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PATTERNS OF OCCURRENCE OF *CAMPYLOBACTER* IN DAIRY FARMS IN MIDWESTERN AND NORTHEASTERN UNITED STATES

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PATTERNS OF OCCURENCE OF *CAMPYLOBACTER* IN DAIRY FARMS IN MIDWESTERN AND NORTHEASTERN UNITED STATES

By

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ABSTRACT

PATTERNS OF OCCURRENCE OF *CAMPYLOBACTER* ON DAIRY FARMS IN MIDWESTERN AND NORTHEASTERN UNITED STATES

By

Amy M. Campbell

Campylobacter is the leading cause of bacterial gastroenteritis in the United States. Because dairy cattle can be a source of *Campylobacter* in humans, more research is needed to describe the epidemiology of *Campylobacter* in dairy cattle and to identify the risk factors associated with the prevalence *Campylobacter* in dairy cattle.

In this work, three major objectives were addressed. First, the patterns of occurrence of *Campylobacter* on dairy farms in Michigan, Minnesota, New York, and Wisconsin were identified. Secondly, any differences in the prevalence of *Campylobacter* by cattle age group, health status, and season were determined. Finally, specific risk factors that contributed to the prevalence of *Campylobacter* on dairy farms were identified.

The overall prevalence of *Campylobacter* of dairy farms in Michigan, Minnesota, and Wisconsin was 12%. Calves had a higher prevalence of *Campylobacter* than adults, sick adults had a higher prevalence than healthy adults, and *Campylobacter* prevalence was the highest in winter and lowest in summer. Risk factors associated with an increased prevalence of *Campylobacter* on dairy farms were those that increased risk of fecal contamination and increased exposure to infected animals.

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INTRODUCTION

Purpose

Campylobacter is the leading cause of bacterial gastroenteritis in the United States. Foods of animal origin are considered to be the greatest sources of these pathogens to humans. Since cattle are known to be intestinal carriers of *Campylobacter*, consumption of meat and milk from cattle has been identified as a major risk to humans. There have been limited studies on the epidemiology of *Campylobacter* in dairy cattle on the farm, which have described the prevalence of *Campylobacter* and identified some risk factors that affect *Campylobacter* prevalence on the farm. More extensive research is needed to describe the epidemiology of *Campylobacter* in cattle on the farm in greater detail, and to confirm the effects of previously reported risk factors and identify new risk factors associated with *Campylobacter* in cattle. This information will aid dairy producers in reducing the potential for contamination of meat and milk used for human consumption.

Hypotheses Tested

1. *Campylobacter* is prevalent in cattle on dairy farms.
2. The prevalence of *Campylobacter* on dairy farms varies by cattle age groups (calves and adult cows), and by season (hypothesis tested in Chapter 2 and 3).
3. Specific dairy herd management practices (including health management, feeding and housing, biosecurity, and sanitation) affect *Campylobacter* prevalence on dairy farms (hypothesis tested in Chapter 2 and 3).

Objectives

1. Identify patterns of occurrence of *Campylobacter* on dairy farms in Michigan, Minnesota, New York, and Wisconsin.
2. Determine whether there are differences in the prevalence of *Campylobacter* by cattle age group, location, and season. Identify specific risk factors that contribute to the prevalence of *Campylobacter* on dairy farms.

Overview

Chapter 1 is a literature review of *Campylobacter* in cattle and the role cattle play in human *Campylobacter* infection. Particular attention is paid to the risk factors that contribute to *Campylobacter* prevalence in cattle. Chapter 2 is a herd level analysis of risk factors for *Campylobacter* prevalence in cattle on Midwestern and Northeastern dairy farms in the United States. Chapter 3 is an individual animal level analysis of risk factors for the occurrence of *Campylobacter* in cattle on Midwestern and Northeastern dairy farms in the United States. The importance of both analysis approaches is discussed in the conclusion.

CHAPTER 1

A Review of Campylobacter spp. in Cattle and Its Importance in Human infection

1. Introduction

Campylobacter is considered the current leading cause of bacterial gastroenteritis in the United States (Altekruse et al, 1999). The usual consequences of *Campylobacter* infection are not serious and do not need medical intervention. It is only when campylobacterosis causes severe illness, sequela such as Guillian-Barré Syndrome, antimicrobial resistance, and in rare instances, death that this organism becomes an issue for the human population both medically and economically (McDowell and McElvaine, 1997; Wegener, 1999).

The sources of human infection are linked mainly to food animals. Poultry, cattle, pigs, and sheep are known to be reservoirs for *Campylobacter jejuni* and *Campylobacter coli*. These organisms do not usually cause disease for these animals but function as commensals (Blaser et al, 1983). Humans acquire *Campylobacter* infection from these animals in an indirect way. Preparation of contaminated meat is primarily the cause for humans to become infected. Either the contaminated meat is not fully cooked or cross-contamination occurs in the kitchen while preparing the contaminated meat (Skirrow, 1982). Poultry is known to be the most important source of infection for humans and is estimated to cause from 50 to 70% of all human infections (Allos, 2001). Whereas poultry remains the most important source of human infection, the importance of cattle in the transmission to humans should not be overlooked.

Campylobacter spp. have been reviewed in a number of papers. Altekruse et al (1998) summarize the epidemiology of *Campylobacter jejuni* in relation to its many reservoirs and its role in the human population. They reported that the greatest concern to humans is the contamination of food, mainly meat, with *Campylobacter jejuni*. Griffiths and Park (1990) describe Camylobacters and the association with human disease. They summarize the epidemiology, pathogenicity, and microbiology of Campylobacters. The main conclusion from this report is that human disease mainly occurs because of food contaminated with *Campylobacter*. To date, no review has been written on the *Campylobacter* in cattle and its importance in humans. None of these reviews or other reviews emphasized the possible areas where the research could be improved; therefore, the purpose of this paper is not only to give background information on *Campylobacter*, describe *Campylobacter* in cattle, and underline the important role of cattle in human campylobacteriosis, but also identify the gaps that exist in the previous literature in order to better direct future research.

2. **Background of Campylobacter**

Campylobacter was originally known as *Vibrio.* in the early part of the 20th century. It was first known to cause abortion in sheep. By the 1930s, this *Vibrio sp.* was known as the cause of jejunitis in cattle and then named *V. jejuni*. It wasn't until the 1960s that the classification of this organism changed from *Vibrio spp.* to *Campylobacter spp.* (Sebald and Véron, 1963). Even though the importance of this organism was described in many

animals previously, it was not until the 1970s that *Campylobacter spp.* was recognized as a human pathogen associated with acute diarrhea in humans (Skirrow, 1982). Today, it is known that *C. jejuni* is commonly isolated from cattle and most commonly the cause of human gastroenteritis (Blaser et al, 1983 and Altekruse, 1999).

Campylobacter spp. are gram-negative, spiral shaped rods. This group of organisms possess a single flagella or polar flagella that create a distinct cork screw movement (Griffiths et al, 1990). The environment that is best suited for this organism is one that is thermophilic and microaerophilic. The thermophilic and microaerophilic environment that best supports the survival of *Campylobacter spp.* is at 42°C and from 5-10% CO_2, respectively. The combination of these two environments allows this organism to be easily found in the gut of many mammals (Ketley, 1997). Another important characteristic of *Campylobacter spp.* is that this organism can transform from a viable rod to a non-culturable coccoid form when exposed to oxygen or temperatures higher than 42°C (Skirrow, 1994 and Hazeleger et al, 1998). Even though *Campylobacter spp.* can undergo this transformation in unfavorable conditions, it has a difficult time surviving in temperatures below 30°C and in dry or acidic environments (Altekruse et al, 1998). Finally, differentiation between *C. jejuni* and other *Campylobacter spp.* can be achieved by serotyping, resistance to cephalothin, pulse-field gel electrophoresis, randomly amplified polymorphic DNA, and hippurate hydrolysis. (Griffiths et al, 1990; Altekruse et al, 1999; Skirrow, 1994; Allos, 2001).

3. Pathology in Cattle

The pathogenesis of *Campylobacter* in cattle is not well understood (Luechtefeld and Lou Lang, 1982). It has been reported to cause a multitude of disease; however, *Campylobacter* organisms are frequently isolated from healthy cattle (Myers et al, 1984; Skirrow, 1994; Wesley et al, 2000). Cattle are known to be good reservoirs for *Campylobacter* because of the ability of this organism to establish itself as a commensal in the gastrointestinal tract without causing any apparent disease (Stanely et al, 1998; Stern, 1992; Warner et al, 1986). Although cattle are carriers of *Campylobacter*, it continues to be a potential causative agent of disease in cattle when conditions are favorable (Skirrow, 1994).

Most of the diseases linked to *Campylobacter* carriage are associated with the gastrointestinal tract. Experimental inoculations of cattle were shown to result in enteritis with blood and mucus present in the feces (Firehammer and Myers, 1981). In another study conducted by Al-Mashat and Taylor, *Campylobacter* was isolated from enteric lesions of cattle and calves (1980). Bacteremia was another anomaly observed in neonatal calves following infection with *Campylobacter* (Warner and Bryner, 1984). Yet, another gastrointestinal illness linked to *Campylobacter* in calves was acute colitis (Morgan et al, 1983; Terzolo et al, 1987).

Other diseases not associated with the gastrointestinal tract, but associated with reproduction, can also be linked to *Campylobacter* infection. One such disease is bovine abortion. Bovine abortion may occasionally be due to *C. jejuni* but is commonly due to *C. fetus* (Berg et al, 1971; Welsh, 1984; Van Donkersgoed et al, 1990, Skirrow, 1994).

Infectious infertility was another disease caused by *Campylobacter* in cattle that was first described by Plastridge et al (1947). *Vibrio fetus,* now called *C. fetus,* was the causative agent of this lowered conception rate in cows. This was evident from isolation of *C. fetus* in cows with infertility along with the evidence of serologically positive bulls. Another disease associated with *Campylobacter* is mastitis. Experimental studies have demonstrated that *Campylobacter* can cause clinical mastitis (Lander and Gill, 1980; Logan et al, 1982). According to Lander and Baskerville (1983), experimental infection of cows' udders with *Campylobacter* can produce a range of symptoms from no disease to severe clinical mastitis. There is very little evidence that nonexperimental clinical mastitis is caused by *Campylobacter.* In one report, (Logan et al, 1982), *Campylobacter* was isolated from the udder of an infected cow. In their report, Logan et al (1982) went on to explain the difficulty of isolating this organism from clinical mastitis cases.

