Up to five sick cows and up to five cows scheduled for culling (within 7 days of leaving the operation) were sampled, with the remainder (up to 35) being from cows with saleable milk.
	260 dairy operations/ 17 States	Feb. 28 to Aug. 30, 2007	6	Manure/slurry (composite fecal) samples from six different adult cow areas where manure accumulates were taken (area samples). Each area sample was composed of about 4 oz of manure/slurry from each of six sites within the area. Areas recommended for sampling included common alleyways, common pens, exits from parlors, floors of holding pens, flush water, gutter cleaner, lagoons or manure pits, and manure spreaders.

*Operations with 30 or more dairy cows.

2. Prevalence

The table below presents both herd- and animal-level *Salmonella* prevalence estimates from the three NAHMS dairy studies. For purposes of comparison, estimates are limited to healthy cows because these sample numbers remained relatively consistent across the three studies. Culture methods were similar for the three NAHMS dairy studies. In 2007, the percentage of *Salmonella*-positive operations was almost double that in 1996, and the percentage of *Salmonella*-positive cows more than doubled over the same time period. Differences in types of operations sampled by region and herd size might account for some of the differences among the three studies; however, it is possible that *Salmonella* is becoming more common on U.S. dairies.

a. Percentage of operations and percentage of cows fecal-culture positive for *Salmonella*[1]

Study	Operations[2]	Cows
Dairy 1996	20.0 (18/90)	5.4 (194/3,585)
Dairy 2002	30.9 (30/97)	7.1 (259/3,645)
Dairy 2007	39.7 (48/121)	13.7 (523/3,804)

[1]Only cows healthy at the time of collection are included.
[2]Operations with at least one culture-positive cow were considered positive.

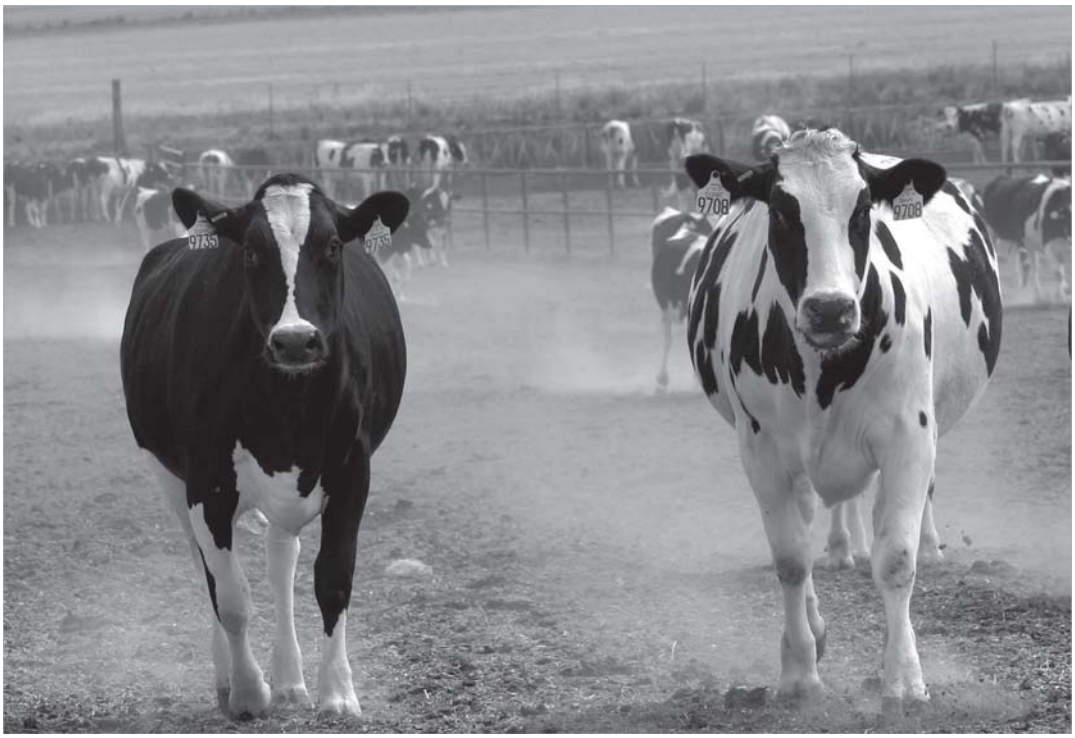
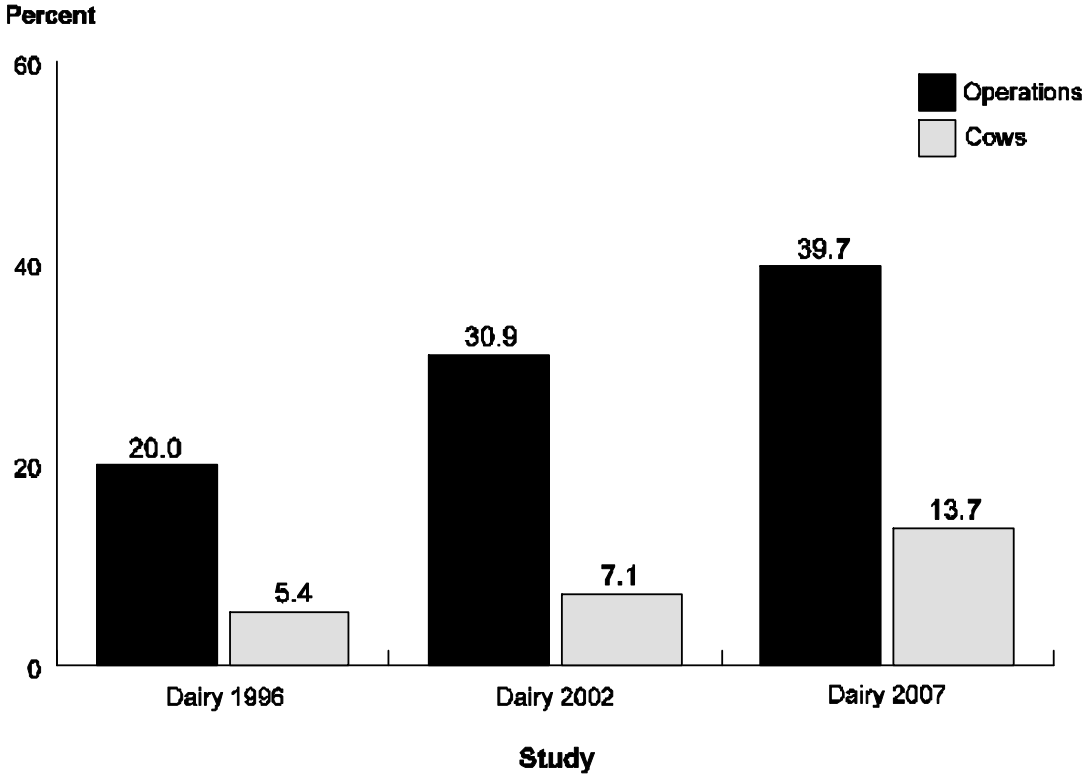


Photo courtesy of Judy Rodriguez

Percentage of Operations and Percentage of Cows Fecal-culture Positive for *Salmonella*



In several previous studies, operations with 100 or more dairy cows have been more likely than operations with fewer than 100 cows to be *Salmonella* positive (Warnick et al., 2001; Wells et al., 2001; Huston et al., 2002; Fossler et al., 2004; Blau et al., 2005; Davison et al., 2006; Cummings et al., 2009a). This finding was true in all three NAHMS dairy studies: the percentage of large operations culture positive for *Salmonella* was at least double that of small operations.

b. Percentage of operations[1] fecal-culture positive for *Salmonella*, by herd size[2]

	Herd Size (Number of Cows)		
Study	**Small** (Fewer than 100)	**Medium** (100–499)	**Large** (500 or More)
Dairy 1996	4.8 (2/42)	29.0 (9/31)	41.2 (7/17)
Dairy 2002	18.2 (6/33)	28.2 (11/39)	52.0 (13/25)
Dairy 2007	24.3 (9/37)	44.7 (21/47)	48.7 (18/37)

[1]Operations with at least one culture-positive cow were considered positive.
[2]Only cows healthy at the time of collection are included.

Salmonella has been found more commonly during summer months than winter months (Evans and Davies, 1996; Wells et al., 2001; Fossler et al., 2005b), although this finding has not been as consistently observed as the herd size differences described previously. In all three NAHMS studies, a higher percentage of operations sampled during summer (June–September) were positive compared with operations sampled during spring (February–May).

c. Percentage of operations[1] fecal-culture positive for *Salmonella*, by season[2]

	Season	
Study	**Spring** (February–May)	**Summer** (June–September)
Dairy 1996	16.2 (12/74)	37.5 (6/16)
Dairy 2002	23.5 (12/51)	39.1 (18/46)
Dairy 2007	29.6 (16/54)	47.8 (32/67)

[1]Operations with at least one culture-positive cow were considered positive.
[2]Only cows healthy at the time of collection are included.

There were no consistent trends in the percentage of *Salmonella*-positive operations by region. In 1996 and 2002, a higher percentage of operations in the West region than in the East region were *Salmonella* positive. In contrast, in 2007 a higher percentage of operations in the East region than in the West region were *Salmonella* positive (43.6 and 20.0 percent, respectively). It is difficult to draw any conclusions with regard to regional differences while ignoring herd size differences. As shown in table on the next page, during Dairy 1996 and Dairy 2002 there were fewer participating operations with 500 or more cows in the East region than there were in Dairy 2007. Thus, any apparent trends with regard to regional differences are likely due to herd sizes within each region.

d. Percentage of operations[1] fecal-culture positive for *Salmonella*, by region[2]

	Region	
Study	**East**	**West**
Dairy 1996	13.8 (9/65)	36.0 (9/25)
Dairy 2002	26.1 (18/69)	42.9 (12/28)
Dairy 2007	43.6 (44/101)	20.0 (4/20)

[1]Operations with at least one culture-positive cow were considered positive.
[2]Only cows healthy at the time of collection are included.

For the West region in 1996 and 2002, a higher percentage of operations with 500 or more cows were *Salmonella* positive compared with operations with fewer than 500 cows. For the East region in 2007, a higher percentage of operations with 500 or more cows were *Salmonella* positive compared with operations with fewer than 500 cows (65.2 and 37.2 percent, respectively). In the West region in 2007, there was little difference by herd size in the percentage of *Salmonella*-positive operations, which was also true in the East region in 1996 and 2002, although there were relatively few participating operations with 500 or more cows in the East region in 1996 and 2002.

e. Percentage of operations[1] fecal-culture positive for *Salmonella*, by herd size and by region[2]

	Dairy 1996		Dairy 2002		Dairy 2007	
	Region					
Herd size (Number of Cows)	**East**	**West**	**East**	**West**	**East**	**West**
Fewer than 500	13.3 (8/60)	23.1 (3/13)	25.8 (16/62)	10.0 (1/10)	37.2 (29/78)	16.7 (1/6)
500 or more	20.0 (1/5)	50.0 (6/12)	28.6 (2/7)	61.1 (11/18)	65.2 (15/23)	21.4 (3/14)

[1]Operations with at least one culture-positive cow were considered positive.
[2]Only cows healthy at the time of collection are included.