Even though these diseases do not always occur or are not routinely apparent in cattle and calves that are colonized with *Campylobacter*, this organism is still considered an important pathological possibility. These bovine diseases caused by *Campylobacter* need to be further defined and understood, especially in regards to clinical mastitis. Many times these diseases are benign and not highly infectious in a herd, but they still warrant concern when the bovine population becomes a common carrier of *Campylobacter.*

4. Frequency in Cattle

Campylobacter is commonly isolated from the feces of cattle; therefore, cattle are

considered common intestinal carriers of this organism (Manser and Dalziel, 1985). Previous studies that reported on the prevalence of *Campylobacter* in cattle usually focused on *Campylobacter* carriage rates from healthy and sick adult cattle and/or healthy and sick calves. In addition to the health status and age of the cattle, many reports provided information on the species of *Campylobacter* that were isolated. The majority research found *C. jejuni* to be most prevalent in cattle. Other pertinent information included in these studies were type of cattle, herd information, number of samples collected, type of sample, and how the samples were processed. Even though this detailed information was provided, great variation in the reported carriage rate of *Campylobacter* in cattle exist.

The reported *Campylobacter* prevalence in healthy adult cattle, either beef or dairy cattle, ranged from 2.5% to 60% (Wesley et al, 2000; Giacoboni et al, 1993; Meanger and Marshall, 1989; Atabay and Corry, 1998; Humphrey and Beckett, 1987; Hoar et al, 2001; Manser and Dalziel, 1985; Munroe et al, 1983; Prescott and Bruin-Mosch, 1981; Waterman et al, 1984; Doyle and Roman, 1982). Some studies only reported the occurrence of *C. jejuni* (Doyle and Roman, 1982; Waterman et al, 1984; Prescott and Bruin-Mosch, 1981; Humphrey and Beckett, 1987; Munroe et al, 1983) whereas other studies reported the occurrence of all *Campylobacter spp.* (Wesley et al, 2000; Giacoboni et al, 1993; Meanger and Marshall, 1989; Atabay and Corry, 1998; Hoar et al, 2001; Manser and Dalziel, 1985). If the studies looked at all thermophilic *Campylobacter*, the major isolate identified was *C. jejuni.* The number of fecal samples and the number of herds in the study varied between studies and some studies were unclear as to how many herds were included (Waterman et al, 1984; Prescott and Bruin-

Mosch, 1981; Manser and Dalziel, 1985; Munroe et al, 1983). The largest number of herds enrolled in the 10 studies was 31 (Wesley et al, 2000) and the smallest number was only one herd (Doyle and Roman, 1982; Meanger and Marshall, 1989). All studies reported the total number of samples that were collected. Although all samples were fecal samples collected rectally, all studies had some variations in the *Campylobacter* isolation techniques. This may partially explain the differences in the prevalences between studies, along with the other apparent differences observed between studies.

Three of ten studies reported on the difference in *Campylobacter* prevalence between healthy and sick adult cattle (Manser and Dalziel, 1985; Munroe, 1983; Prescott, 1981). The sick cattle were considered sick because of the presence of diarrhea. The frequency of *Campylobacter* among these sick cattle in the three studies was from 1.5% to 26%. The frequency of *Campylobacter* found in the diarrheic cattle did not significantly differ from the frequency of *Campylobacter* found in the healthy animals. Among these studies sample numbers from diarrheic animals ranged from 198 (Manser and Dalziel, 1985) to 314 samples (Munroe, 1983). Sample numbers from healthy cattle ranged from 107 samples (Munroe, 1983) to 202 samples (Prescott, 1981). Two of the three studies only reported *C. jejuni* rates (Munroe, 1983; Prescott, 1981) and one study reported on all thermophilic *Campylobacter* (Manser and Dalziel, 1985). All fecal samples in these three studies were obtained from submissions to veterinary teaching hospitals or from submissions to diagnostic centers. Again, variation in isolation techniques occurred in the three studies.

Comparing seven studies that examined the prevalence in healthy calves, the reported *Campylobacter* prevalence ranged from 19% to 100% (Snodgrass et al, 1986;

Giacoboni et al, 1993; Busato et al, 1999; Atabay and Corry, 1998; Myers et al, 1984; Firehammer and Myers, 1981; Rycke et al, 1986). The number of rectal fecal samples in these studies ranged from 3 to 1521. The study that only sampled from 3 healthy calves found a prevalence of 100% (Firehammer and Myers, 1981) whereas the study that sampled 395 calves reported a prevalence of 42.9% (Busato et al, 1999). The number of samples may be another reason prevalence varies greatly. Two studies reported only *C. jejuni* rates (Firehammer and Myers, 1981; Rycke et al, 1986) and the other studies reported thermophilic *Campylobacter* rates but included the breakdown by *Campylobacter* species.

Of the seven studies that reported prevalence in calves, three of those studies (Rycke et al, 1986; Snodgrass et al, 1986; Firehammer and Myers, 1981) compared the prevalence of *Campylobacter* between healthy calves and sick calves. Sick calves were defined as sick due to enteric disease resulting in diarrhea. The prevalences of *Campylobacter* in those sick ranged from 19% to 50%. One study sampled for two years and reported the prevalence of *Campylobacter* from those two years separately (Firehammer and Myers, 1981). The least number of samples collected from sick calves in these three studies was 32 (Rycke et al, 1986) and the greatest number of samples was 156 (Snodgrass et al, 1986). Two of the studies statistically compared the prevalence of *Campylobacter* between healthy and sick calves and found no statistical difference between the two groups (Rycke et al, 1986; Snodgrass et al, 1986). One study did not examine the significance between the healthy and sick calves (Firehammer and Myers, 1981), probably due to the low number of samples collected from the healthy calves. The two groups were not comparable based on the sample size so no further conclusions

could be made in regards to differences between the groups. Also, two studies reported only *C. jejuni* rates (Firehammer and Myers, 1981; Rycke et al, 1986) while the remaining study reported the thermophilic *Campylobacter* prevalence along with giving the *C. jejuni* rate (Snodgrass et al, 1986).

In all the cited studies in cattle some *Campylobacter* was isolated from the feces of these animals, regardless of age. There was variation among all studies due to at least one of the following reasons: isolation technique, number of animals studied, the species of *Campylobacter* isolated, the health status of the animal, or the age of the animal. There were probably many other factors that could contribute to the variations including transportation of the samples to the laboratory, climate where the samples were collected, and size of the sample collected. Even though variation was observed between all studies, the information on the prevalence of *Campylobacter* in cattle is still useful for other researchers and for farmers.

A number of studies examined milk from cattle for the occurrence of *Campylobacter* (Waterman et al, 1984; Oosterom et al, 1982; Desmasures et al, 1997; Beumer et al, 1985; Beumer et al, 1988; Orr et al, 1995; Davidson et al, 1989; Doyle and Roman, 1982; Rohrbach et al, 1992). Milk was either collected from bulk tanks or directly from the individual cows. The majority of the studies reported only *C. jejuni* rates (Waterman et al, 1984; Oosterom et al, 1982; Beumer et al, 1985; Beumer et al, 1988; Orr et al, 1995; Davidson et al, 1989; Doyle and Roman, 1982; Rohrbach et al, 1992) whereas only one study did not report on the species of *Campylobacter* isolated (Desmasures et al, 1997). The prevalence of *Campylobacter* ranged from 0% to 95%. Most herds had a very low prevalence of *Campylobacter* between 0.9% and 3.2%. The

one study that found 95% prevalence was an investigational study that only looked at 19 raw milk samples (Orr et al, 1995). The number of total samples investigated ranged from 19 (Orr et al, 1995) to 1,501 (Waterman et al, 1984). It was very difficult to find *Campylobacter* in milk according to these studies. Again, these studies had different transport and culture techniques that could explain the variation in the prevalence. For the most part, the reported prevalences in milk were low. One study (Beumer, 1988) found that an enzyme found in milk, lactoperoxidase, was the reason for the low recovery rates in the samples. They experimentally inactivated this enzyme and found that the rate of recovery of *Campylobacter* was much greater. This may be the major reason for the difficulty in isolated *Campylobacter* from milk.

Only seven looked at potential risk factors associated with the prevalence of *Campylobacter* (Wesley, 2000; Busato, 1999; Hoar, 2001; Rohrbach, 1992; Meanger and Marshall 1989; Waterman et al, 1984; Humphrey and Beckett, 1987). Four of the seven studies examined many different risk factors that could contribute to the frequency of *Campylobacter* (Wesley, 2000; Busato, 1999; Hoar, 2001; Rohrbach, 1992).

Wesley and colleagues (2000) found broadcast spreaders for manure disposal, feed containing alfalfa, feed containing cottonseed or hulls, and nuisance birds to be significantly associated with the prevalence of *Campylobacter* on the herd-level. On the cow-level, they reported that large herds and cows fed brewer's by-products were significantly associated with the presence of *Campylobacter* in the feces of cows. They also found that lactating cows had significantly higher prevalence rates of *Campylobacter* than cull cows.

In a longitudinal study (Busato et al, 1999), age, open barns, feeding a dry matter that consisted of more than 50% grass or corn silage were found to be significantly associated with increased prevalence of *Campylobacter spp.* in beef cattle. On the other hand, the number of cows, crossbreed animals, antiparasitics, and feeding 50% dry matter other than silage was significantly associated with the decreased prevalence of *Campylobacter.*

In a cross-sectional study, Hoar et al (2001) used a multivariable model and found the number of female cattle present to be the only significant factor for the increased prevalence on the beef farms. The authors explained, however, that the number of female cattle on these farms was an indirect indicator of herd size.

In another cross-sectional study, Rohrbach and colleagues (1992) examined the associations between risk factors and *Campylobacter* prevalence. This study was different because it looked at risk factors associated with *Campylobacter* prevalence in bulk tank milk. They found no significant difference between any of the risk factors they examined and the prevalence of *Campylobacter* in milk despite the 12.3% prevalence found in the 292 milk samples collected. Some of the risk factors included were milking hygiene, grade classification of dairy, mean cow number, number of clinical mastitis cases, facilities for milking, or the percent of replacement stock on the farm.

The remaining three studies that looked at risk factors and the occurrence of *Campylobacter* did not include formal statistics to analyze the risk factors that were included on those studies (Meanger and Marshall, 1989; Waterman, 1984; Humphrey and Beckett, 1987). In one longitudinal study conducted by Meanger and Marshall (1989), they found that the highest prevalence rate of *Campylobacter* was in the autumn months.

Another study examined the risk of season in the association of *Campylobacter* occurrence in cattle and found that cattle excreted more *Campylobacter* in the winter than in the summer (Waterman, 1984). A third study found that herds exposed to river water were more likely to shed *Campylobacter* than herds that drank from mains water (Humphrey and Beckett, 1987). Without true statistical analysis only speculations can be made in regards to these potential risk factors and the prevalence of *Campylobacter* in cattle.

More than half of the studies that reported on the prevalence of *Campylobacter* in cattle or in milk did not report on the possible risk factors contributing to the prevalence rate. Prevalence, alone, only gives a partial picture of the occurrence of *Campylobacter* in cattle. More extensive research needs to be done to help answer the questions of where these isolates are originating from, why these isolates are persisting, and how farm management practices can contribute to the increased or decreased rates of *Campylobacter* found in cattle.

V. Significance of Cattle as sources of infection for humans.

Currently, the most common cause of bacterial diarrhea in most industrialized countries is *Campylobacter* (Tauxe, 1992; Skirrow, 1994). *Campylobacter jejuni* is the major source of *Campylobacter* enteritis in humans but other *Campylobacter* species such as *C. hyointestinalis, C. coli, C. lardis,* and *C. pylori* are known to be associated with human infection (Skirrow, 1994; Penner, 1988). The incubation period of *Campylobacter* enteritis is from one to seven days (Andrews, 1998). The majority of

those that experience this type of illness have symptoms such as diarrhea, fever, and severe abdominal pain. Some cases may even experience bloody diarrhea (Morris, 1996). By 24 to 48 hours after symptoms develop, the illness usually peaks and gradually resolves itself within one week (Blaser, 1997). Treatment is not usually needed, but if signs and symptoms persist or worsen antimicrobials may be prescribed (Altekruse, et al. 1999).

After the initial infection with *Campylobacter*, complications are known to occur but are infrequent. Reactive arthritis, Reiter's syndrome, pancreatitis, or Guillain-Barré Syndrome (GBS) are possible sequelae to the initial gastroenteritis. The most important of these sequelae is GBS. GBS affects approximately 1 out of every 1000 people infected with *Campylobacter*. The onset of GBS occurs from 10 days to 3 weeks after onset of diarrhea (Allos, 1997). GBS can range from mild demyelinating neuropathy to severe axonal neuropathy that leads to residual disability (Rees et al, 1995). The clinical presentation associated with this syndrome includes paralysis, pain, and wasting muscles (Ropper, 1992; Miller, 1985). Most patients recover from GBS, but approximately 20% will be left with some form of disability. Another 5% of GBS patients will not survive this disease (Altekruse, 1999). GBS and the other sequelae of *Campylobacter* infection are rare, but can cause significant hardship for those patients that experience such complications.

An additional concern associated with *Campylobacter* is antimicrobial resistance. *Campylobacter* resistance is reported to be on the rise. A marked resistance to fluoroquinolones along with a number of other antimicrobial agents has been reported (Aarestrup, 1999; Sanchez et al, 1994; Velazquez, 1995). There has also been

speculation as to why there is resistance and where it may be coming from. One possible answer is that the increased resistance is due to the use of antimicrobial agents in humans and in animals (Acar, 2001), however the problem is being focused on the use of antimicrobials in food animals (Nicholls, 2001). This issue remains undefined because of the limited research conducted in this area.

The initial step in combating the problem of *Campylobacter* disease in humans along with the problem of *Campylobacter* resistance is to identify the potential risk factors for *Campylobacter* infection. There are four commonly reported risk factors associated with human campylobacterosis. The greatest risk to humans is reported to be contact with contaminated food (Skirrow, 1994). Included in this category, is the consumption of raw or undercooked meat including poultry, the consumption of raw or inadequately pasteurized milk, and the cross-contamination from food items infected with *Campylobacter* (Adak, 1995; Hopkins, 1984; Peabody, 1997; Frost, 2001). Contact with animals is another potential risk factor for human illness (Blaser, 1980). This not only includes contact with pets but also contact with food animals. The risk of animal contact greatly increases if the animals are experiencing diarrhea (Tenkate, 2001; Saeed, 1993). A third risk factor is the consumption of untreated water (Vogt, 1982). A final major risk factor includes travel abroad (Rodrigues, 2000). Defining these risk factors helps to create guidelines to minimize potential problems for humans.

VI. Preventive Measures

Preventive measures need to be applied to eliminate or decrease the potential risks of human *Campylobacter* illness. Prevention must be placed into practice not only on the human level but also on the animal level (Altekruse, 1994). One place to begin prevention is the farm. Good livestock management practices must be put into effect in order to reduce or eliminate the spread of *Campylobacter* among farm animals (Altekruse, 1998a). One possible way to do so is by chlorinating the water supply provided for the food animals (Kapperud, 1993). Additional farm risk factors must first be defined before other farm management practices are put into effect.

Human behavior is another area that can be altered in order to control campylobacterosis. People must exercise good food safety technique. This includes separately preparing raw meat in an area apart from other foods, properly sanitizing hands and cookware before and after food preparation, and thoroughly cooking meat (CDC, 1998). Next, people that come in contact with animals should properly wash hands after contact especially when diarrheic animals are handled (Blaser, 1983). Unpasteurized milk should also be avoided (Skirrow, 1994). Finally, establishment of surveillance systems will also insure proper monitoring of the disease along with providing risk analysis and education geared toward prevention practices (Altekruse, 1998b).

VII. Discussion

Much of the literature and research has been focused on poultry and poultry meat as the major source of *Campylobacter* in humans. These reports compare prevalence of *Campylobacter* between poultry meat and beef and report that beef products are a minute problem. Only briefly does this literature mention the occurrence in live food animals (Harris et al, 1986, Kotloff, 1999; Peterson, 1994, Humphrey, 1995; Dawkins, 1984; Skirrow and Blaser, 1992). According to the articles reviewed in this paper, live cattle should be of concern to the human population because of the high prevalence rate in cattle. The literature does not focus much on cattle and beef products. This may be due to the limited number of studies conducted in this area. Much greater research has been conducted on poultry and poultry meat, which would influence many authors to report on such trends. Serotyping helps in finding the possible origin of human *Campylobacter* isolates. In one study by Nielson and colleagues (1997), both poultry and cattle were identified as major sources of human *Campylobacter* due to serotyping. Poultry are a very important source of human illness, but cattle are likely an underestimated source of human campylobacterosis. More research is needed to examine the role of cattle in human *Campylobacter* infection.

Along with the need to conduct further research on the association cattle play in human *Campylobacter* cases, better research is needed to more accurately identify the prevalence rate on the farms and also to better identify risk factors that contribute to the prevalence on the farm. In previously reviewed literature huge variations exist in the prevalence rates and risks factors of *Campylobacter* in cattle between each study. This is

due mainly to the isolation techniques and the number of herds or animals involved in the studies. Because *Campylobacter* is believed to be shed intermittently, larger and longer prospective studies need to be conducted to find truer prevalence rates. This, in turn, will help to find additional risk factors in cattle and on the farm. Better studies will provide a better basis for the potential risks to humans along with finding preventive measures on the farms. Only one study has really met these objectives. The prospective study by Wesley and colleagues (2000) was a large study that examined numerous risk factors associated with *Campylobacter* prevalence in cattle. This study found a herd prevalence rate of 80.6% (n = 31 herds) and a cow prevalence rate of 37.7% (n=2,085 cows). Wesley and colleagues also found that the use of broadcast feeders, feed, dietary supplements, and accessibility of feed to birds to be potential risks for the increased prevalence in dairy cattle. Other studies like this will help reinforce their findings and get to the root of the problem.

Chapter 2

Patterns of Occurrence of *Campylobacter* on Dairy Farms in the Midwestern and Northeastern United States: A Herd-Level Analysis

STRUCTURED ABSTRACT

OBJECTIVES: 1) Identify patterns of occurrence of *Campylobacter spp.* over time; 2) Investigate risk factors that contribute to prevalence of *Campylobacter spp.* on dairy farms.

DESIGN: Longitudinal

SAMPLE POPULATION: 25,155 cattle from 128 randomly selected dairy farms from Michigan, Minnesota, New York and Wisconsin. Herds were stratified by state and herd size. Cattle were sampled based on age and health/lactation status.

PROCEDURE: Management data and biological samples were collected from each herd bimonthly for 10 months, and *Campylobacter spp.* were isolated from these samples. Apparent period prevalences (APP) were computed, and multivariable Poisson regression was used to assess associations between management factors and herd-level APPs.

RESULTS: The overall APP of *Campylobacter spp.* was 12%, and over 97% were *C. jejuni*. Higher APPs were seen in calves versus adults. Sick adults had higher APPs than

healthy adults, while healthy calves had higher APPs than sick calves. APPs were highest in winter and lowest in summer. Factors associated with higher APPs included higher levels of calf diarrhea, the use of inorganic cattle bedding, and poor feed storage. Factors associated with lower APPs included housing lactating cows on dry lots, and washing calf housing.

CONCLUSIONS AND CLINICAL RELEVANCE: Management factors which increased risk of fecal contamination and exposure to infected animals were associated with higher APPs. Factors associated with decreased APPs were those that reduced animal stress, and reduced environmental survival of *Campylobacter spp.* Specific factors identified in this study can be used to develop programs to reduce *Campylobacter spp.* on farms.

INTRODUCTION

Campylobacter is the most frequently identified cause of foodborne bacterial gastroenteritis in the United States (Allos, 2001; Altekruse et al., 1999). Most cases are mild, self-limiting episodes of vomiting, cramping and diarrhea, but serious illness can occur in immune suppressed individuals (Tauxe et al., 1992; Acheson, 2001). Antimicrobial resistance has been recognized as an emerging global health issue (Neu, 1992; Moore et al., 2001), and drug resistance has been found in *Campylobacter* from humans (Allos, 2001; Moore et al., 2001). Long-term sequelae of *Campylobacter* infection, including arthritis and the neuropathic Guillian-Barré syndrome, have been identified (Rees et al., 1995; Mead et al., 1999). Foods of animal origin, including poultry (Smith et al., 1999; Harris et al., 1986) and raw milk (Lehner et al., 2000), have been associated with *Campylobacter* gastroenteritis. Dairy cattle are sources of foods (milk and meat) that have been recognized as sources of *Campylobacter* for consumers (Evans et al., 1996; Dilworth et al., 1988). Human infection can also occur through contact with contaminated farm environments, ground water, and other farm animals (Piddock et al., 2000).

Since *Campylobacter* can colonize the gastrointestinal tracts of mammals and birds without causing disease (Manser and Dalziel, 1985), these animals can serve as reservoirs of *Campylobacter* (Wesley et al., 2000). Cattle, poultry, swine, and sheep are known to be intestinal carriers of *Campylobacter spp* (Blaser et al., 1983; Harvey et al., 1989; Engvall et al., 1986; Penner and Hennessy, 1980). To date, only a limited number of studies have been reported on *Campylobacter* in cattle. From these studies, reported

Campylobacter prevalence rates in cattle ranged from 5% (Oosterom, 1982) to 65% (Atabay and Corry, 1998; Giacoboni et al., 1993), with most prevalence rates around 20% (Manser and Dalziel, 1985; Beumer et al., Humphrey and Beckett, 1987).