In all three dairy studies, a lower percentage of healthy cows were *Salmonella* positive on small operations than on medium and large operations. Large operations had the highest percentage of *Salmonella*-positive cows in 1996 and 2002.

f. Percentage of healthy cows fecal-culture positive for *Salmonella*, by herd size

Study	Herd Size (Number of Cows) Small (Fewer than 100)	Medium (100–499)	Large (500 or More)
Dairy 1996	0.6 (9/1,494)	6.3 (81/1,292)	13.0 (104/799)
Dairy 2002	1.8 (21/1,152)	7.7 (118/1,535)	12.5 (120/958)
Dairy 2007	5.5 (66/1,209)	17.9 (270/1,508)	17.2 (187/1,087)

In 1996 and 2002, a higher percentage of healthy cows were positive for *Salmonella* in the West region than in the East region. In contrast, in 2007 a higher percentage of cows were positive for *Salmonella* in the East region than in the West region; however, a much smaller number of cows were sampled in the West region than in the East region in 2007 (580 and 3,224, respectively).

g. Percentage of healthy cows fecal-culture positive for *Salmonella*, by region

Study	Region East	West
Dairy 1996	1.6 (39/2,429)	13.4 (155/1,156)
Dairy 2002	5.3 (136/2,569)	11.4 (123/1,076)
Dairy 2007	15.5 (499/3,224)	4.1 (24/580)

One of the goals of the Dairy 1996 study was to evaluate whether cows scheduled for culling were more likely to be *Salmonella* positive than other cows on the operation. Aside from cows scheduled for culling, other cows sampled were to be representative of all cows on the operation on the day of sampling, including sick cows, dry cows, and cows in the milking string. It was noted at the time of sampling whether a cow was sick, dry, from the milking string, or scheduled for culling, but there were no requirements for sampling a specified number of sick cows. Likewise, for Dairy 2002 there were no requirements for sampling different types of cows, but it was noted at the time of sampling whether a cow was sick, scheduled for culling within 7 days, dry, or from the milking string. In contrast, in the Dairy 2007 study there were specific instructions to collect samples from up to 5 sick cows and up to 5 cows scheduled for culling, with the remainder of samples—up to 35—taken from cows with saleable milk.

The following results should be interpreted with these sampling differences in mind. For all three NAHMS studies, a higher percentage of cows designated as sick on the day of the visit were culture positive for *Salmonella* compared with cows designated as healthy. These results are supported by a previous study which collected samples from preweaned calves, sick cows, cows scheduled to be culled, periparturient cows (within 14 days of calving), and healthy cows and found that sick cows had the highest odds of being *Salmonella* positive (Fossler, 2005a). It is possible that the primary cause of illness in the sick cattle was salmonellosis, or that battling another illness or condition may make the animals more susceptible to secondary infections with *Salmonella.*

In 1996, a higher percentage of cows scheduled for culling were culture positive for *Salmonella* compared with healthy cows. In Dairy 2007, there was no difference in *Salmonella* prevalence between cows scheduled for culling and healthy cows.

h. Percentage of cows fecal-culture positive for *Salmonella*, by cow status

Cow Status	Dairy 1996	Dairy 2002	Dairy 2007
Healthy	5.4 (194/3,585)	7.1 (259/3,645)	13.7 (523/3,804)
Sick	7.3 (4/55)	30.8 (8/26)	18.2 (40/220)
Scheduled for culling	18.1 (121/668)	0.0 (0/17)	12.6 (17/135)
All	7.4 (319/4,308)	7.2 (267/3,688)	13.9 (580/4,159)

Longitudinal studies with repeated sampling suggest that *Salmonella* can be found on almost all dairy operations. A study in which 110 dairies were sampled 5 times over the course of 1 year found 92.7 percent of operations to be culture positive for *Salmonella* (Fossler et al., 2004). In that study, between 31 and 55 percent of farms were positive on a per-visit basis and 25.0 percent of dairies accounted for 75 percent of the *Salmonella*-positive samples, implying that a relatively small percentage of dairy operations account for a majority of *Salmonella*-positive cattle.

Each of the three NAHMS dairy studies sampled operations at a single point in time, and the majority of operations were negative when tested for *Salmonella.* On many of the operations that tested positive for *Salmonella,* less than 10 percent of the cows sampled tested positive. Among culture-positive operations in 1996, 2002, and 2007, the median within-herd prevalence was 6.4, 10.3, and 21.9 percent, respectively. Among culture-positive operations in 1996, 2002, and 2007, the 75th percentile for within-herd prevalence was 40.0, 34.6, and 60.0 percent, respectively.

In each of the NAHMS dairy studies, approximately 10 percent of the sampled operations accounted for 75 percent or more of the positive samples from healthy cows. In 1996, 4 of the 90 operations accounted for 77.8 percent of the positive samples from healthy cows; in 2002, 9 of the 97 operations accounted for 74.5 percent of the positive samples from healthy cows; and in 2007, 16 of the 121 operations accounted for 74.6 percent of the positive samples from healthy cows.

i. Number of operations by within-herd prevalence of *Salmonella**

Within-herd Prevalence	Dairy 1996	Dairy 2002	Dairy 2007
0.0	72	67	73
0.1 to 10.0	11	16	20
10.1 to 20.0	1	2	4
20.1 to 30.0	1	3	4
30.1 to 40.0	1	3	3
40.1 to 50.0	0	1	2
50.1 to 60.0	0	3	4
60.1 to 70.0	1	0	2
70.1 to 80.0	0	0	1
80.1 to 90.0	3	1	4
90.1 to 100.0	0	1	4
Total	90	97	121

*Only cows healthy at the time of collection were included.

3. Serotypes

The following table shows the number of operations on which each serotype was identified from at least one cow (i.e., herd-level results). Cerro and Kentucky were the most common serotypes isolated from operations in Dairy 2007. *S.* Montevideo, one of the most common serotypes identified in the 1996, 2002, and 2007 NAHMS studies, has been among the top 10 identified from humans every year from 1996 through 2006 (CDC, 2008b).

a. Number of operations on which the following *Salmonella* serotypes were identified[1]

Serotype[2]	Dairy 1996 Number of Operations (90 Sampled)	Dairy 2002 Number of Operations (97 Sampled)	Dairy 2007 Number of Operations (121 Sampled)
Cerro	2	2	14
Kentucky	3	8	14
Muenster	2	3	8
Meleagridis	4	5	6
Montevideo	5	8	6
Untypable	1	5	6
Typhimurium[3]	2	3	4
Mbandaka	4	5	3
Anatum[3]	3	2	2
Agona	1	3	2
Bovismorbificans	1	0	2
Newport	0	5	2
Senftenberg	1	4	2
Derby	0	0	1
Fresno	0	0	1
Infantis	0	0	1
Muenchen	3	0	1
Saintpaul	0	0	1
Thompson	0	1	1
Give	2	2	0
Barranquilla	0	1	0
Cubana	0	1	0
Hartford	0	1	0
Livingstone	0	1	0
Newington	1	1	0
Ohio	0	1	0
Oranienburg	0	1	0
Reading	0	1	0
San Diego	0	1	0
Tennessee	0	1	0
Uganda	0	1	0
Worthington	2	0	0
Enteritidis	1	0	0
Menhaden	1	0	0
New Brunswick	1	0	0
Albany	1	0	0
Havana	1	0	0
Niakhar	1	0	0
Dublin	1	0	0

[1]Only cows healthy at the time of collection are included.
[2]Listed in order by rank for Dairy 2007 study.
[3]Includes variant species (e.g., Typhimurium var. 5-, formerly Typhimurium var. Copenhagen).

The top 10 serotypes identified from *Salmonella* isolates for each of the three NAHMS dairy studies are listed in the following table. Serotypes not among the top 10 were grouped into the "other" category. Three serotypes—Meleagridis, Montevideo, and Kentucky—ranked in the top five serotypes indentified in 1996, 2002, and 2007. The top 10 account for 81.1, 82.7, and 94.6 percent of total isolates in 1996, 2002, and 2007, respectively.

b. Number of *Salmonella* isolates from healthy cows, by serotype

Dairy 1996		Dairy 2002		Dairy 2007	
Serotype	**No. Isolates** (n=228)	**Serotype**	**No. Isolates** (n=283)	**Serotype**	**No. Isolates** (n=556)
Montevideo	49	Meleagridis	71	Cerro	157
Kentucky	29	Montevideo	34	Kentucky	130
Menhaden	27	Typhimurium*	29	Montevideo	66
Cerro	17	Kentucky	28	Mbandaka	47
Meleagridis	16	Agona	21	Meleagridis	40
Mbandaka	12	Mbandaka	12	Derby	27
Anatum	11	Ohio	12	Muenster	18
New Brunswick	9	Senftenberg	11	Anatum	17
Muenster	8	Cerro	8	Senftenberg	13
Albany	7	Newport	8	Newport	11
Other	43	Other	49	Other	30

*Includes variant species (e.g., Typhimurium var. 5-, formerly Typhimurium var. Copenhagen).

4. Comparison of serotypes isolated from cattle and humans

The following tables (a. through c.) compare *Salmonella* serotypes identified from cattle and humans in 1996, 2002, and 2007. Serotype data on healthy cows were taken from the respective NAHMS studies. Data on clinically ill cows originated from diagnostic samples submitted to the National Veterinary Services Laboratories (NVSL). Serotype data on humans were provided by the CDC through the Public Health Laboratory Information System. Salmonellosis is on the CDC's list of Nationally Notifiable Infectious Diseases.

The two most common serotypes identified in humans were *S.* Typhimurium and *S.* Enteritidis in 1996, 2002, and 2007. Sources of individual cases of salmonellosis in humans are often not identified, and the role of livestock in human cases of salmonellosis is unknown. There are many avenues other than food of animal origin, such as produce, by which people can get sick. Poultry is generally considered the most common source of salmonellosis in humans from *S.* Enteritidis. Hogs were the most common source of *S.* Typhimurium isolates among clinical animal submissions to NVSL in the most recent report in 2006. *S.* Montevideo was the only serotype that ranked among the 10 most common serotypes found in healthy cows, clinically ill cows, and humans in 1996, 2002, and 2007. However, *S.* Montevideo was a relatively uncommon serotype in humans, making up only 2 to 3 percent of isolates identified from humans in 1996, 2002, and 2007. *S.* Typhimurium was among the two most common serotypes identified in clinically ill cattle and humans in all 3 years but was uncommon among healthy cows. A recent study in which dairy herds were monitored for approximately 1 year for clinical signs of salmonellosis found *S*. Newport and *S*. Typhimurium to be the most common serotypes identified (Cummings, 2009b), which coincides with the NVSL results on clinically ill cattle. *S*. Typhimurium and *S*. Newport were among the four most common serotypes identified from humans in all 3 years. Clinically affected cattle may pose a greater threat to public health than healthy cattle (Cummings et al., 2009b) However, these serotype data alone do not provide sufficient evidence of transmission of *Salmonella* from cattle to humans.

a. Number of *Salmonella* isolates from healthy dairy cows, clinically affected cattle, and humans in 1996, by serotype

Healthy Cows (NAHMS)		Clinical Cattle (NVSL)[1 2]		Humans (CDC)[2]	
Serotype	**No. Isolates** (n=228)	**Serotype**	**No. Isolates** (n=4,183)	**Serotype**	**No. Isolates** (n=39,035)
Montevideo	49	Typhimurium[3]	1,081	Enteritidis	9,570
Kentucky	29	Montevideo	589	Typhimurium[3]	9,501
Menhaden	27	Cerro	239	Heidelberg	1,998
Cerro	17	Kentucky	230	Newport	1,985
Meleagridis	16	Anatum	228	Montevideo	1,227
Mbandaka	12	Dublin	213	Javiana	749
Anatum	11	Muenster	201	Oranienburg	690
New Brunswick	9	Meleagridis	172	Hadar	658
Muenster	8	Menhaden	118	Agona	606
Albany	7	Give	118	Muenchen	595
Other	43	Other	994	Other	11,456