Several factors have been associated with increased *Campylobacter* prevalence in food animals, including animal age, health status, season, and environmental contamination. Unfortunately, there have been very few studies (Wesley et al., 2000) looking at dairy cattle management practices and their associations with herd *Campylobacter* levels.

The relationship between *Campylobacter* occurrence and host age is not clear. Chickens younger than two weeks were not colonized with *Campylobacter*, but the rate of infection increased as the age of the flock increased (Jacobs-Reitsma et al., 1995). However, the prevalence of *C. jejuni* was greater in calves than in adult cows (Giacoboni et al., 1993), and the rate of *Campylobacter* prevalence decreased as pigs aged (Weijtens et al., 1993). In mammals, it appears that the *Campylobacter* shedding decreases with age, but further research is needed to confirm this.

It is possible that associations between animal health and increased isolation of *Campylobacter* may be due to other conditions that result in reduced immune response in an animal, which could lead to increased *Campylobacter* burdens and subsequent shedding. One study found that pigs with diarrhea had a higher isolation rate of *C. coli* than healthy pigs (Nielsen et al., 1997). However, other studies have found no differences in the isolation rate between healthy and sick calves (Rycke et al., 1986; Snodgrass et al., 1986), and healthy and sick cattle (Manser and Dalziel, 1985).

Additional work is needed to determine the influence of animal health on the prevalence of *Campylobacter* shedding.

Seasonal patterns in *Campylobacter* prevalence have been observed. Higher rates of *Campylobacter* in cattle have been reported for winter (Waterman et al., 1984), and spring and autumn (Stanley et al., 1998). Another study found that *Campylobacter* prevalence was highest in autumn and lowest in winter (Meanger and Marshall, 1989). In poultry, *Campylobacter spp.* were shown to occur at higher rates in May and October (Atanassova and Ring, 1999), and in autumn (Kapperud et al., 1993). However, no seasonal trends were seen in the rate of colonization of *Campylobacter spp.* in broiler chickens (Gregory et al., 1997), or recovery rates for *C. jejuni* from dairy cattle in the U.S (Wesley et al., 2000).

Since human infection has been linked with contact with contaminated farm environments, ground water, and infected animals (Piddock et al., 2000), these sources could also be sources of *Campylobacter* for cattle. One study suggests that the high rates of *C. jejuni* in cattle may be due to feed and water contamination, and found increasing recovery of *C. jejuni* on farms that used broadcast spreaders for manure disposal (Wesley et al., 2000). Unfortunately, *Campylobacter* are difficult to detect in environmental samples due to the effects of temperature and desiccation (Hoar et al., 1999; Waage et al., 1999), and other competitive microflora on samples (Waage et al., 1999). Consequently, the effects of environmental sources of *Campylobacter* on the prevalence in cattle remain unclear.

The studies conducted on the prevalence of *Campylobacter spp.* in food animals suggest that there are multiple factors that contribute to the frequency of isolation in these

animals. Differences seen in results from these studies may be due to low numbers of herds or animals or both, and the cross-sectional nature of these studies. The purpose of this study was to use a longitudinal study design to determine the prevalence of *Campylobacter* on Midwestern and Northeastern dairy farms, and major risk factors associated with prevalence. The specific objectives of this study were to: 1) identify patterns of occurrence of *Campylobacter spp.* over time; and 2) investigate specific risk factors that contribute to prevalence of *Campylobacter spp.* on dairy farms.

MATERIALS AND METHODS

Study design -This study is part of a larger study, with the long-term goals to describe the ecology of *Campylobacter* on Midwestern and Northeastern dairy farms, and to understand the dynamics of shedding of these bacteria.

A longitudinal approach was used to collect specimens and corresponding data relating to potential risk factors. Data collection and sampling occurred bimonthly over a 10-month period, resulting in 5 to 6 data collection points over one year.

Study population -Dairy herds from Michigan, Minnesota, New York, and Wisconsin were recruited for the study. Each farm needed to have at least 30 milking cows, provide good records, and allow samples to be collected randomly from the animals on the farm and from specific areas of the farm. A pool of farms was identified based on travel time and distance to research facilities within each state, stratified by herd size (Table 1), and farms were then randomly selected and recruited for participation in the study.

Animals in each herd were classified into different age/status classes, including pre-weaned calves; healthy lactating cows; cull cows (identified by the producer as selected to leave the herd within 7 days, regardless of reason); periparturient (within 14 days of calving) cows; and sick cows (as reported by the producer). The number of animals to sample within a herd differed in each animal class: up to 15 calves, up to 5 cull cows, up to 10 periparturient cows, and up to 5 sick cows were sampled. The number of healthy lactating cows to sample was dependent on herd size: 20 from herds with 30-49 cows, 25 from herds with 50-99 cows, and 30 from herds with 100 or more milking cows.

Sample size -A sample size of 128 dairy herds was established for the study, based on the ability to evaluate herd level prevalence rates of *Campylobacter*. Collection of 50 samples in large herds (200 cattle or more) would provide 95% confidence of detecting at least one positive animal per visit if the within-herd prevalence is $\geq$ 5%, which should allow sensitivity of sampling given reported prevalence rates vary from 5% to 37% in individual dairy cattle (Oosterom et al., 1982; Hoar et al., 2001).

Data collection -Data were collected using initial and bimonthly pre-tested questionnaires administered in person. On the initial questionnaires, data collectors asked detailed questions about herd management practices, herd inventory, animal housing, feed, water systems, production, milk quality, cattle health, manure management, and antimicrobial use on the farm being studied. On the bimonthly questionnaire, data collectors asked questions about any changes in the herd management practices, herd inventory, and antimicrobial use that may have occurred after the previous sampling visit.

An 'other animal' index was developed as a measure of the presence of other animals on the farm that can carry *Campylobacter:*

$$Index_H = \sum Swine_H + Poultry_H + Geese_H$$

where:

$Swine_H$ = 1 if swine present on the farm, 0 if no swine present

$Poultry_H$ = 1 if chickens, ducks, turkeys or other poultry present on farm, 0 if not present

$Geese_H$ = 1 if wild geese present on farm, 0 if not present

Values for the index ranged from 0 to 3.

Sample collection - All samples collected were identified by farm code, sample date, sample container number and type of sample (animal identification or type of environmental sample). Cattle were systematically selected for sample collection, and approximately 5 grams of feces were collected per rectum and placed in sterile Whirl-pak® containers. Approximately 30 ml of milk were collected from the bulk tank, and the milk filter was collected after the morning milking on the sampling day and stored in a plastic bag for shipping. Sterile cotton swabs of each environmental sample were taken from several locations: floors in the sick and/or calving pens; calf housing, hides of cull cows, feed alley, lagoon sludge or manure pile, and any bird droppings in the areas where cattle may have come into contact. Swabs were saturated with sterile skim milk for transport, and placed in a Whirl-pak® for shipment. A 100 ml sample of water from a cattle watering tank was collected in a sterile specimen cup. All samples were shipped in a Styrofoam cooler, packed with ice, and sent to a central laboratory at Michigan State

University. The samples were shipped within 24 hours of collection and processed immediately upon arrival at the laboratory.

Sample processing -Fecal and bulk tank milk samples were prepared by the addition of approximately 30 ml of phosphate buffered saline solution. Milk filters and environmental samples were prepared by enrichment with 30 ml of Bolton broth (Oxoid) supplemented with 5% laked horse blood and antimicrobial agents (20 mg/l cefoperazone, 20 mg/l vancomycin, 20 mg/l trimethoprim, 50 mg/l cycloheximide), and incubated at 42°C in 5-10% CO_2 for 48 h. After preparation or enrichment, samples were then directly plated onto selective *Campylobacter* Blaser agar with Supplement B (BD Bioscience), streaked for isolation, and incubated at 42°C in 5-10% CO_2 for 48 h. If growth was observed after 2 days, the isolate was subcultured onto a sheep blood agar (SBA) plate and incubated at 42°C in 5-10% CO_2 for 48 h. Gram staining, oxidase testing (BD Bioscience), and hippurate (Remel) testing were performed. Motility testing was performed by inoculating Mueller Hinton Broth with a heavy inoculum of the suspect *Campylobacter*, incubating for 48 h at 42°C in 5-10% CO_2 , and examining the suspension under bright field microscopy for characteristic darting motility. Isolates that were gram negative rods with spiral shaped morphology, demonstrated darting motility, and were oxidase positive were classified as *Campylobacter spp.* Hippurate testing was performed to distinguish hippurase positive isolates as *C. jejuni* while hippurase negative isolates were classified as non-*jejuni Campylobacter spp.* If the gram-stain, oxidase test, or motility test were not indicative of *Campylobacter*, the sample was recorded as negative.

STATISTICAL ANALYSIS

Calculation of apparent period prevalence -Because animals from each farm were tested every other month for a period of ten months and results were from a sample of cattle population in the four states, apparent period prevalence was computed. A positive animal was defined as an animal from which *Campylobacter* was isolated from any sample during the 10-month period. Because different numbers of animals were tested from each herd within a herd size category, weighted prevalence was computed, using a previously reported method (Kaneene and Hurd, 1990). In order to describe the patterns of this infection on dairy farms, apparent period prevalence was calculated in three ways; one for the whole herd (cows and calves), one for cows only, and one for calves only. The general formula used to calculate the apparent period prevalence (APP) in each animal category was:

$$APP = \frac{Positive\ Animals_{GH}}{Number\ of\ cattle\ tested\ in\ study\ period_{GH}} \times 100.0$$

where $Positive\ Animals_{G}$ was the number of *Campylobacter*-positive animals in group *G* (whole herd, cows, or calves) tested from herd *H*, and $Number\ of\ cattle\ tested\ in\ study\ period_{G}$ was the total number of cattle in group *G* from herd *H*.

Analysis of risk factors -Associations between the prevalence of *Campylobacter spp.* and herd size, animal health status and age, location, season, and other possible on-farm risk factors were tested using the non-parametric Wilcoxon rank-sum test for association.

Risk factors which were associated with *Campylobacter* prevalence at $p \leq 0.2$ were considered for further analysis.

Multivariable analysis of risk factors was then conducted. Three separate models were developed; one for the cow population, one for calf population, and one for both cows and calves (herd model), since cows and calves are managed differently on dairy farms, and the apparent period prevalence of *Campylobacter* was found to differ between cows and calves during preliminary analysis of the data. It was hoped that this approach would provide specific information that could be used in reducing the risk of this infection in each age group.

Since the APP followed a Poisson distribution, multivariable Poisson regression modeling (SAS PROC LOGISTIC) was used to identify the major risk factors associated with the APP for each animal age class category. Herd size and state were included in the multivariable model to control for confounding, as both were confounders of several of the risk factors in the analysis. A backward elimination procedure was used to find the best fitting model in each case: if removal of a potential confounder resulted in a 10% or more change in the odds ratios of the remaining risk factors of interest, the variable was retained in the model to control for confounding.

RESULTS

Study population -A total of 128 dairy herds from Michigan, Minnesota, New York, and Wisconsin were enrolled in the study (Table 1). The average number of milking cows per herd in Michigan, Minnesota, New York, and Wisconsin were 217, 169, 198,

and 177, respectively. A total of 25,155 samples from dairy cattle were studied, of which 19,727 (80.3%) were healthy lactating cows.

Apparent period prevalence -The overall APP of *Campylobacter* was 12% from all animals sampled (Table 2). The majority of the herds had prevalence values ranging from 5% to 15% (Figure 1). There were significant associations ($p \leq .0001$) between season and *Campylobacter* APP: winter was found to have higher APP, while the summer had lower *Campylobacter* APP (Table 2). Calves had greater *Campylobacter* APPs than adult cows ($p \leq .0001$) (Table 2). Sick cows had significantly higher levels of *Campylobacter* than healthy adult cows ($p = .0021$) (Table 2), but healthy calves had greater APPs than sick calves ($p \leq .0001$) (Table 2). The APP of *Campylobacter* from milk samples was 2.0%, with higher levels of *Campylobacter* in milk filters compared to bulk tank milk samples (Table 3).

The APP of *Campylobacter* from environmental samples was very low, with an overall prevalence of 1.3% (Table 3). The highest rates of isolation were from cull cow hide swabs (2.5%), while the lowest rates were from swabs of the feed alley (.5%) (Table 3). A significant difference was observed between the APPs of *Campylobacter* in animal samples (12.0%) and environmental samples (1.3%) ($p \leq 0.0001$).

Identification of *C. jejuni* – Based on hippurate testing, almost all *Campylobacter* isolates identified in this study were *C. jejuni*: 97.8% from animal samples (2,950 of 3,016 isolates) and 97.1% of environmental samples (68 of 70 isolates) were identified as *C. jejuni.*

Multivariable analysis of risk factors -From 55 risk factors available, a total of 28 risk factors met the criteria for inclusion in the multivariable analyses (Table 4). Data from one herd was omitted from analysis due to missing data on herd milk production.

Herd animal model -For the herd model (including both cows and calves), risk factors associated with increased *Campylobacter* APP included not protecting feed from wild birds or rodents, increased levels of calf scours within 60 days prior to the beginning of the study, group-housing calves, the use of inorganic bedding for lactating cows, using a bucket loader for feed, availability of sick animal housing, and higher percentages of the milking herd coming from off-farm sources (Table 5). Risk factors associated with reduced levels of *Campylobacter* included the use of dry lot housing for lactating cows, and washing calf housing.

Cow population model -For healthy lactating cows, risk factors associated with increased odds for *Campylobacter* included not protecting feed from wild birds or rodents, increased levels of calf scours, group-housing calves, the use of inorganic bedding for lactating cows, using a bucket loader for feed, availability of sick animal housing, higher percentages of the milking herd coming from off-farm sources, and if a manure pack was used for manure disposal (Table 6). Risk factors associated with the reduced odds for *Campylobacter* included the use of dry lot housing for lactating cows, pasture access for cattle, washing calf housing, washing feed loader buckets between uses, and the use of combined sick/maternity cattle housing.

Calf population model -In the model for pre-weaned calves, factors associated with increased *Campylobacter* prevalence included increasing adult herd APP, increased levels of calf scours, group housing of lactating cows, pasture access, the use of inorganic

bedding for calves, and high bacterial counts in bulk tank milk (Table 7). Risk factors associated with the reduced *Campylobacter* prevalence in calves included cleaning milk buckets between feedings, lactating cow access to dry lots, use of manure packs, and possible contact with other animals on the farm.

Comparison of cow and calf population models -Risk factors that were retained in both the cow and calf population models were increased levels of calf scours within 6 months prior to beginning of the study, cattle access to pasture, lactating cow access to dry lots, use of manure pack, and the use of inorganic bedding (Table 8). Several risk factors had similar affects on the APPS in both the cow and calf models. The use of inorganic bedding and increasing levels of calf scours were associated with increasing odds in both models, while keeping lactating cows on a dry lot was associated with reduced prevalence in both models. However, there were risk factors that showed differing effects between the models. Herd pasture access decreased the odds of cow prevalence, but was associated to increased risk for calves, while the use of manure packs was associated with increased risk for cows, but decreasing risk for calves.

DISCUSSION

Study population -We were able to recruit 128 herds for the study, and were able to collect over 25,000 samples for bacterial isolation. While the initial target for herd enrollment was to enroll equal numbers of herds in each size classification, there were very few small herds available for enrollment in the study. Despite this limitation, the number of herds that were enrolled and the number of samples collected were sufficient for the purposes of this study.

Apparent Period Prevalence -The overall herd APP reported in this study was 12%, which was somewhat lower than the prevalence reported for other studies of *Campylobacter* in cattle of 20% (Manser and Dalziel, 1985; Beumer et al, 1988; Humphrey and Beckett, 1987). Factors that may account for differences between our current work and previous authors include: study design (longitudinal in this study versus cross-sectional in other studies), number of cattle sampled (25,155 cattle samples versus 94 (Giacoboni et al., 1993) and 904 (Beumer et al., 1988)), and measure of prevalence (period prevalence versus point prevalence).

In this study, *Campylobacter* prevalence was significantly associated with season. Winter had the highest *Campylobacter* prevalence (15.5%), which agrees with results from other studies (Waterman et al., 1984). Other studies have reported peaks in *Campylobacter* incidence in fall (Meanger and Marshall, 1989; Stanley et al., 1998) and spring (Stanley et al., 1996). Samples collected in the summer had the lowest seasonal prevalence in this study (6.7%), which was also observed by other investigators (Waterman et al., 1984). These differences in the effect of season on *Campylobacter*

prevalence could be due to cattle densities being increased in the winter months when cattle are often confined indoors during inclement weather in the upper Midwestern and Northeastern United States. The other studies were conducted in New Zealand (Meanger and Marshall, 1989) and the United Kingdom (Stanley et al., 1996), which have climates that may not necessitate protecting cattle from harsh weather.

Calves in this study had a significantly higher *Campylobacter* prevalence (14.4%) than adult cows (11.4%), which has also been reported in the literature (Giacoboni et al., 1993). This may be due to the naïve immune systems to *Campylobacter* in calves. As cattle age, they have more opportunities to become exposed to *Campylobacter*, develop immunity from these exposures over time, and become less likely to shed the organism.

When comparing *Campylobacter* APP by animal health status, differences were seen between sick and healthy cattle according to age. Sick adult cows were found to have an APP of 17.9%, while healthy adults were found to have an 11.3% APP ($p < .0001$). Conversely, healthy calves had an APP of 14.6%, and sick calves had APP of 12.0%, which did not statistically differ. These findings differ from reports in the literature, which found no differences in prevalence based on health status (Manser and Dalziel, 1985; Rycke et al., 1986; Snodgrass et al., 1986).

A fundamental reason for this difference in findings with prior research may be in how 'illness' was defined in the study. In several studies, illness was limited to enteric disease (Blaser et al., 1983; Rycke et al.). 1986In the current study, diarrhea was the most commonly reported illness in calves, which would suggest that results of this study should be similar to the other studies. One explanation for the differences in calf APPs may be that the pathogens responsible for diarrhea in calves (e.g., *E. coli, Salmonella,*

Coccidia) out-compete *Campylobacter* in the intestines, making it much more difficult to detect *Campylobacter* in fecal specimens from sick calves. In adult cattle, illness was defined by diagnoses which could include diarrhea, metritis, respiratory illnesses, ketosis, displaced abomasum, milk fever, and peritonitis. These illnesses may suppress the animal's immune system, which may allow *Campylobacter* to flourish, and when levels in the gut become high enough, become detectable in fecal specimens.

Campylobacter from milk filters and bulk tank milk samples were isolated in this study. There have been reports of *Campylobacter* isolated from milk filters, especially in food-related disease outbreak situations (Robinson and Jones, 1981). Farms that had *Campylobacter* isolated from milk filters had higher levels of *Campylobacter*, with a mean APP of 17% on these farms. This may reflect less hygienic procedures during milking, and levels fecal contamination in the milking parlor.

Although the APP of *Campylobacter* from environmental samples was low (1.3%) compared to animal samples (12%), we were able to isolate the organism from a variety of environmental sources. The highest APP from environmental samples was from cull cow hide swabs: these swabs were taken from the flank and rump of culled animals, which are areas on cattle where fecal contamination would be likely. This is a significant finding since *Campylobacter* on cull cattle hides may contaminate beef during the slaughtering process.

In contrast, swabs from the manure lagoon or manure pile did not yield higher levels of *Campylobacter* than other environmental samples. These swabs were taken at the edge of the manure lagoon or the surface of the manure pile, and any *Campylobacter* in

these areas would have been exposed to sunlight and open air, which would decrease their likelihood of survival.

Campylobacter jejuni was the most frequently isolated species in this study. In cattle, *C. jejuni* has been reported as the most common *Campylobacter* isolated (Nielsen et al., 1997), and the most common species of *Campylobacter* associated with human enteric disease is *C. jejuni* (Smith et al., 1999).

Risk factors associated with the APP –When examining risk factors associated with the prevalence of *Campylobacter* on dairy farms, it is helpful to view them in an epidemiological context, as being measures of host (cattle), agent (*Campylobacter spp.*), or environmental factors.

The Herd Model

Host-Related Risk Factors –In general, host-related factors are those that are indicative of cattle health and susceptibility to colonization and shedding of *Campylobacter* due to illness or age. Factors associated with increasing risk of *Campylobacter* identified in the herd model included increased levels of calf scours within 60 days prior to beginning of the study, the availability of sick animal housing on the farm, group housing of calves, the percent of the herd not raised on the farm, and housing of lactating cows in dry lots.

Reported high levels of scouring calves may be indicative of higher levels of pathogens on the farm. Since the immune systems of calves are not as well developed as those of adult cattle, calves are more likely to shed *Campylobacter* than adults. Farm maintenance of separate sick animal housing may be an indirect indicator of herd health status. Maintenance of separate facilities for sick animals requires an investment on the

part of the dairy operation: space must be made available, pens maintained and cleaned, etc. Consequently, farms that make the investment in sick animal facilities may have a relatively constant level of sick animals that require segregation from the healthy milking herd.