[1]Serotypes are from beef and dairy sources. NVSL typically receives diagnostic samples from clinically ill cattle, but they may not be exclusively from ill cattle.
[2]From the *Salmonella* Annual Summaries published by the CDC at http://www.cdc.gov/ncidod/dbmd/phlisdata/*Salmonella*.htm
[3]Includes variant species (e.g., Typhimurium var. 5-, formerly Typhimurium var. Copenhagen).

b. Number of *Salmonella* isolates from healthy dairy cows, clinically affected cattle, and humans in 2002, by serotype

Healthy Cows (NAHMS)		Clinical Cattle (NVSL)[1,2]		Humans (CDC)[2]	
Serotype	**No. Isolates** (n=283)	**Serotype**	**No. Isolates** (n=2,674)	**Serotype**	**No. Isolates** (n=32,308)
Meleagridis	71	Newport	769	Typhimurium[3]	7,062
Montevideo	34	Typhimurium[3]	583	Enteritidis	5,116
Typhimurium[3]	29	Dublin	136	Newport	4,204
Kentucky	28	Agona	124	Heidelberg	1,957
Agona	21	Montevideo	115	Javiana	1,188
Mbandaka	12	Uganda	91	Montevideo	717
Ohio	12	Anatum	89	Muenchen	591
Senftenberg	11	Muenster	87	Oranienburg	585
Cerro	8	Kentucky	70	Saintpaul	535
Newport	8	Mbandaka	54	Infantis	463
Other	49	Other	556	Other	9,890

[1]Serotypes are from beef and dairy sources. NVSL typically receives diagnostic samples from clinically ill cattle, but they may not be exclusively from ill cattle.
[2]From the *Salmonella* Annual Summaries published by the CDC at http://www.cdc.gov/ncidod/dbmd/phlisdata/*Salmonella*.htm
[3]Includes variant species (e.g., Typhimurium var. 5-, formerly Typhimurium var. Copenhagen).

c. Number of *Salmonella* isolates from healthy dairy cows, clinically affected cattle, and humans in 2007, by serotype

Healthy Cows (NAHMS)		Clinical Cattle (NVSL)[1 2]		Humans (CDC)[2]	
Serotype	**No. Isolates** (n=556)	**Serotype**	**No. Isolates** (n=3,770)	**Serotype**	**No. Isolates** (n=40,666)
Cerro	157	Newport	436	Typhimurium[3]	6,872
Kentucky	130	Typhimurium[3]	425	Enteritidis	6,740
Montevideo	66	Orion var. 15+,34+	365	Newport	3,373
Mbandaka	47	Dublin	335	Heidelberg	1,495
Meleagridis	40	Montevideo	293	Javiana	1,433
Derby	27	Agona	239	I 4,[5],12:i-	1,200
Muenster	18	Anatum	210	Montevideo	1,061
Anatum	17	Kentucky	164	Muenchen	753
Senftenberg	13	Muenster	163	Oranienburg	719
Newport	11	Cerro	155	Mississippi	604
Other	30	Other	985	Other	16,416

[1]Serotypes are from beef and dairy sources. NVSL typically receives diagnostic samples from clinically ill cattle, but they may not be exclusively from ill cattle.
[2]From the *Salmonella* Annual Summaries published by the CDC at http://www.cdc.gov/ncidod/dbmd/phlisdata/*Salmonella*.htm
[3]Includes variant species (e.g., Typhimurium var. 5-, formerly Typhimurium var. Copenhagen).

5. Antimicrobial susceptibility

Salmonella isolates from healthy cows showed relatively little resistance to antimicrobial agents in 1996, 2002, and 2007. Of all *Salmonella* isolates found in healthy cows and tested for antimicrobial susceptibility in 1996, 2002, and 2007, 92.3, 82.3, and 96.6 percent, respectively, were susceptible to all antimicrobials tested. In each of the studies, no more than 5 percent of *Salmonella* isolates from healthy cows were resistant to two or more antimicrobials.

a. Percentage of *Salmonella* isolates by number of antimicrobials in which antimicrobial resistance[1] was observed[2]

Study (n=Number of Isolates)	Susceptible to All Antimicrobials	Resistant to a Single Antimicrobial	Resistant to Two or More Antimicrobials	Total
Dairy 1996 (n=220)	92.3	3.6	4.1	100.0
Dairy 2002 (n=283)	82.3	12.7	5.0	100.0
Dairy 2007 (n=556)	96.6	0.7	2.7	100.0

[1]Intermediate isolates were classified as susceptible.
[2]Only cows healthy at the time of sample collection are included.

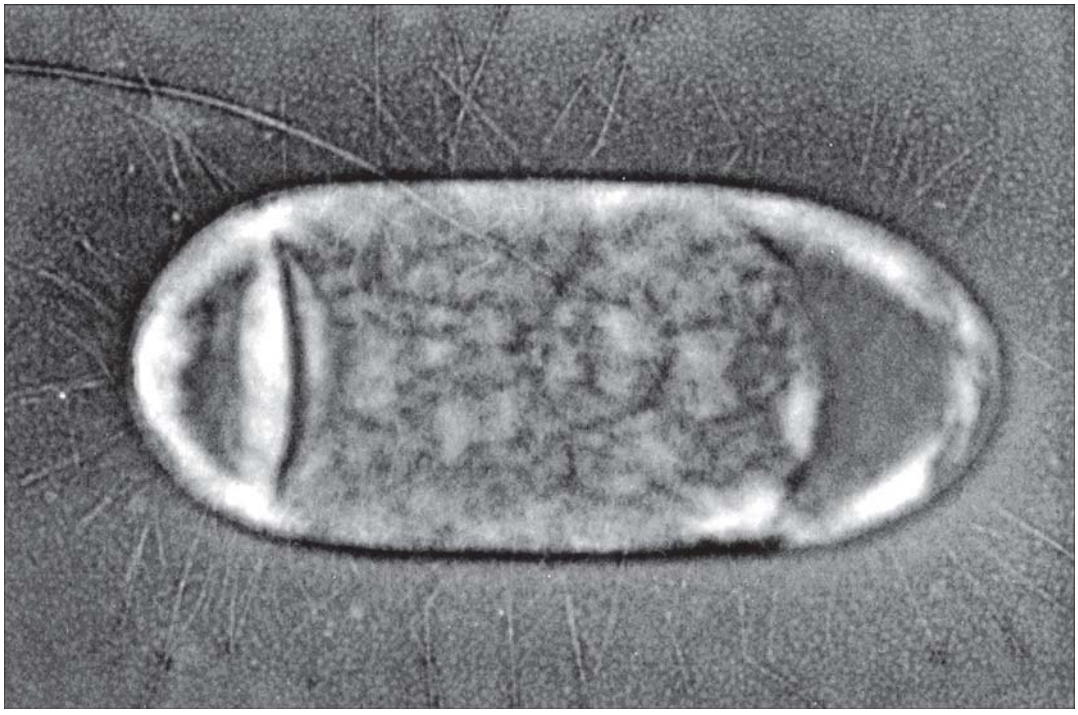


Photo of *S.* Typhimurium courtesy of Agriculture Research Service

Resistance to amikacin, ciprofloxacin, nalidixic acid, and trimethoprim-sulfamethoxazole was not observed in any of the three dairy studies. Resistance to ceftriaxone was observed in Dairy 2002 and Dairy 2007, but it was observed in only one isolate in Dairy 2007. *Salmonella* resistance to ceftriaxone is of interest because it is commonly used to treat severe *Salmonella* infections in children (Zhao et al., 2003).

b. Percentage of resistant[1] isolates from all *Salmonella* isolates tested for antimicrobial susceptibility, by antimicrobial[2]

Antimicrobial	Dairy 1996 (n=220)	Dairy 2002 (n=283)	Dairy 2007 (n=556)
Amikacin (AMI)	0.0	0.0	0.0
Amoxicillin-clavulanic acid (AMO)	0.9	4.9	1.8
Ampicillin (AMP)	3.6	4.6	2.2
Apramycin (APR)	0.0	NA	NA
Cefoxitin (FOX)	NA	3.9	1.6
Ceftiofur (TIO)	0.0	4.6	2.0
Ceftriaxone (AXO)	0.0	2.5	0.2
Cephalothin (CEP)	2.3	4.9	NA
Chloramphenicol (CHL)	1.4	4.6	2.5
Ciprofloxacin (CIP)	0.0	0.0	0.0
Gentamicin (GEN)	0.0	0.7	0.2
Kanamycin (KAN)	1.4	0.7	0.0
Nalidixic acid (NAL)	0.0	0.0	0.0
Streptomycin (STR)	4.1	9.9	2.7
Sulfamethoxazole[3] (SUL)	1.8	3.9	2.3
Tetracycline (TET)	2.3	12.4	3.1
Ticarcillin (TIC)	3.2	NA	NA
Trimethoprim-sulfamethoxazole (TRI)	0.0	0.0	0.0

[1]Intermediate isolates were classified as susceptible. Resistance break points were those current at the time of sample collection. Break points for extended spectrum cephalosporins changed in 2010 and testing was done prior to this change.
[2]Only cows healthy at the time of collection are included.
[3]Sulfisoxazole replaced sulfamethoxazole in 2007.

6. Multidrug resistance patterns

There were 53 multidrug-resistant isolates identified over the 3 study years. In 1996 and 2002, more *S.* Typhimurium isolates were resistant to multiple drugs compared with other serotypes. In 2007, however, no multidrug-resistant *S.* Typhimurium was observed. Dairy 2007 was the first NAHMS dairy study in which multidrug resistance was observed in *S.* Montevideo, which was one of the top three serotypes identified in each of the previous NAHMS studies.

Number of multidrug-resistant isolates by serotype and by resistance pattern[1]

Serotype	Resistance Pattern[2]	Dairy 1996 Isolates (n=356)	Dairy 2002 Isolates (n=291)	Dairy 2007 Isolates (n=620)
Agona	AMO, AMP, FOX, TIO, CHL, KAN, STR, SUL, TET, TRI	0	0	1
	CHL, STR, SUL, TET	0	0	2
Albany	AMO, AMP, CEP, TIC	1	0	0
Anatum	AMO, CEP	1	0	0
Cerro	CHL, TET	0	0	1
Dublin	AMP, CHL, KAN, STR, SUL, TET, TIC	2	0	0
Kentucky	AMP, CEP, TIC	1	0	0
Mbandaka	AMO, CEP, TET	0	1	0
	AMP, CEP, TIC	1	0	0
Menhaden	AMO, CEP	1	0	0
Montevideo	AMO, AMP, FOX, TIO, CHL, STR, SUL, TET	0	0	8
	AMO, AMP, TIO, CHL, STR, SUL, TET	0	0	1
	AMP, TIO, CHL, STR, SUL, TET	0	0	1
Muenster	AMP, CEP, TIC	1	0	0
Newport	AMO, AMP, FOX, TIO, AXO, CHL, STR, SUL, TET	0	0	1
	AMO, AMP, FOX, TIO, CEP, CHL, GEN, KAN, STR, SUL, TET	0	1	0
	AMO, AMP, FOX, TIO, CEP, CHL, STR, SUL, TET	0	5	0
	AMO, AMP, FOX, TIO, CEP, CHL, STR, TET	0	1	0
	AMO, AMP, FOX, TIO, CHL, STR, SUL, TET	0	0	8
Reading	AMO, AMP, FOX, TIO, CEP, CHL, GEN, KAN, STR, SUL, TET	0	1	0
	AMO, AMP, FOX, TIO, CHL, STR, SUL, TET	0	0	4
Saintpaul	AMP, GEN, TET	0	0	1
Typhimurium	AMO, AMP, CEP, CHL, GEN, KAN, STR, SUL, TET, TIC, TRI	1	0	0
	AMO, AMP, FOX, TIO, CEP, CHL, STR, SUL, TET	0	2	0
	AMO, AMP, FOX, TIO, CEP, CHL, STR, TET	0	1	0
	AMO, AMP, TIO, CEP, CHL, STR, SUL, TET	0	2	0
	AMP, CHL, SUL, TET, TIC	1	0	0
	AMP, KAN, STR, SUL, TET, TIC	1	0	0
Total multidrug-resistant isolates		11	14	28

[1]Healthy, sick, and to-be-culled cows are included.
[2]See previous table for the full name of the antimicrobial corresponding to the abbreviations listed here.

B. *Campylobacter*

1. Background

Campylobacter is recognized as a major cause of acute bacterial gastroenteritis in humans worldwide, comparable with or even surpassing *Salmonella* (Friedman et al., 2000). Mead et al. (1999) estimated that in the United States there are approximately 2.5 million cases of *Campylobacter jejuni* (*C. jejuni*) infections each year, 80 percent of which are food related. *Campylobacter coli* (*C. coli*) was estimated to cause approximately 26,000 cases in 2000 (Tam et al., 2003). Human cases of campylobacteriosis in the United States are estimated to cost $18.8 billion annually (Scharff, 2010).

Typical signs of *Campylobacter* infection in humans include abdominal cramping, vomiting, fever, and diarrhea (with or without blood), lasting from several days to more than a week (Skirrow and Blaser, 2000). Of individuals that recover from the disease, 20 percent may relapse or experience prolonged or severe illness requiring antimicrobial treatment. The disease is rarely fatal, and only about 10 percent of infected individuals are hospitalized.

The recently recognized association between development of Guillain-Barré syndrome in humans and prior *C. jejuni* infection, along with other sequelae, has increased the level of public health concern for this pathogen. Guillain-Barré syndrome is an autoimmune disease of the nervous system that can result in paralysis, pain, and muscle wasting; it has an annual incidence of about 2 in 100,000 persons in the United States (Allos, 2001). An estimated 0.1 percent of reported *Campylobacter* illnesses result in Guillain-Barré syndrome (CDC, 2010).

C. jejuni and *C. coli*, commonly found in the intestinal tracts of food animals, are the most frequently isolated *Campylobacter* species found in cases of human infection (Engberg et al., 2000). Poultry and poultry products have been documented as a major source of *Campylobacter* infection in humans (Corry and Atabay, 2001). Beef and dairy cattle are also common carriers of *Campylobacter* (Atabay and Corry, 1998; Wesley et al., 2000; Stanley and Jones, 2003; Bae et al., 2005). Young animals are more often colonized than older animals (Sato et al., 2004). Feedlot cattle are more likely than grazing cattle to carry *Campylobacter* (Giacoboni et al., 1993; Beach et al., 2002).

Although *Campylobacter* species can be considered commensal organisms or normal flora in livestock, they can produce clinical disease with diarrhea in neonatal calves and may cause abortion, infertility, and early embryonic death (Wesley et al., 2000; Smith, 2002). *Campylobacter* spp. has been identified from many livestock species. Although cattle can be colonized by *C. coli*, *C. jejuni* is the most common *Campylobacter* species isolated in cattle (Wesley et al., 2000; Harvey et al., 2004; Bae et al., 2005).

Foodborne transmission of *Campylobacter* can occur through fecal contamination of carcasses at slaughter, although *Campylobacter* is not frequently isolated from cattle carcasses or fresh beef (Minihan et al., 2004; Whyte et al., 2004; Hakkinen et al., 2007). Fecal contamination of milk or water is another potential route of human exposure (CDC, 2002; Clark et al., 2003). Unpasteurized milk has emerged as a risk

factor for human campylobacteriosis in epidemiological studies (Jacobs-Reitsma, 2000; Studahl and Andersson, 2000; Neimann et al., 2003), and numerous outbreaks of human *Campylobacter* infection have occurred through consumption of raw dairy products (Evans et al., 1996; Altekruse et al., 1999; Schildt et al., 2006).

2. Sampling and testing overview

NAHMS has examined *Campylobacter* occurrence using individual fecal samples from dairy cows in three separate studies: Dairy 1996, Dairy 2002, and Dairy 2007. Typically, NAHMS studies generate population-based estimates, and appropriate sample sizes are used to arrive at such estimates. Field resources, laboratory capacity, and the expense of culturing samples make it difficult to provide a national estimate of *Campylobacter* prevalence based on fecal culturing of individual animals. Therefore, for the *Campylobacter* estimates in this section, all three NAHMS dairy studies used a sample of approximately 100 operations, which is not an optimal sample size for providing national estimates of prevalence. Despite this limitation, the NAHMS studies provide valuable information on *Campylobacter* occurrence and antimicrobial susceptibility patterns on U.S. dairies and represent the only national examination of *Campylobacter* on dairy operations in the United States. Other research studies have examined *Campylobacter* occurrence in dairy cattle but have been limited to specific regions of the United States.

Campylobacter monitoring in the NAHMS studies focused on *C. jejuni* and *C. coli* because these species are most commonly associated with human disease. Each of the three studies investigated the prevalence of *Campylobacter* on U.S. dairy operations. Antimicrobial susceptibility patterns of the *Campylobacter* isolates were evaluated in 2002 and 2007. At the time of sampling, records were kept as to whether each cow was sick, healthy (from the milking string), scheduled for culling (within 7 days of leaving the operation), or dry (dry cows were sampled only in Dairy 1996 and Dairy 2002; dry cows were grouped with healthy cows in the following estimates). Dairy 1996 compared the prevalence of *Campylobacter* in milk cows on-farm to that of milk cows on-farm scheduled for culling within 7 days, and to cull cows at markets. To allow for comparisons across the three studies, results presented in this report focused primarily on healthy cows.

The methods used to identify samples as *Campylobacter* positive varied across the three NAHMS studies. Dairy 1996 used PCR methods, and Dairy 2002 and Dairy 2007 used culture and PCR methods. A different PCR was used in Dairy 1996 than was used in Dairy 2002 and Dairy 2007. These differences in identification methods should be noted when interpreting *Campylobacter* results for the NAHMS dairy studies. Because Dairy 1996 identification methods were limited to PCR only, no antimicrobial susceptibility testing was performed. In addition, the PCR test from Dairy 1996 identified isolates with a 460-bp fragment as *C. coli* and isolates with both 160- and

460-bp fragments as *C. jejuni*. Because of the overlap between species at 460 bp, it could not be determined whether any samples were positive for both *C. jejuni* and *C. coli*. There were only 14 *C. coli* isolates in Dairy 1996, and for the purposes of this report it was assumed that no samples were positive for both *C. jejuni* and *C. coli*. For Dairy 2002, isolates were characterized as presumptive positive based on culture and microscopy, with PCR being used to confirm isolates as *Campylobacter* and to determine species. However, PCR was performed on only a subset of the presumptive-positive isolates. For Dairy 2007, species identification was performed on all positive isolates, and antimicrobial susceptibility testing was performed on all viable *C. jejuni* and *C. coli* isolates.

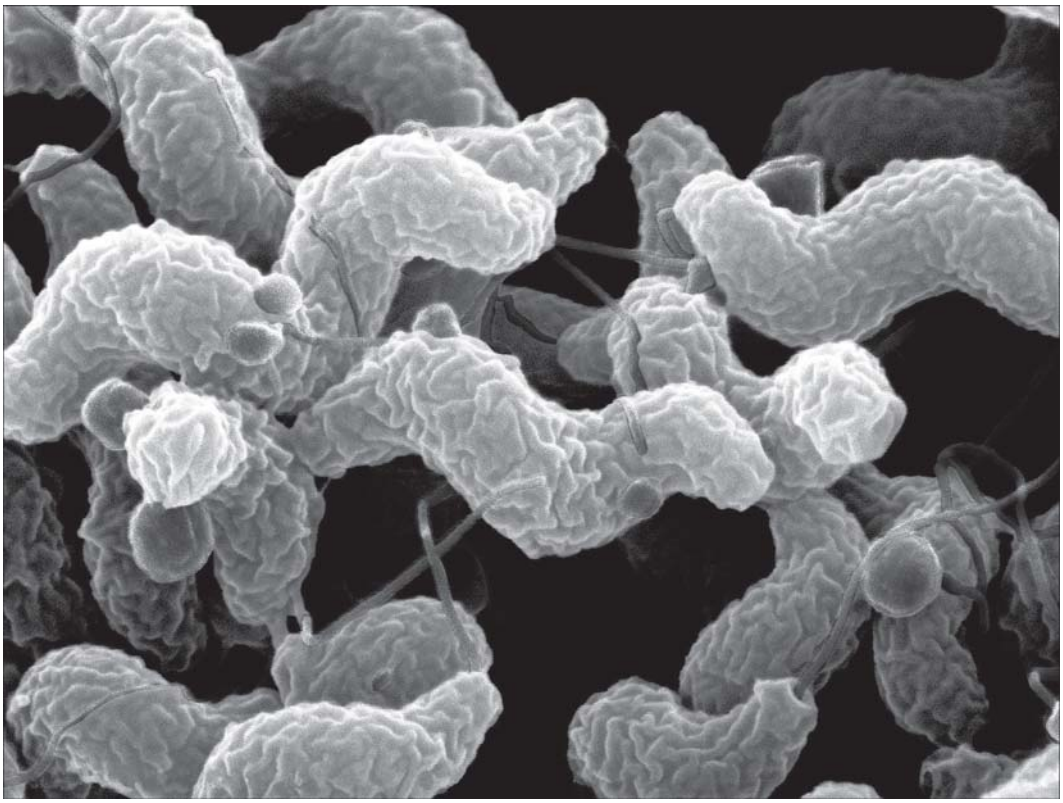


Photo of *C. jejuni* courtesy of CDC.