Stress is a known cause of decreased immune function in cattle (Jones, et al., 1999), and several risk factors identified in this study are associated with sources of stress in cattle. Group housing can be a source of competitive stress for calves, which would result in increasing *Campylobacter* shedding. The increasing percentage of the milking herd coming from off-farm sources is another area in which stress can influence the APP. The stresses associated with transporting cattle have been associated with increased bacterial shedding (CEAH, 1995),which may increase the number of cows actively shedding at the time of sampling, and provide more opportunities for spread of *Campylobacter* through fecal contamination by increasing the quantities of the organism in the fecal material.

Dry lot housing for cows, a form of group housing, was associated with decreased levels of *Campylobacter*. Dry lot housing for adults allows animals a lower density of housing and may be less stressful than confining cattle to indoor housing, which can result in lower APP in cows on dry lots. Group housing adults would allow direct contact between cows, which would provide exposure to the entire population to any bacteria carried by a single animal. Over time, the entire group would develop herd-wide immunity to these bacteria, resulting in overall resistance to infection, which would decrease the likelihood for *Campylobacter* shedding.

Agent-Related Risk Factors –Risk factors associated with the agent, specifically *Campylobacter*, are those which influence the quality or quantity or both of the agent present and available for exposure to hosts. In the herd level model, the increasing percentage of the milking herd coming from off-farm sources was associated with increased *Campylobacter* prevalence. Cattle coming from off-farm sources may be naïve to the flora endemic on a farm or these introduced animals may bring new bacteria, including *Campylobacter,* which may spread quickly within members of a herd.

Environment-Related Risk Factors –Epidemiological environment-related risk factors are those associated with the conditions in which the hosts become exposed to the agent, and factors that affect the agent's ability to survive outside the host during transmission. Host exposure can occur in one of two general ways: either direct exposure of uninfected hosts to infected hosts, or through indirect exposure of uninfected hosts through contaminated feed, water, or housing. One risk factor for direct exposure was group housing calves (increasing risk). Animals that are housed together have direct contact with each other, and are more likely to spread disease within the group, which would explain why group housing calves increases the APP of *Campylobacter.*

Risk factors for indirect exposure which were associated with increased risk of *Campylobacter* were feed storage which protects from moisture but not from pests, using inorganic bedding for lactating cows, and using a bucket loader for feed. Risk factors for indirect exposure of animals to *Campylobacter* which were associated with decreased odds of prevalence were washing calf housing and lactating cow access to dry lots.

Potential exposure to *Campylobacter* through contamination of feed was found in this study. Not protecting stored feed from rodents, wild birds, and other pests was

associated with increasing prevalence, which agrees with one study which found that pest access to feed stores could be responsible for the persistence of *Campylobacter* (Wesley et al., 2000). In another study, the presence of rats on the farm was an indicator of increased *Campylobacter* in broiler flocks (Kapperud et al., 1993). In another study of lambs and beef cattle, temporal trends in antimicrobial resistance patterns of *C. jejuni* coincided with peaks in bird activity in farm outbuildings: high levels of metronidazole resistance were seen in isolates from starlings and gulls, and peaks in metronidazole resistance in beef calves occurred when these birds were present in large numbers on the farm (Stanley and Jones, 1998), indicating that wild birds and other pests may be sources of *Campylobacter* for livestock.

In addition to risks of feed contamination by pests, using a loader for handling feed was associated with increase risks for *Campylobacter.* Farms that used a loader for moving feed often used the loader for moving manure, which would provide opportunity for the loader bucket to become a vehicle for contamination of *Campylobacter.*

Conditions that reduced the survivability of *Campylobacter* in the environment were associated with reduced prevalences in this study. In a dry lot, any environmental contamination by fecal material would be exposed to sunlight, wind, and other environmental factors that would reduce the survival of *Campylobacter* outside the host, and consequently reduce the risk of a cow being infected through fecal contamination of the environment. Also, there may be other factors associated with dry lot housing that more directly decrease *Campylobacter* prevalence, but which were outside the scope of this study.

The hygiene of cattle housing was associated with the APP of *Campylobacter* in this study. Herds that used inorganic bedding for lactating cows showed an increased risk for *Campylobacter*. In this study, inorganic bedding was defined as any inorganic material used for bedding such as sand, rubber tires or mats, mattresses, crushed limestone, etc. While not significant in the analysis, these materials were washed or changed much less frequently than organic bedding, which would provide conditions in which fecal contamination of cattle housing would pose a greater risk for exposure to *Campylobacter*. Washing calf housing will remove manure and any contaminated material in the calf environment, which will decrease the chance of exposure to *Campylobacter* and other enteric bacteria. High levels of calf diarrhea may also be an indicator of poor farm hygiene. Infectious scours is difficult to contain, but basic cleaning and sanitizing may help control and possibly prevent such outbreaks (Etgen et al., 1987). High levels of scours would provide opportunities for spread of fecal contamination and subsequent increases in levels of *Campylobacter* on the farm.

The Cow Population Model –Since over 80% of the samples evaluated in the Herd model were from the cow population, there is considerable overlap in the results of the two models.

Host-related Risk Factors –The use of shared maternity and sick cattle housing facilities was associated with decreased APP of *Campylobacter*, which is in opposition to the association between any sick cattle housing and increasing *Campylobacter* risk. The reasons for the differences between these associations are not clear. The use of combined

sick and maternity facilities may reflect overall lower level of disease of a farm if separate sick animal facilities do not need to be maintained.

The use of pastures for cattle was also associated with decreased risk of *Campylobacter*. As described for the use of dry lot housing for milking cattle in the herd model, pasture access reduces animal density and may decrease animal stress, while still providing opportunity for improved herd immunity.

Environment-related Risk Factors –In the cow model, risk factors associated with *Campylobacter* prevalence were the washing of loader buckets (decreasing risk), herd pasture access (decreasing risk), and the use of manure packs for manure storage (increasing risk). As described in the herd model, use of a loader for feed and manure handling may increase the risk of fecal contamination of feed. In this scenario, the washing of loader buckets would then remove fecal materials and any microorganisms present, and therefore reduce the risk of feed contamination with *Campylobacter* or other bacteria. When evaluating the decreased risk of *Campylobacter* associated with pasture use, housing cattle outdoors would decrease the survival of *Campylobacter* outside the host, due to the effects of sunlight and desiccation in this open environment. On the other hand, storing manure in manure packs increased risk for *Campylobacter* in this study. Manure packs were defined as piles of manure stored inside barns, which would be protected from sunlight, desiccation, and severe temperature changes. These piles would constitute a risk for exposure to any cattle coming in contact with the manure pile, and result in increased *Campylobacter* prevalence.

The Calf Population Model –

Host-related Risk Factors – The animal index was developed to measure the presence of other species known to be intestinal carriers of *Campylobacter* on the farm, including swine, chickens, turkeys, and other poultry (Harvey et al., 1999; Engvall, et al., 1986; Penner and Hennessey, 1980). Since these animals commonly harbor *Campylobacter,* it was expected that *Campylobacter* prevalence would increase if these animals were also kept on the farm premises, but this effect was not seen. It is difficult to determine why the presence of these animals was associated with reduced *Campylobacter* prevalence. It is possible that the index, generated from whether these species were present or absent on the farm, was not sufficiently sensitive to the risk that these other species may have posed to the cattle herd. A more likely explanation of this finding is that the presence of various other livestock species on a dairy farm did not measure dairy cattle exposure to these other potential reservoirs of *Campylobacter.* Raising other species may also be associated with other styles of overall farm management that function to reduce the prevalence *Campylobacter.*

Agent-related Risk Factors – The adult cow APP and high bacteria counts in bulk tank milk were associated with increased APP of *Campylobacter* in calves. Increased levels of *Campylobacter* in adult cattle, which make up the majority of animals on the farm, will result in increasing levels of the bacteria available for exposure to calves. If milk with high bacterial counts is fed to calves, this provides a direct source of infection for calves through the consumption of *Campylobacter* and other organisms in milk. *Campylobacter* was isolated from both milk filters and bulk tank milk in this study (Table 3), making this scenario highly likely. The association between feeding waste milk and

Campylobacter prevalence was assessed in this study, but was not found to be significant. These high bacteria counts may be an indicator of the milking and general hygiene practiced on a particular farm.

Environment-related Risk Factors –There were several environment-related risk factors present in the Calf Population model that were not retained in the Herd or Cow Population models. Risk factors associated with increased *Campylobacter* included herd access to pasture, housing calves on inorganic bedding, and keeping lactating cows in group housing. Risk factors associated with reduced *Campylobacter* prevalence were washing calf milk buckets and use of a manure pack. Almost all farms that used manure packs (95%) kept cattle in dry lots, a reduced density group housing situation. It is likely that the use of manure packs is another indicator of low density housing in the adult cattle, and does not directly protect calves from exposure to *Campylobacter*.

Herd access to pasture was associated with increased APP of *Campylobacter* in the Calf Population model, but was associated with reduced APP in the Cow Population model. The rationale for the differences in the effect of this risk factor on the two populations is not clear, but may be similar to factors which contribute to the development of herd immunity in adults may actually increase expression of disease in calves.

There were several risk factors present in the Calf Population model, which captured information on calf exposure to *Campylobacter* through fecal contamination. As described in the Cow Population model, the effect of using inorganic bedding for calves probably increases risks for *Campylobacter* exposure due to increased fecal contamination since the bedding is changed or washed less frequently than organic

bedding. Group housing of lactating cows was associated with increase prevalence in calves, presumably by increasing the adult herd reservoir of the organism. Conversely, a group housing situation with decreased density of adult cattle and lowered stress, such as in dry lots, was associated with reduced prevalence in calves. Finally, washing calf milk buckets between feedings would reduce the chance of spreading infection through milk that may become contaminated from environmental sources while in the bucket.

Comparison of Cow and Calf Population Models – Risk factors present and consistent in effect both models were the levels of scouring calves in the 60 days prior to the beginning of the study, the use of inorganic bedding, and keeping lactating cows in dry lot housing. While the strength of association for the level of scouring calves was not large (odds ratios ranging from 1.02 to 1.04), the consistency of the strength of the association indicates that this is an important component of *Campylobacter* prevalence on the farm. Levels of calf diarrhea are probably not specifically a cause of increased *Campylobacter* prevalence, but can be used by farm managers as an indicator of increased herd risk for *Campylobacter* due to poor hygiene practices.