***Campylobacter* fecal sampling methods, by study**

Study	Number of Operations Sampled	Sampling Period	Number of Samples per Operation	Notes
Dairy 1996	31 dairy operations/ 17 States	Feb. 26 to July 10, 1996	40 or 50, depending on herd size, plus all to-be-culled cows	All samples were taken rectally from individual cows. There were no specific targets as to the number of sick, dry, or milking string cows, other than the sample was to be representative of the cows on hand on the day of the visit. Cow type was noted at the time of collection. **Dairies with 30–99 cows**: Operations were visited once. Up to 40 fecal samples were collected, which included samples from all cows scheduled for culling present on the day of the visit. **Dairies with 100 or more cows:** Operations were visited three times. During one visit, 50 cows (from milking string, dry, or sick) were sampled along with up to 20 cows scheduled for culling in the next 7 days. On 2 other visits, up to 20 samples were taken from cows scheduled for culling in the next 7 days.
	36 dairy cull cow markets/ 14 States	Feb. 26 to July 10, 1996	25	Twenty-five fresh fecal samples per market—either by rectal retrieval from individual cows or from pen floors if restraining facilities were not available.
Dairy 2002	97 dairy operations/ 21 States	Mar. 27 to Sept. 25, 2002	15	The goal was to collect 15 individual fecal samples during a single visit, all via rectal retrieval. There were no specific targets for number of sick, dry, or milking string cows to be sampled, but cow type was noted at the time of collection.
Dairy 2007	121 dairy operations/ 17 States	Feb. 28 to Aug. 30, 2007	17	All samples were taken via rectal retrieval from individual cows at a single visit. The goal was to collect 17 to 18 samples per operation. Sampling for *Salmonella* was performed at the same time, with samples numbered from 1 to 35. While all 35 samples were tested for *Salmonella*, either the odd or even sample numbers were tested for *Campylobacter*. There were no specific targets as to the number of sick cows or cow scheduled for culling to be tested per operation for *Campylobacter*. However, because there were specific goals for testing these cow groups for *Salmonella*, in general 2 to 3 sick cows and 2 to 3 cows scheduled for culling (within 7 days of leaving the operation) were tested for *Campylobacter*, with the remainder up to 18 being from cows with saleable milk

3. Prevalence

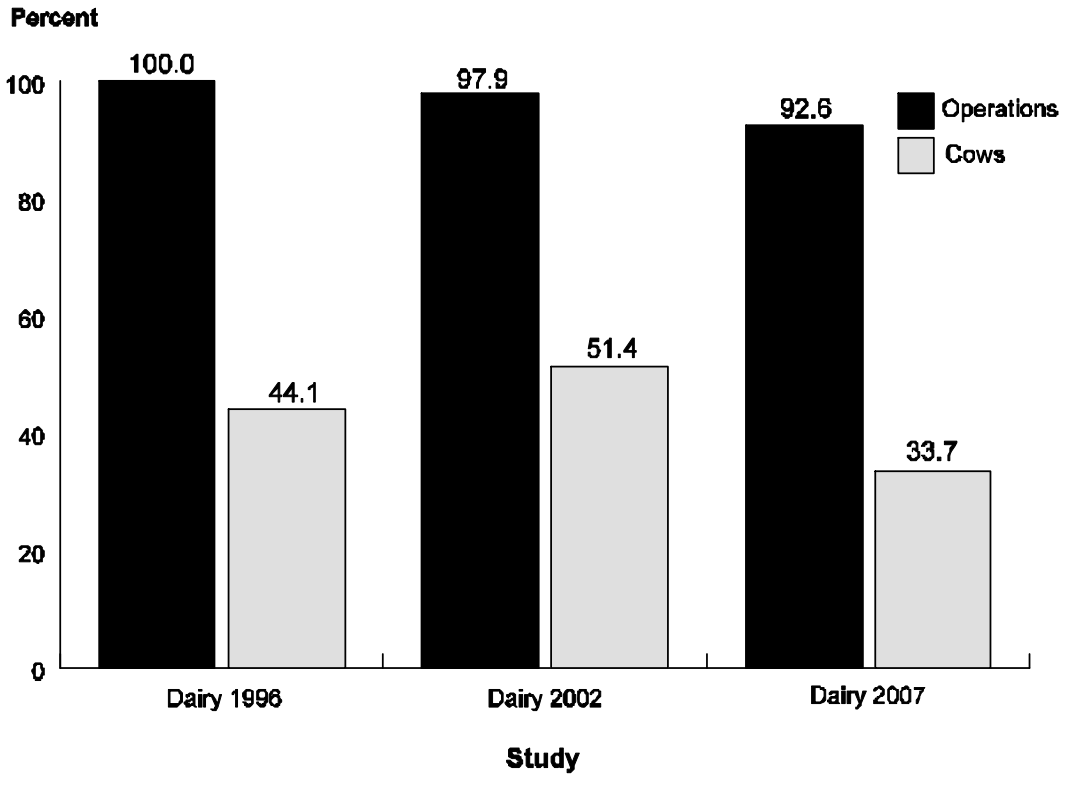
In 1996, *Campylobacter* was detected in at least one cow on all 31 sampled operations. In 2002, 97.9 percent of operations sampled had at least one cow shedding *Campylobacter* in feces. In 2007, 92.6 percent of operations had at least one cow shedding *Campylobacter* in feces.

a. Percentage of operations and percentage of healthy cows fecal-culture positive for *Campylobacter*

Study	Operations[1]	Cows
Dairy 1996[2]	100.0 (31/31)	44.1 (505/1,144)
Dairy 2002[3]	97.9 (95/97)	51.4 (732/1,424)
Dairy 2007	92.6 (112/121)	33.7 (635/1,885)

[1]Operations with at least one positive cow were considered positive.
[2]Only milk cow or cull cow was recorded for *Campylobacter* results for Dairy 1996, so a few operations might have had a small number of sick cows sampled.
[3]Data for 2002 were presumptive positives based on culture and microscopy. Confirmatory testing was performed only on a subset of these presumptive-positive isolates.

Percentage of Operations and Percentge of Healthy Cows Fecal-culture Positive for *Campylobacter*



C. jejuni was found on all of the *Campylobacter*-positive operations from the 1996, 2002, and 2007 studies for which species identification was performed. In contrast, *C. coli* was found on 19.4 to 39.8 percent of *Campylobacter*-positive operations during the three NAHMS studies.

b. Of the *Campylobacter* isolates tested for species identification, percentage of operations[1] and percentage of healthy cows fecal-culture positive for *C. jejuni* or *C. coli*

	C. jejuni		*C. coli*	
Study	**Percent**	**Cows**	**Percent**	**Cows**
Dairy 1996[2]	100.0 (31/31)	97.2 (491/505)	19.4 (6/31)	2.8 (14/505)
Dairy 2002[3]	100.0 (93/93)	89.1 (465/522)	39.8 (37/93)	10.9 (57/522)
Dairy 2007	100.0 (112/112)	90.1 (554/615[4])	25.0 (28/112)	9.3 (57/615[4])

[1]Operations with at least one positive cow were considered positive.
[2]Only milk cow or cull cow was recorded for *Campylobacter* results for Dairy 1996, so a few operations may have had a small number of sick cows sampled.
[3]Species identification was performed on a subset of presumptive-positive isolates from Dairy 2002.
[4]Four of the 615 isolates in 2007 were *C. lari*. Twenty isolates were nonviable at the time of species identification, and these are not included in the isolates listed here.

For Dairy 2002 and Dairy 2007, a slightly higher percentage of sick cows were fecal-culture positive for *Campylobacter* compared with other cow types.

c. Percentage of cows fecal-culture positive for *Campylobacter,* by cow type

	Cow Type			
Study	**Healthy**	**Sick**	**Scheduled for Culling**	**All Cows**
Dairy 2002	51.4 (732/1,424)	56.3 (9/16)	40.0 (2/5)	51.4 (743/1,445)
Dairy 2007	33.7 (635/1,885)	46.4 (51/110)	35.4 (28/79)	34.4 (714/2,074)

During all three study years, a lower percentage of cows on small operations than on large operations were fecal-culture positive for *Campylobacter*.

d. Percentage of healthy cows fecal-culture positive for *Campylobacter*, by herd size

Study	Herd Size (Number of Cows) Small (Fewer than 100)	Medium (100–499)	Large (500 or More)
Dairy 1996*	38.5 (150/390)	45.2 (208/460)	50.0 (147/294)
Dairy 2002	43.7 (211/483)	49.2 (287/583)	65.4 (234/358)
Dairy 2007	22.1 (133/603)	36.3 (269/742)	43.1 (233/540)

*Only milk cow or cull cow was recorded for *Campylobacter* results for Dairy 1996, so a few operations may have had a small number of sick cows sampled.

In 1996, the percentage of cows PCR positive for *Campylobacter* was similar in the East and West regions. In 2002 and 2007, a slightly higher percentage of cows in the West region were culture positive for *Campylobacter* compared with cows in the East region.

e. Percentage of healthy cows fecal-culture positive for *Campylobacter*, by region

Study	Region East	West
Dairy 1996*	44.6 (323/724)	43.3 (182/420)
Dairy 2002	46.9 (477/1,018)	62.8 (255/406)
Dairy 2007	31.7 (507/1,597)	44.4 (128/288)

*Only milk cow or cull cow was recorded for *Campylobacter* results for Dairy 1996, so a few operations may have had a small number of sick cows sampled.

In 1996, 2002, and 2007 over 90 percent of operations were positive for *Campylobacter*. Among all operations tested, the median within-herd prevalence in 1996, 2002, and 2007 was 42.9, 58.3, and 30.8 percent, respectively. Among all operations, the top quartile within-herd prevalence was 64.0, 73.3, and 52.9 percent in 1996, 2002, and 2007, respectively.

The within-herd prevalence for *Salmonella* and *Campylobacter* in healthy cows differed greatly on dairy operations. The majority of operations were *Salmonella* negative, and the highest percentage of positive herds had a within-herd prevalence of 10 percent or less. In contrast, for *Campylobacter* most operations were *Campylobacter* positive, and most positive herds had a within-herd prevalence of over 10 percent.

f. Number of operations by within-herd prevalence of *Campylobacter*[1]

Within-herd Prevalence	Dairy 1996	Dairy 2002	Dairy 2007
0.0	0	2	9
0.1–10.0	1	4	7
10.1–20.0	3	7	24
20.1–30.0	6	7	20
30.1–40.0	5	17	18
40.1–50.0	5	6	14
50.1–60.0	4	22	11
60.1–70.0	6	7	8
70.1–80.0	0	15	7
80.1–90.0	0	4	2
90.1–100.0	1	6	1
Total	31	97	121

4. Antimicrobial susceptibility

Antimicrobial susceptibility testing was conducted on a subset of *Campylobacter* isolates from Dairy 2002 and on all isolates from Dairy 2007. Antimicrobial susceptibility testing was not performed in Dairy 1996. In Dairy 2002, 49.2 percent of the *C. jejuni* isolates from healthy cows were susceptible to all antimicrobials against which they were tested. In Dairy 2007, 37.1 percent of the *C. jejuni* isolates from healthy cows were susceptible to all antimicrobials. A relatively low percentage of isolates were resistant to two or more antimicrobials.

a. Percentage of *C. jejuni* isolates by number of antimicrobials in which antimicrobial resistance[1] was observed[2]

Study (n=Number of Isolates)	Susceptible to All Antimicrobials	Resistant to a Single Antimicrobial	Resistant to Two or More Antimicrobials	Total
Dairy 2002 (n=465)	49.2	47.4	3.4	100.0
Dairy 2007 (n=553)	37.1	60.9	2.0	100.0

[1] Intermediate isolates were classified as susceptible.
[2] Only cows healthy at the time of collection are included.

Of the antimicrobials in the table below, ciprofloxacin and erythromycin are especially important because they are often used when treatment is indicated for *Campylobacter* infection in humans (Gupta et al., 2004). Very few of the *C. jejuni* isolates were resistant to ciprofloxacin or erythromycin in 2002 and 2007. The highest percentages of *C. jejuni* isolates were resistant to tetracycline in 2002 and 2007 (47.5 and 62.4 percent, respectively).

b. Percentage of resistant[1] isolates from all *C. jejuni* isolates tested for antimicrobial susceptibility, by antimicrobial[2]

Antimicrobial	Dairy 2002 (n=465)	Dairy 2007 (n=553)
Azithromycin (AZI)	0.9	0.4
Ciprofloxacin (CIP)	2.6	1.3
Chloramphenicol (CHL)	0.0	NA
Clindamycin (CLI)	0.6	0.2
Erythromycin (ERY)	0.4	0.4
Florfenicol (FLO)	NA	0.0
Gentamicin (GEN)	0.2	0.0
Nalidixic acid (NAL)	4.1	1.6
Telithromycin (TEL)	NA	0.0
Tetracycline (TET)	47.5	62.4

[1]Intermediate isolates were classified as susceptible.
[2]Only cows healthy at the time of collection are included.

5. Multidrug resistance patterns

The table below shows resistance patterns for *C. jejuni* and *C. coli* isolates from all cow types for 2002 and 2007. No isolates were resistant to more than four antimicrobials. No isolates were resistant to both ciprofloxacin and erythromycin.

Number of *Campylobacter* isolates, by resistance pattern[1]

Species	Resistance Pattern2	Dairy 2002 Isolates	Dairy 2007 Isolates
jejuni	AZI, CLI, ERY, TET	0	1
	AZI, CLI, ERY, NAL	1	0
	AZI, CLI, TET	1	0
	AZI, ERY, TET	1	1
	CIP, NAL, TET	6	7
	CIP, NAL	6	3
	NAL, TET	0	2
	AZI, CLI	1	0
	NAL	6	0
	GEN	1	0
	TET	214	381
	Pansusceptible	234	228
	Total	471	623
coli	AZI, CLI, ERY, NAL	1	0
	AZI, CLI, ERY, TET	2	1
	AZI, ERY, TET	2	1
	AZI, CLI, NAL	1	0
	CIP, NAL, TET	0	6
	CLI, TET	1	0
	GEN, TET	2	0
	NAL, TET	3	0
	TET	29	32
	Pansusceptible	18	24
	Total	59	64

[1]Healthy, sick, and to-be-culled cows are included.
[2]See preceding table for the full name of the antimicrobials.

Section III: Sampling and Diagnostic Testing

A. Farm Selection

Dairy 1996

A stratified random sample of dairy operations from the USDA National Agricultural Statistics Service (NASS) list frame in each of 20 selected States,[1] representing 80.2 percent of U.S. dairy operations and 83.1 percent of U.S. dairy cows, was the basis for selecting participating operations in the Dairy 1996 study. More than 2,500 and 1,200 dairy producers participated in Phase I and Phase II of the study, respectively. A convenience sample of 100 of the 1,200 dairies was selected for participation in *Salmonella* sampling. This sample included 50 dairies with 30 to 99 dairy cows, and 50 dairies with 100 or more dairy cows. The number of small and large operations allocated to each State was proportional to the number of small and large operations in the State. Cull-cow markets were also selected for fecal sampling in these 20 States, allocated based on the number of cull dairy-cow markets within the State. Previous history of salmonellosis was not a selection factor. Samples were collected from February 26 to July 10, 1996.

Dairy 2002

A stratified random sample of dairies was chosen based on herd size from the NASS listing for each of 21 selected States.[2] This sample represented 85.5 percent of the U.S. dairy cows and 82.8 percent of U.S. dairy operations. Dairy operations reporting one or more milk cows in inventory on January 1, 2002, were eligible for Phase I of the study, and operations with at least 30 dairy cows were eligible for Phase II. Participation in the study included over 2,400 dairy producers in Phase I and 1,000 producers in Phase II. Of the Phase II operations, bulk-tank milk samples from 861 operations were collected by Federal and State veterinary medical officers or animal health technicians. Samples were collected from February 27 to July 1, 2002. A convenience sample of 100 of these operations with at least 30 milk cows was selected for fecal sampling. Approximately five operations per State were selected. Previous history of salmonellosis was not a selection factor. Samples were collected from March 27 to September 25, 2002.

[1]California, Florida, Idaho, Illinois, Indiana, Iowa, Kentucky, Michigan, Missouri, New Mexico, New York, Minnesota, Ohio, Oregon, Pennsylvania, Tennessee, Texas, Vermont, Washington, and Wisconsin.

[2]California, Colorado, Florida, Idaho, Illinois, Indiana, Iowa, Kentucky, Michigan, Minnesota, Missouri, New Mexico, New York, Pennsylvania, Ohio, Tennessee, Texas, Vermont, Virginia, Washington, and Wisconsin.

Dairy 2007

Data were collected during the NAHMS Dairy 2007 study from dairy operations in 17 major dairy States[3] representing 79.5 percent of U.S. dairy operations and 82.5 percent of U.S. dairy cows. The survey design was a stratified random sample with unequal selection probabilities within each stratum to ensure that large dairy operations were well represented in the sample. Dairy operations reporting one or more milk cows in inventory on January 1, 2007, were eligible for Phase I of the study, and operations with at least 30 dairy cows were eligible for Phase II. Participation in the study included over 2,194 dairy producers in Phase I and 582 producers in Phase II. Of the Phase II operations, bulk-tank milk and in-line milk filter samples from 538 operations were collected by Federal and State veterinary medical officers or animal health technicians. A convenience sample of 121 of these operations with at least 30 milk cows was selected for individual cow fecal sampling. Previous history of salmonellosis was not a selection factor. Samples were collected from February 28 to August 29, 2007.

Photo courtesy of Agriculture Research Service

[3]California, Idaho, Indiana, Iowa, Kentucky, Michigan, Minnesota, Missouri, New Mexico, New York, Ohio, Pennsylvania, Texas, Vermont, Virginia, Washington, and Wisconsin.

B. Sampling Methods

1. Bulk-tank milk and milk filter sampling

A single bulk-tank milk sample from each participating operation was collected during the Dairy 2002 and Dairy 2007 studies and tested for *Listeria* and *Salmonella*. Bulk-tank milk was not tested for *Salmonella* or *Listeria* in Dairy 1996. Milk filters were collected only in 2007. Dairy 2002 used both culture and PCR methods for *Salmonella* detection, but only PCR was used for Dairy 2007. Bulk-tank milk samples were aseptically collected only when milk from at least 70 percent of the operation's lactating cows was represented in the sample. Additionally, for Dairy 2007, milk filters were collected at the time of sampling. If the milk filter was not available for removal from the milk line during the sample visit, farm operators were requested ahead of time to place the filter from the most recent milking in a clean plastic bag and store in the refrigerator. For Dairy 2007, sample collectors were instructed not to freeze samples. In some cases for Dairy 2002, the samples were frozen prior to shipping. Bulk-tank milk and milk filters were shipped overnight with ice packs to the USDA–Agricultural Research Service (ARS) Environmental Microbial Safety Laboratory (EMSL) in Beltsville, MD.

2. Fecal sampling

Each of the three dairy studies had slightly different objectives with regard to *Salmonella* sampling, which led to differences in the types of cattle sampled. Although the numbers of samples taken on each operation were similar across studies, they were not identical. A subset of samples taken for *Salmonella* testing during the three dairy studies was tested for *Campylobacter*. Thus, the type of cattle sampled, the number of cattle sampled, and the sampling collection methods were the same for *Salmonella* and for *Campylobacter*, but fewer samples were tested for *Campylobacter* than for *Salmonella*.

Dairy 1996

The Dairy 1996 study set out to determine if *Salmonella* prevalence differed among milk cows on the farm, cows scheduled for culling within 7 days, and cull cows at livestock markets. Small dairies (30 to 99 cows) were visited once for fecal sampling, and up to 40 samples were collected at this single visit. All cows scheduled for culling within 7 days were sampled, and the remainder of samples—up to 40—were taken from other cows, including healthy milking cows, dry cows, and sick cows. Dairies with 100 or more cows were visited 3 times. At one visit, 50 cows (milking string, dry, or sick) were sampled along with up to 20 cows scheduled for culling. During the other two visits, up to 20 samples were taken from cows scheduled for culling. There were no specific target numbers for sick, dry, or milking

string cows, other than samples were to be representative of the cows on hand on the day of the visit. Cow type was recorded at the time of collection. At each livestock market, 25 fresh fecal samples were taken, either by rectal retrieval or from pen floors if restraining facilities were not available. Samples were taken by rectal retrieval, and a separate glove was used to collect each fecal sample to avoid cross-contamination during sampling. Samples were placed in sterile screw-top vials. Fecal samples were approximately golf-ball sized and were kept on ice and shipped to NVSL. *Salmonella*-positive samples were sent to the USDA–ARS Bacterial Epidemiology and Antimicrobial Resistance Unit (BEAR) [formerly the Antimicrobial Resistance Research Unit] in Athens, GA, for antimicrobial susceptibility testing. At 50 samples per herd (40 per herd for operations with fewer than 100 cows), this sample provided 95-percent confidence of detecting at least 1 positive animal if the within-herd prevalence was greater than or equal to 5 percent, assuming an equal risk of fecal shedding for each cow sampled. *Campylobacter* testing was done on all samples from 31 of the operations.

Dairy 2002

The Dairy 2002 study set out to estimate *Salmonella* prevalence and describe antimicrobial resistance on U.S. dairies. Operations were visited once. The goal was to collect 40 samples per operation regardless of herd size, or from all cows if the operation had fewer than 40 cows. There were no specific targets for numbers of sick, dry, or milking string cows to be sampled, but cow type was recorded at the time of collection. Samples were taken by rectal retrieval, and a separate glove was used to collect each fecal sample to avoid cross-contamination during sampling. Samples were placed in sterile Whirl-pak® bags. Fecal samples were approximately golf-ball sized and were shipped on ice to BEAR for culturing and antimicrobial susceptibility testing. *Campylobacter* testing was done on 15 of the samples per operation.

Dairy 2007

The Dairy 2007 study set out to estimate *Salmonella* prevalence and describe antimicrobial resistance on U.S. dairies. An additional goal was to evaluate testing strategies for detecting *Salmonella* using fecal samples from individual cows, pooled fecal samples, and composite fecal samples. Operations were visited once. Up to 35 fecal samples per operation were taken via rectal retrieval. The goal was to collect 35 fecal samples from every operation, regardless of operation size. Up to 5 sick cows and up to 5 cows scheduled for culling were sampled, with the remainder (up to 35) taken from cows with saleable milk. A separate glove was used to collect each fecal sample to avoid cross-contamination during sampling. Fecal samples were approximately golf-ball sized. Samples were placed in sterile Whirl-pak bags. Samples were kept on ice and shipped to BEAR for culturing and antimicrobial susceptibility testing. Samples from individual cows were pooled at the laboratory, with each pool representing five cows.

C. Laboratory Methods

1. *Salmonella* testing of bulk-tank milk and milk filters

Dairy 2002

Culture was one of the testing methods used to detect *Salmonella* in milk samples (Van Kessel et al., 2004). Briefly, milk (250 µL) was plated in triplicate directly onto XLT4 agar (XLT4 agar base with XLT4 supplement; BD Diagnostics) using an Autoplate 4000. Plates were incubated at 37°C and scored for presumptive *Salmonella* colonies (black colonies) at 24 and 48 h. For enrichment of *Salmonella*, 5 to 10 mL of milk was added to 90 mL of tetrathionate broth. The variation in volume was due to variation in available sample volume. Enrichment bottles were incubated at 37°C for 24 h and then the broth was streaked (10 µL) onto XLT4 agar. Plates were incubated at 37°C and examined at 24 and 48 h for the presence of black colonies. Isolated, presumptive *Salmonella* colonies were transferred from XLT4 plates onto XLT4, brilliant green, and L-agar. Colonies that exhibited the *Salmonella* phenotype (black on XLT4 and pink on brilliant green) were preserved for future analysis. Colony biomass was transferred from the L-agar plates to a vial containing 0.5 mL of a 1:1 mixture of Lennox broth and the 2x freezing medium for cells (Schleif and Wensink, 1981). The isolates were stored at -80°C. L-agar slants were inoculated and, after incubation at 37°C for 24 h, sent to NVSL for serotyping.