Two risk factors were present in the Cow Population and Calf Population models, but had differing effects on the *Campylobacter* APP. Herd access to pasture increased the odds of *Campylobacter* prevalence in the Calf Population model, but decreased the odds of *Campylobacter* prevalence in the Cow Population model, while the use of manure packs on the farm was associated with decreased prevalence in the Calf Population model and increased prevalence in the Cow Population model. As described above, the differences seen in the effects in the two models may be a reflection of the difference in the immune status of calves and cows. Calves and cows are managed very

differently on the farm, so the effects of risk factors may function differently in the two age groups. Additional research is needed in this area to determine whether the effects of these and other potential risk factors for *Campylobacter* shedding are influenced by population immune status.

CONCLUSIONS

As previously mentioned, this study is one part of a larger study looking at the ecology and dynamics of shedding of *Campylobacter* on dairy farms. We were able to collect over 25,000 fecal samples for bacterial isolation from 128 dairy farms in 4 states over a 10 month period, which provided the opportunity examine patterns in the APP of *Campylobacter* in dairy calves and milking cows. Although there may have been areas in the collection of data on risk factors that may have been more extensive, this study provided information that can be used to direct future research on specific herd management factors that can reduce the prevalence of *Campylobacter* on the farm.

In summary, *Campylobacter* was commonly found on dairy farms, particularly in healthy calves and sick adult cattle. There were seasonal patterns seen in prevalence of *Campylobacter*, with the highest rates seen in the winter and the lowest rates seen in the summer. Results of multivariable analyses in this study found that factors associated with reduced health of the herd were associated with increasing prevalence of *Campylobacter*. Herd management risk factors associated with increased *Campylobacter* prevalence were those which increased risk of fecal contamination and exposure for cattle, and increased calf exposure to infected animals. Management risk factors

associated with decreased *Campylobacter* were those which reduced fecal contamination risks, and increased the opportunities for adult animals to develop natural immunity to *Campylobacter* through exposure to infected animals. The findings of this study are by no means exhaustive, but the specific risk factors identified in this study can be used to develop programs aimed at reducing the risk of infection on farms. Additional targeted research on management strategies to reduce *Campylobacter* shedding on dairy farms will provide the industry with the tools necessary to provide a safer milk supply to the food chain.

Table 1. Description of farms by herd size, state, and farm management style

Herd Size[a]	Michigan	Minnesota	New York	Wisconsin
30 – 49	0	6	4	6
50 – 99	10	9	9	9
100 – 199	11	8	9	8
200 +	11	9	10	9
Total	32	32	32	32

a – Number of cows in milking herd

Table 2. Apparent Period Prevalence of *Campylobacter* from Animals

Category	Number of animals tested	Percent positive
Overall	25,155	12.0
State		
Michigan	6,887	11.9
Minnesota	5,624	15.2
New York	6,678	11.0
Wisconsin	5,933	10.2
Age Group		
Adult cows	20,377	11.4
Calves	4,745	14.4
Health Status		
Cows only – Healthy	19,303	11.3
Cows only – Sick	606	17.0
Calves only – Healthy	4,380	14.6
Calves only – Sick	357	12.0
Season		
Fall	4,701	14.1
Winter	5,906	15.5
Spring	6,480	13.8
Summer	8,036	6.7

Table 3. Apparent Period Prevalence of *Campylobacter* from Milk and Environmental Samples

Category	Number of samples tested	Percent positive
Overall	5,127	1.3
Milk Samples		
Bulk tank milk	562	1.1
Milk filters	560	2.9
Environmental Samples		
Feed Alley	614	.49
Calf housing	606	.99
Sick pens	182	1.6
Maternity pens	451	1.6
Water tank	616	1.1
Manure Lagoon	614	1.3
Bird droppings	596	.84
Cull cow hide swabs	326	2.45

Table 4. Risk Factors used in Herd, Cow, and Calf Population Multivariable Analyses

Risk Factor	Coding Used in Analysis
General Herd Management	
% milking herd imported	Continuous (0 - 100%)
General Cattle Housing	
Lactating cows in dry lot housing	1 = yes; 0 = no
Any cattle kept in dry lot housing	1 = yes; 0 = no
Calves group housed	1 = yes; 0 = no
Calves housed on organic bedding	1 = yes; 0 = no
Calves housed on inorganic bedding	1 = yes; 0 = no
Lactating cows on organic bedding	1 = yes; 0 = no
Lactating cows on inorganic bedding	1 = yes; 0 = no
Frequency of calf organic bedding changes	1) daily; 2) weekly; 3) monthly
Frequency of cow organic bedding changes	1) daily; 2) weekly; 3) monthly
Herd has pasture access	1 = yes; 0 = no
Herd has access to surface water	1 = yes; 0 = no
Special Cattle Housing	
Sick animal housing available	1 = yes; 0 = no
Maternity pens available	1 = yes; 0 = no
Shared maternity / sick pens	1 = yes; 0 = no

Table 4. Risk Factors used in Herd, Cow, and Calf Population Multivariable Analyses (cont.)

Risk Factor	Coding Used in Analysis
Biosecurity and Sanitation	
Feed protected from moisture	1 = yes; 0 = no
Feed protected from animal pests	1 = yes; 0 = no
Calf housing washed with water	1 = yes; 0 = no
Calf milk buckets washed	1 = yes; 0 = no
Loader used for feed	1 = yes; 0 = no
Wash feed loader buckets	1 = yes; 0 = no
Contact with other animals	Categorical index (0 - 4)
Manure pack used	1 = yes; 0 = no
Slurry spread on fields	1 = yes; 0 = no
High somatic cell count (> 30,000)	1 = yes; 0 = no
High bacteria counts in milk (> 300,000)	1 = yes; 0 = no
Percent of herd calves scouring	Continuous (0 - 100%)
APP for adult cows (calf model only)	Continuous (0 – 100)

Table 5. Final multivariable Poisson regression model for prevalence of *Campylobacter*, for herd (n = 127), controlling for herd size, state, and season

Risk Factor	Odds Ratio *	95% C.I.
Feed protected from moisture	1.20	1.04 - 1.39
Percent of herd calves scouring	1.03	1.01 - 1.04
Lactating cows in dry lot housing	.81	.73 - .90
Calves group housed	1.10	1.01 - 1.20
Lactating cows on inorganic bedding	1.36	1.24 - 1.50
Calf housing washed/w water	.74	.66 - .83
Loader used for feed	1.20	1.05 - 1.36
Sick animal housing available	1.48	1.34 - 1.63
% Milking herd imported	1.06	1.04 - 1.07

Model -2 log L = 17,644.7

Likelihood ratio = 708.6, 19 d.f., $p < .0001$

Estimated $R^2 = 2.81$ %

* - all odds ratios significant at $p \leq 0.05$

Table 6. Final multivariable Poisson regression model for prevalence of *Campylobacter*, for Cow Population (n=127), controlling for herd size, state, and season

Risk Factor	Odds Ratio *	95% C.I.
Protecting feed from moisture only	1.36	1.15 – 1.61
Percent of herd calves scouring	1.02	1.00 – 1.03
Calves in group housing	1.13	1.03 – 1.25
Lactating cows in dry lot housing	.87	.77 – .97
Herd has pasture access	.80	.71 - .90
Inorganic bedding for lactating cows	1.44	1.28 – 1.62
Calf housing washed	.76	.67 - .87
Manure pack used	1.17	1.01 – 1.35
Bucket loader used for handling feed	1.17	1.01 – 1.36
Loader bucket washed	.79	.70 - .90
Sick animal housing available	1.78	1.51 – 2.09
Combined sick/maternity facility	.83	.72 - .96
% Milking herd imported	1.06	1.04 – 1.08

$-2 \log L = 13{,}756.74$

Likelihood ratio = 648.38 d.f., $p < .0001$

est. $R^2 = 3.16\ \%$

* - all odds ratios significant at $p \leq 0.05$

Table 7. Final multivariable Poisson regression model for prevalence of *Campylobacter*, for Calf Population (n=127), controlling for herd size, state, and season

Risk Factor	Odds Ratio *	95% C.I.
Adult Cow APP (5% change)	1.12	1.09 – 1.17
Percent of herd calves scouring (10%)	1.05	1.03 – 1.08
Lactating cows in group housing	1.49	1.01 – 2.19
Lactating cows in dry lots	.75	.60 – .94
Herd has pasture access	2.17	1.67 – 2.80
Inorganic bedding for calves	2.54	1.69 – 3.82
Calf milk buckets washed	.68	.56 – .83
Manure pack used	.65	.49 – .86
High bacteria counts in milk	1.89	1.45 – 2.47
Other Animal Index	.61	.49 - .75
-2 log L = 3,490.49 Likelihood ratio = 353.49, 18 d.f., $p < .0001$ est. $R^2 = 7.29$ %		

* - all odds ratios significant at $p \leq 0.05$

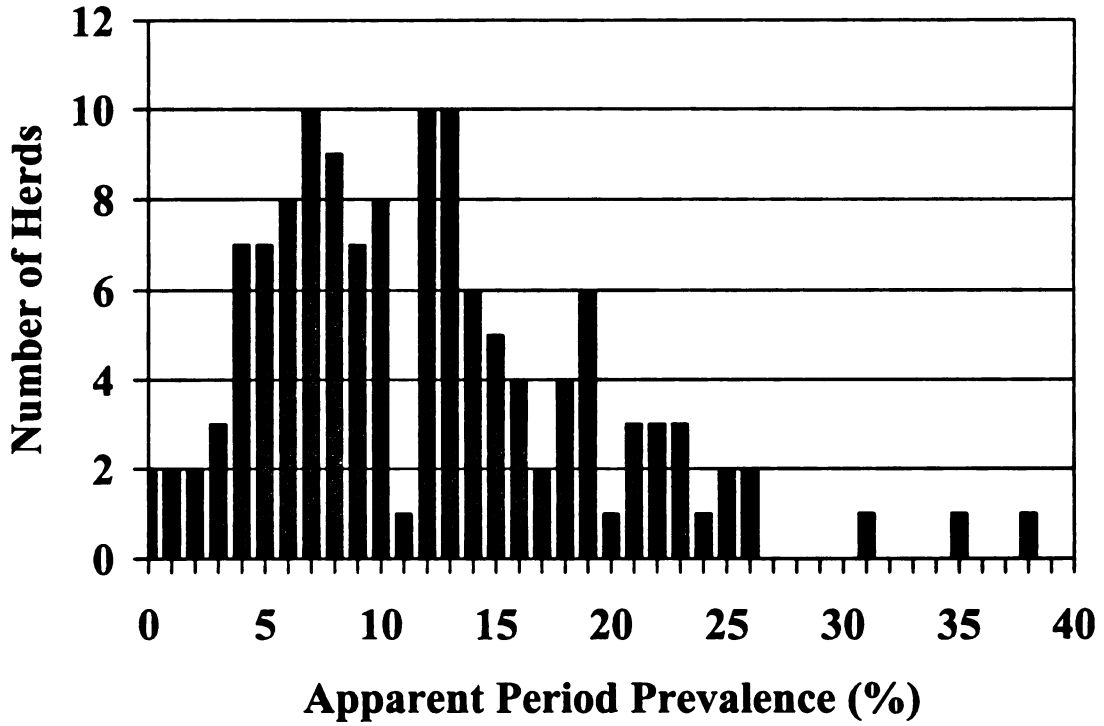
Table 8. Comparison of risk factors between Cow Population and Calf Population Poisson regression model for *Campylobacter* prevalence, controlling for herd size, state, and season