PCR was also used to detect *Salmonella* in milk samples using RT-PCR, as described by Van Kessel, et al. (2003). Briefly, 5 to 10 mL of milk was added to 95 mL of tetrathionate broth. The variation in volume was due to variation in available sample volume. Enrichment bottles were incubated at 37°C for 24 h. After incubation, enriched samples (1.5 mL) were centrifuged (13,000 x *g*) in microcentrifuge tubes, the supernatants were discarded, and the pellets were stored at -20°C. DNA was extracted from bacterial pellets using 200 µL of InstaGene Matrix (Bio-Rad Laboratories, Hercules, CA) following the manufacturer's directions. The DNA preparations were stored at -20°C and later analyzed for the presence or absence of *Salmonella* via RT-PCR . RT-PCR was carried out using the Ruggedized Advanced Pathogen Identification Device (RAPID) [Idaho Technology Inc., Salt Lake City, Utah]. Premixed, freeze-dried PCR reagents that target the *spa*Q gene on the chromosome of *Salmonella* were used according to the manufacturer's directions using 2 µL of sample. Preincubation was at 94°C for 60 s. Forty-five PCR cycles were run under the following conditions: 95°C for 0 s (the cuvettes are heated to 95°C but not held there), followed by 60°C for 20 s with a temperature transition rate of 20°C/s. Other variable parameters included: channel 2, gain 8, and mode 1. The RAPID system, in conjunction with the *Salmonella* detection kit, has the capability of running melting point curves on the PCR reaction products. Melting point curves were run on all samples that were identified as *Salmonella*-positive by the RAPID software. The initial temperature was 94°C for 1 min; the temperature was reduced to 50°C, and then increased from 50 to 94°C at a rate of 0.2°C/s. The fluorescence in the sample was read at each stage of the temperature gradient and a first derivative plot of fluorescence vs. temperature was used to determine the melting point of any

PCR products present. The software supplied by the manufacturer provided a score for each reaction based upon the degree that the maximum level of fluorescence recorded during the PCR run differed from the baseline calculated in the early stages of the run. Thus, the score depended upon the magnitude of fluorescent signal generated and the quality of the baseline. The higher the score, the more the maximum fluorescent signal varied from the baseline. For samples with a very low PCR score, a subjective analysis of the melting point curve and the RT-PCR amplification curve was used to decide if a sample was finally considered *Salmonella*-positive or *Salmonella*-negative (Van Kessel et al., 2003). Logistic regression analysis of the relationship between RT-PCR signal and the likelihood of obtaining a positive culture was done using the PROC PROBIT procedure in SAS 9.1 (SAS Inst. Inc., Cary, NC). Samples that gave a positive result in the real-time assay were subjected to two rounds of conventional PCR using primer set 139-141 targeting the *inv*A gene as described by Rahn et al. (1992) and shown by Malorny et al. (2003) to detect a wide range of *Salmonella*. The conditions for the first round of PCR were those described by Malorny et al. (2003) except that 1 U of Amplitaq Gold (Applied Biosystems, Foster City, CA) was used per 25 µL reaction, a 10-min incubation at 95°C was added to activate the enzyme at the beginning of the reaction, and the PCR was run for 40 cycles. A portion (1 to 3 µL) of the InstaGene preparation from the tetrathionate broth enrichments of raw milk samples was added to each reaction. For the second round of PCR, 5 µL of first-round product was added to 20 µL of fresh PCR mix to give the same final composition as the first-round reactions. Amplification was done on a Biometra Personal Cycler (Biometra, Göttingen, Germany). The PCR products were separated by electrophoresis on a 2-percent horizontal agarose gel in Tris-borate buffer as described by Maniatis et al. (1982). The gel contained 0.5 µg/mL ethidium bromide; bands were visualized on a UV transilluminator, and documented with a video camera. Detection of a band in the region of 284 bp indicated the presence of *Salmonella*.

Dairy 2007

Bulk-tank milk and/or in-line milk filter samples were analyzed by RT-PCR. Ten mL of milk were added to 10 mL of 2X tetrathionate broth and incubated overnight at 37°C. Milk filters were cut into pieces and mixed with buffered peptone water in a stomacher bag and pummeled for 2 min. Five mL of the liquid of the stomacher bag were added to 5 mL of 2X tetrathionate broth and incubated at 37°C overnight. After incubation, 1.5 mL of the broth was centrifuged (16,000 x *g*) for 2 min in microcentrifuge tubes. The supernatant was discarded, and the DNA was extracted from the pellet biomass using 200 µL of InstageneGene Matrix (Bio-Rad Laboratories, Hercules, CA) following manufacturer's instructions. The DNA preparations were stored at -20°C and analyzed for presence of *Salmonella* via PCR for the *invA* gene using the primers described by Rahn et al. (1992) and shown by Malorny et al. (2003) to be effective for the detection of multiple serotypes of *Salmonella*. The PCR reactions were run at EMSL and monitored in real time through the addition of EVAGreen dye (Biotium, Inc., Hayward, CA). PCR-positive samples were then cultured to allow for *Salmonella* serotyping.

2. *Listeria* testing of bulk-tank milk and milk filters

Dairy 2002

Milk (250 µL) was plated in triplicate directly onto Modified Oxford Medium (MOX) agar (BD Diagnostics) using an Autoplate 4000. Plates were incubated at 37°C and scored for presumptive *Listeria* colonies (esculin hydrolysis, black colonies) at 24 and 48 h. For enrichment of *Listeria*, 5 to 10 mL of milk were added to 90 mL of Modified *Listeria* Enrichment Broth (BD Diagnostics). Enrichment bottles were incubated at 37°C for 48 h, and then the broth was streaked (10µL) onto MOX agar. Plates were incubated and scored as described previously. Isolated, presumptive *Listeria* colonies were transferred from MOX plates onto MOX, PALCAM (BD Diagnostics), and trypticase soy agar with 0.6 percent yeast extract (TSA-YE). Colonies that exhibited the *Listeria* phenotype (black on MOX and gray-green with esculin hydrolysis on PALCAM) were preserved for future analysis. Colony biomass was transferred from the TSA-YE plates to 1.5 mL of tryptic soy broth and incubated at 37°C for 48 h. The enriched broth was centrifuged (16,000 x *g*), and the supernatants were discarded. The bacterial pellet was resuspended in 0.5 mL of 1x freezing medium for cells of Schleif and Wensink (1981), and the isolates were stored at -80°C. Presumptive *Listeria* isolates were grown on TSA-YE for further testing. Isolates were tested for oxidase with 1-percent tetramethyl-p-phenylenediamine dihydrochloride (BD Diagnostics), catalase with 3 percent hydrogen peroxide, and gram-stained using a 3-step staining kit (BD Diagnostics). Hemolytic activity was determined by stabbing blood agar (Columbia with 5 percent sheep blood; Remel, Lenexa, KS) and incubating at 37°C for 48 hours. The Christie-Atkins-Munch-Peterson test was performed on each isolate using *Staphylococcus aureus* Beta Lysin Disks (Remel) and *Rhodococcus equi* (ATCC 6939; American Type Culture Collection, Manassas, VA) on sheep blood agar. Additionally, RT-PCR was run on DNA extracts of the presumptive *Listeria* isolates. Isolates were grown in 1.0 mL of tryptic soy broth at 37°C for 48 h. The enriched broth was centrifuged (16,000 x *g*), and the supernatants were discarded. The DNA was extracted from the bacterial pellets using a commercially prepared extraction preparation (InstaGene Matrix; Bio- Rad Laboratories, Hercules, CA) following the manufacturer's directions. The DNA preparations (200 µL) were stored at -20°C prior to analysis. RT-PCR was run according to the method described by Nogva et al. (2000) using a Mx4000 Multiplex Quantitative PCR System (Stratagene, La Jolla, CA). Amplification reactions (50 µL) contained 300 nM of each primer, 250 nM probe, 12.6 µg of BSA, 25 µL of TaqMan Master Mix (Applied Biosystems, Foster City, CA), and 5 µL of extracted DNA product. The thermal profile used for PCR was 50°C for 2 min followed by 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. Serotyping of the *L. monocytyogenes* isolates was conducted using a previously described ELISA (Palumbo et al., 2003).

Dairy 2007

Ten mL of milk was mixed with 90 mL 1X modified *Listeria* Enrichment Broth (mLEB) for enrichment of *Listeria* and incubated at 37°C for 40 to 48 h. Milk filters were cut into pieces and mixed with 2 parts (w/w) buffered peptone water in a stomacher bag and pummeled for 2 min. Then 5 mL of liquid from stomacher bag was mixed with 5 mL 2X mLEB and incubated at 37°C for 40 to 48 h. After 48 h, 2 mL of each enrichment was harvested by centrifuging at 16K x *g* for 2 min in a 2-mL cryovial. The supernatant was removed and the pelleted biomass was suspended in 0.5 mL of preservation medium and frozen at -80°C to archive live cells (Preservations). Biomass was harvested from 1.5 mL of each enrichment in a 1.7-mL microcentrifuge tube. The supernatant was removed and the pellet was saved for DNA extraction by freezing at -20°C. A 10-µL loop was used to streak 10 µL of each enrichment onto Modified Oxford Agar plates (MOX). Plates were incubated at 37°C for 48 h and examined for colonies with morphology resembling *Listeria*. Identity of colonies was confirmed as *Listeria* and determined to be *L. monocytyogenes* vs. non-*L. monocytyogenes* by patching suspect colonies onto PALCAM and BCM media. Any phospholipase-positive isolates were further characterized with a CAMP test to distinguish *L. ivanovii* from *L. monocytyogenes*.

3. *Salmonella* testing of fecal samples

Various diagnostic testing methods are available for detecting *Salmonella*, including culture, PCR, and ELISA. Culture methods must be used if antimicrobial susceptibility testing is to be performed. Culture was the diagnostic method used in the Dairy 1996, Dairy 2002, and Dairy 2007 studies, and culture methods were similar across studies. The following culture methods apply to Dairy 1996, Dairy 2002, and Dairy 2007, unless noted otherwise.

Approximately 1 g of feces from each sample was placed into each of two culture media—gram-negative Hajna broth and tetrathionite broth—which were incubated at 37°C for 24 and 48 h, respectively. Following primary enrichments, 100 µL culture aliquots from each broth enrichment were transferred into Rappaport R-10 medium for secondary enrichment, giving two Rappaport secondary enrichments per sample. In each case, Rappaport R-10 medium was incubated overnight at 37°C and then streaked onto brilliant green agar with sulfadiazine and xylosine-lysine-tergitol-4 (XLT-4) plates, resulting in four plates per sample. All plates were incubated overnight at 37°C. At least three (Dairy 1996) or four (Dairy 2002 and 2007) colonies having the typical appearance of *Salmonella* were inoculated into triple sugar iron and lysine iron agar slants. All slants were incubated overnight at 37°C. All isolates presumed to be *Salmonella* were serogrouped using serogroup-specific sera and sent to NVSL for serotyping. Isolates with different serogroups from each sample were kept. If all four colonies from a sample had the same serogroup, only one isolate was kept.

Salmonella isolates were tested for antimicrobial drug susceptibility at BEAR. For Dairy 1996, Dairy 2002, and Dairy 2007, susceptibility testing was conducted with a custom-designed panel of antimicrobial drugs using a Sensititre semi-automated testing system (TREK Diagnostic Systems, Inc.). Antimicrobial agents included in the custom designed panel differed slightly for each of the three NAHMS studies. The minimum inhibitory concentration (MIC) for each isolate was determined, and each isolate was classified as susceptible, intermediate, or resistant, according to guidelines published by the National Committee on Clinical Laboratory Standards for broth-microdilution susceptibility testing, when available. When guidelines were not available, breakpoint interpretations were determined using National Antimicrobial Resistance Monitoring System (NARMS) guidelines. The antimicrobials included for all studies included amikacin, amoxicillin-clavulanic acid, ampicillin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, tetracycline, and trimethoprim-sulfamethoxazole. Apramycin was included in Dairy 1996 only. Cefoxitin was included in Dairy 2002 and 2007 but not Dairy 1996. Cephalothin was included in Dairy 1996 and 2002 but not 2007. Ticarcillin was included in Dairy 1996 only. Sulfamethoxazole was included in Dairy 1996 and 2002, and then a similar sulfa antimicrobial, sulfisoxazole, replaced it in Dairy 2007.

Breakpoints used for susceptibility testing of *Salmonella*[1,2]

Antimicrobial Class	Antimicrobial Agent	Breakpoints (µg/mL) Susceptible (less than or equal)	Intermediate	Resistant (greater than or equal)
Aminoglycosides	Amikacin	16	32	64
	Gentamicin	4	8	16
	Kanamycin	16	32	64
	Streptomycin	32	NA	64
β-lactam/β-lactamase inhibitor combinations	Amoxicillin-clavulanic acid	8/4	16/8	32/16
Cephems	Cefoxitin	8	16	32
	Ceftiofur	2	4	8
	Ceftriaxone[2]	8	16–32	64
Folate pathway inhibitors	Sulfamethoxazole/ sulfisoxazole[3]	256	NA	512
	Trimethoprin-sulfamethoxazole	2/38	NA	4/76
Penicillin	Ampicillin	8	16	32
Phenicols	Chloramphenicol	8	16	32
Quinolones	Ciprofloxacin	1	2	4
	Nalidixic acid	16	NA	32
Tetracyclines	Tetracycline	4	8	16

[1]Breakpoints were adopted from CLSI (Clinical and Laboratory Standards Institute), except for streptomycin, which has no CLSI breakpoints.
[2]CLSI revised the breakpoints for ceftriaxone in its M100-S20 document published in January 2010. The old breakpoints were used for the data in this report.
[3]Sulfamethoxazole was tested from 1996 through 2003 and was replaced by sulfisoxazole in 2004.

4. *Campylobacter* testing of fecal samples

Various diagnostic testing methods are available for detecting *Campylobacter*. Culture is the traditional identification method, but PCR is also commonly used. Culture methods are required in order to perform antimicrobial susceptibility testing. Specific PCR and culture methods differed among the dairy studies, and diagnostic procedures follow.

Dairy 1996

For *Campylobacter* testing, a multiplex PCR was used that allowed for the simultaneous identification of *C. jejuni* and *C. coli* (Harmon et al., 1997). The assay targeted the *flA* genes of *C. jejuni* and *C. coli*, which yielded a 460-bp product. A second set of primers identified a nucleic acid sequence unique to *C. jejuni* and yielded a 160-bp product. Within 36 h of sample collection, approximately 1 g of feces was diluted (10 percent wt/vol) in buffered peptone water (9 mL). An aliquot (0.4 mL) of the fecal suspension was plated to the surface of modified blood-free charcoal, cefoperazone deoxycholate agar (CM 739; Oxoid Ogdensburg, NY) and incubated microaerobically for 2 to 3 d at 42°C (Ono et al., 1995). After incubation, bacterial growth from the first quadrant was harvested with a bacteriological loop, placed in Tris-EDTA buffer (pH 7.4, 200µL) and frozen (-20°C) prior to PCR analysis. The bacterial suspension in Tris-EDTA (200 µL) was boiled for 5 min prior to PCR analysis and centrifuged (13,000 x g, 1 min at room temperature), and a 5-µL aliquot was used as the PCR template. Samples were subjected to an initial denaturation step (94°C for 4 min), followed by 25 amplification cycles. Each amplification cycle consisted of denaturation (1 min at 94°C), primer annealing (1 min at 45°C), and primer extension (1 min at 72°C). Final primer extension (7 min at 72°C) followed the last amplification cycle. PCR products were electrophoretically separated (120 V, 45–55 min). *C. coli* were identified by the appearance of a 460-bp product, and *C. jejuni* was identified by presence of both a 460-bp and a 160-bp product. Antimicrobial susceptibility testing was not performed on *Campylobacter* isolates from the Dairy 1996 study.

Dairy 2002

Fecal samples were diluted 1:4 and 1:40 in phosphate-buffered saline. 100-µL aliquots of each dilution were spread uniformly on duplicate Campy-Cefex plates (Stern et al., 1992). The plates were placed in zip-top bags and incubated microaerobically (5 percent O_2, 10 percent CO_2, and 85 percent N_2) for 48 h at 42°C. *Campylobacter* was presumptively identified from microscope wet mounts of cells using phase contrast optics at 100x. Samples from 97 operations were tested for *Campylobacter*, and antimicrobial susceptibility testing was performed on isolates from 94 operations. For 26 operations, all available isolates were tested for antimicrobial susceptibility. For cost reasons, 5 isolates (or as many as were available for operations with fewer than 5 isolates) were randomly chosen for antimicrobial susceptibility testing from the remaining 68 operations. From each sample with *Campylobacter* growth, a single colony was selected for antimicrobial susceptibility testing. The isolates were identified to the species level

using the *Campylobacter* BAX® PCR (DuPont Qualicon, Wilmington, DE), a multiplex assay specific for *C. coli* and *C. jejuni* (Englen and Fedorka-Cray, 2002). A total of 532 isolates, including 473 *C. jejuni* and 59 *C. coli*, were selected for susceptibility testing to 8 antimicrobials. The Etest® method (AB-Biodisk, Piscataway, NJ) was used according to the manufacturer's directions as described by Englen et al. (2005). Briefly, 150-mm Mueller Hinton plates containing 5 percent lysed horse blood (B-D Biosciences, Sparks, MD) were inoculated with 100 µL of a cell suspension equal to a 1.0 McFarland standard. The inoculum was swabbed evenly across the entire plate surface, and four Etest strips were laid at right angles onto each plate. The plates were put into zip-top bags and incubated in a microaerobic atmosphere (5 percent O_2, 10 percent CO_2, and 85 percent N_2) for 48 h at 42°C. Following incubation, the point at which the zone of growth inhibition intersected the strip was read as the MIC of the antimicrobial in µg mL^{-1}. Quality control ATCC strains *C. jejuni* 33560, *Escherichia coli* 25922, and *Staphylococcus aureus* 25923 were tested biweekly to confirm susceptibility to all eight antimicrobials. The antimicrobial resistance break points (MICs) used were those established by NARMS in accordance with Clinical and Laboratory Standards Institute guidelines: azithromycin, ≥2 µg mL^{-1}; chloramphenicol, ≥32 µg mL^{-1}; ciprofloxacin, ≥4 µg mL^{-1}; clindamycin, ≥4 µg mL^{-1}; erythromycin, ≥8 µg ml^{-1}; gentamicin, ≥16 µg ml^{-1}; nalidixic acid, ≥32 µg ml^{-1}; tetracycline, ≥16 µg ml^{-1}.

Dairy 2007

Fecal samples were diluted 1:10 in phosphate-buffered saline before being enriched in Bolton's enrichment broth for 48 h at 42°C under microaerophilic conditions (5 percent O_2, 10 percent CO_2, 85 percent N_2). Aliquots (10 µL) were spread onto Campy-Cefex plates (Stern et al., 1992) which were incubated as in 2002. Presumptive *Campylobacter* colonies were selected by observation of cellular morphology and motility using a wet mount under phase-contrast microscopy. Isolates were identified using the *Campylobacter* BAX PCR (DuPont Qualicon, Wilmington, DE), a multiplex assay specific for *C. coli* and *C. jejuni*. The assay was performed according to manufacturer directions as previously described (Englen and Fedorka-Cray, 2002). If a PCR product was not obtained using the BAX PCR, a traditional PCR was used as previously described (Wang et al., 2002). This traditional PCR can identify *C. jejuni, C. coli, C. lari, C. fetus,* and *C. upsaliensis. Campylobacter* isolates were susceptibility tested using broth microdilution in a custom panel of nine antimicrobials: azithromycin, ciprofloxacin, clindamycin, erythromycin, florfenicol, genamicin, nalidixic acid, telithromycin, and tetracycline. The semi-automated Sensititre™ System (TREK Diagnostic Systems, Inc., Cleveland, OH) was used per manufacturer's instruction. MICs were determined for each isolate and classified as susceptible,

intermediate, or resistant according to Clinical and Laboratory Standards Institute standards, where available. Otherwise, breakpoint determinations were based on those used by NARMS (FDA, 2009).

Breakpoints used for susceptibility testing of *Campylobacter*[1]

Antimicrobial Class	Antimicrobial Agent	Breakpoints (µg/mL) Susceptible (less than or equal)	Intermediate	Resistant (greater than or equal)
Aminoglycosides	Gentamicin	2	4	8
Ketolides	Telithromycin	4	8	16
Lincosamides	Clindamycin	2	4	8
Macrolides	Azithromycin	2	4	8
	Erythromycin	8	16	32
Phenicols	Florfenicol[2]	4	NA	8
Quinolones	Ciprofloxacin	1	2	4
	Nalidixic acid	16	32	64
Tetracyclines	Tetracycline	4	8	16

[1]Breakpoints were adopted from CLSI (Clinical and Laboratory Standards Institute) when available.
[2]For florfenicol, only a susceptible breakpoint (=4 µg/mL) has been established. In this report, isolates with an MIC =8 µg/mL are categorized as resistant.

D. Testing Methods Overview

The table below presents a synopsis of the testing methods used on each NAHMS dairy study, by organism and type of sample.

c. Testing method by NAHMS study, organism, and type of sample

	Study Year					
	1996		2002		2007	
Organism/ Sample Type	**Culture**	**PCR**	**Culture**	**PCR**	**Culture**	**PCR**
Salmonella						
Individual cow fecal samples	X		X		X	
Composite fecal (environmental) samples					X	
Bulk-tank milk sample			X	X		X
Milk filter samples						X
Campylobacter						
Individual cow fecal samples		X	X		X	
Listeria						
Bulk-tank milk samples			X		X	
Milk filter samples					X	

Appendix I: NAHMS Study Methodology–Phase II*

NAHMS Dairy Studies			
	1996	2002	2007
Data collection dates	2/26–7/10	3/27–9/25	2/28–8/30
Minimum number of dairy cattle	30	30	30
Number of States	20	21	17
Data collectors	State and Federal veterinary medical officers and animal health technicians		
Participating States as a percentage of U.S. population coverage			
Operations	85.6	86.9	84.7
Cows	82.7	85.7	82.5
Respondent sample profile (herd size)			
Small (fewer than 100 cows)	630	400	233
Medium (100–499 cows)	502	392	215
Large (500 or more cows)	87	221	134
Respondent sample profile (region)			
East	931	805	474
West	288	208	108
Response category			
Survey complete	1,219	1,013	582
Percent of total	76.0	70.4	54.0
Refused	340	335	380
Did not contact	16	76	111
Ineligible	29	14	4
Total	1,604	1,438	1,077

*For more detailed information about the methodology for each study, see methodology section of each descriptive report at: http://nahms.aphis.usda.gov

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Appendix III. Previously Published Material

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