	Calves		Cows	
Risk Factor	**O.R. ***	**95% C.I.**	**O.R. ***	**95% C.I.**
Percent of herd calves scouring	1.05	1.03 – 1.08	1.02	1.00 – 1.03
Lactating cows on dry lots	.75	.60 – .94	.87	.77 – .97
Herd has pasture access	2.17	1.67 – 2.80	.80	.71 – .90
Inorganic bedding used	2.54	1.69 – 3.82	1.44	1.28 – 1.62
Manure pack used	.65	.49 – .86	1.17	1.01 – 1.35

* - all odds ratios significant at $p \leq 0.05$

Figure Legend:

Figure 1. Frequency of Apparent Period Prevalence in Herds



CHAPTER 3

Patterns of occurrence of Campylobacter on dairy farms in Midwestern and Northeastern United States: An Individual Animal Analysis

STRUCTURED ABSTRACT

OBJECTIVE: Investigate specific risk factors that are associated with an animal's *Campylobacter* status on dairy farms

DESIGN: Prospective

SAMPLE POPULATION: 25,121cattle from 128 randomly selected conventionally managed dairy farms and organic dairy farms from Michigan, Minnesota, New York and Wisconsin. Herds were stratified by state, farm management and size. Cattle sampled were divided into six classes based on age and health/lactation status.

PROCEDURE: Herd management data and fecal samples were collected from each herd every other month over a 10-month period, and *Campylobacter* were isolated from fecal samples. Multivariable logistic regression with random effects was used to assess the associations between management risk factors and an individual animal's *Campylobacter* status.

RESULTS: The overall apparent period prevalence (APP) of *Campylobacter* was 12%.

Calves had higher APP than adult cattle, sick adults had higher APP than healthy adults. Factors associated with a greater risk of *Campylobacter* included poor or stressed health status, decreasing age, inorganic cattle bedding, pasture availability, level of milking herd from off-farm sources , feed protected only from moisture, and the presence of sick animal housing. Factors associated with lower risk of *Campylobacter* included washing calf housing, washing calf milk buckets, housing lactating cows on dry lots, and spreading manure slurry on fields rather than on-farm storage.

CONCLUSIONS AND CLINICAL RELEVANCE: Herd management risk factors associated with higher risk of *Campylobacter* were those which increased risk of fecal contamination and exposure for cattle, and increased calf exposure to infected animals. Management risk factors associated with decreased risk of *Campylobacter* were those which reduced fecal contamination and increased the opportunities for adult animals to develop natural immunity to *Campylobacter* through exposure to infected animals. Specific risk factors identified in this study can be used to develop programs aimed at reducing the risk of this infection on farms.

INTRODUCTION

Campylobacter is identified as the leading cause of foodborne bacterial gastroenteritis in the United States (Allos 2001; Altekruse et al., 1999). Most *Campylobacter* enteritis cases are mild and self-limiting, but more serious consequences like arthritis and the neuropathy Guillian-Barré syndrome can occur (Tauxe et al., 1992; Rees et al., 1995; Mead et al., 1999). Antimicrobial resistance is another serious consequence of *Campylobacter* infection and is considered an emerging global health issue (Neu 1992; Moore et al., 2001). These issues pose a major medical and economic concern for the human population (McDowell and McElvaine, 1997; Rees et al., 1995; Wegener, 1999).

The greatest risk of *Campylobacter* infection in humans is from foods of animal origin. (Smith et al., 1999; Harris et al., 1986). Milk and meat from dairy cattle are one of the sources of *Campylobacter* for consumers (Evans et al., 1996: Dilworth et al., 1988; Lehner et al., 2000). Cattle are known to be intestinal carriers of *Campylobacter spp.* (Blaser et al., 1983) since *Campylobacter* can colonize their gastrointestinal tract without causing disease (Manser and Dalziel, 1985). The reported *Campylobacter* prevalence rate in dairy cattle is around 20% (Manser and Dalziel, 1985; Beumer et al., 1988; Humphrey and Beckett, 1987).

Several factors have been associated with the risk of *Campylobacter* in dairy cattle, including animal age, health status, and environment. Calves were reported to have higher prevalence rates of *C. jejuni* than adult cows (Giancoboni et al., 1993). Several studies have seen no increased risk for *Campylobacter* in sick cattle compared to healthy

animals (Rycke et al., 1986; Snodgrass et al., 1986; Manser and Dalziel, 1985). Grazing on pasture was found to be associated with increased rates of *Campylobacter* shedding in contrast to animals fed hay and silage diets (Jones et al., 1999). Also, parturition appeared to increase shedding in animals compared to other periods of gestation (Jones et al., 1999).

From studies conducted on cattle, most have looked at herd-level risk factors that influence *Campylobacter* prevalence (Beumer et al, 1988; Giacoboni et al, 1993; Humphrey and Beckett, 1987; Stanley et al, 1998; Wesley et al, 2000) (Chapter 2). While these types of analyses offer tremendous insight, more information about the dynamics of *Campylobacter* infection on the farm can be gained by evaluating risk factors on the individual animal level. Therefore, the specific objective of this study was to investigate specific animal-level risk factors associated with the shedding of *Campylobacter spp.* in cattle on dairy farms in four Midwestern and Northeastern states.

MATERIALS AND METHODS

Study design

This study is part of a larger study, with the long-term goals to determine the ecology of *Campylobacter* on conventional and organic Midwestern and Northeastern dairy farms, and understand the dynamics of shedding of this bacteria.

A prospective approach was used to collect specimens and corresponding data relating to potential risk factors. Data collection and sampling occurred bimonthly over a 10-month period, resulting in 5-6 data collection points over one year.

Study population

Dairy herds from Michigan, Minnesota, New York, and Wisconsin were recruited for the study. For a farm to be included in the study, it had to meet the following criteria: a farm had to have at least 30 milking cows, provide good records, and allow samples to be collected randomly from the animals on the farm and from specific areas of the farm for a year. A pool of farms was identified based on travel time and distance to research facilities within each state, and farms were then randomly selected and recruited for participation in the study.

The study utilized conventionally-managed dairy farms and organic dairy farms (certified to ship to local organic dairy processors and did not use any antimicrobial therapy for cattle greater than one year old within the previous three years). The herds were stratified into four herd size categories, 30 to 49, 50 to 99, 100 to 199, and more than 200 milking cows. When possible, equal numbers of organic and conventional dairies were enrolled within each size class within each state.

Animals in each herd were classified into different age/status classes, including calves less than 2 months of age (pre-weaning); healthy lactating cows; cull cows (identified by the producer as leaving the herd within 7 days, regardless of reason); periparturient (within 14 days of calving) cows; and sick cows (as reported by the producer). Up to 15 calves, 5 cull cows, 10 periparturient cows, and 5 sick cows were sampled from each herd. The number of healthy lactating cows to sample was dependent on herd size: 20 from herds with 30-49 cows, 25 from herds with 50-99 cows, and 30 from herds with 100 or more milking cows.

Sample size

A sample size of 128 dairy herds was established for the study, based on the ability to evaluate herd level prevalence rates of *Campylobacter*. Taking at least 50 samples per herd would provide 95% confidence of detecting at least one positive animal per visit if the within-herd prevalence is $\geq$ 5%, which agrees with reported prevalence rates, which should allow sensitivity of sampling given reported prevalence rates vary from 5% to 37% in individual dairy cattle (Hoar et al, 2001; Wesley et al., 2000).

Data collection

Data were collected using initial and bimonthly pre-tested questionnaires administered in person. The initial questionnaires were designed to capture data regarding herd management practices, herd inventory, animal housing, feed, water systems, production, health, manure management, and antimicrobial use on the farm being studied. On the bimonthly questionnaires, data collectors recorded information about any changes in herd management practices, herd inventory, and antimicrobial use that may have occurred after the previous sampling visit.

An 'other animal' index was developed as a measure of the presence of other animals on the farm that have been reported as sources of *Campylobacter* (Harvey et al, 1999; Engvall, et al, 1986; Penner and Hennessy, 1980):

$$Index_H = \sum (Swine_H + Poultry_H + Geese_H)$$

where:

$Swine_H$ = 1 if swine present on the farm, 0 if no swine present

$Poultry_H$ = 1 if chickens, ducks, turkeys or other poultry present on farm, 0 if not present

$Geese_H$ = 1 if wild geese present on farm, 0 if not present

Values for the index ranged from 0 to 3.

Sample collection

All samples collected were identified by farm code, sample date, sample container number and type of sample (animal identification or type of environmental sample). Cattle were systematically selected for sample collection, and approximately 5 grams of feces were collected per rectum and placed in sterile Whirl-pak® containers. All samples were shipped in a Styrofoam cooler, packed with ice, and sent to a central laboratory at Michigan State University. The samples were shipped within 24 hours of collection and processed immediately upon arrival at the laboratory.

Sample processing

Fecal samples were prepared by the addition of approximately 30 ml of phosphate buffered solution, and then directly plated onto *Campylobacter* agar (BD Biosciences), streaked for isolation, and incubated at 42°C in 5-10% CO_2 for 48 h. If growth was observed after 2 days, the isolate was subcultured onto a sheep blood agar (SBA) plate

and incubated at 42°C in 5-10% CO_2 for 48 h. Gram staining, oxidase testing (BD Biosciences), and hippurate (Remel) testing were performed. Motility testing was performed by inoculating Mueller Hinton broth with a heavy inoculum of the suspect *Campylobacter*, incubating for 48 h at 42°C in 5-10% CO_2 , and examining the suspension under bright field microscopy for characteristic darting motility. If the gram-stain, oxidase test, or motility test were not indicative of *Campylobacter*, the sample was recorded as negative. Hippurate testing was performed to distinguish *C. jejuni* from other *Campylobacter spp.*

STATISTICAL ANALYSIS

Associations between individual animal *Campylobacter* status and possible risk factors were assessed using logistic regression models with random effects (SAS PROC MIXED, macro GLIMMIX). These models controlled for the effects of state, herd size, and season, and included individual herd as the random effect term, to control for any herd effect in the analysis. Initially, separate models were developed for each risk factor, and factors that showed associations with *Campylobacter* status at $p \leq 0.2$ were considered for inclusion in the multivariable analysis.

Cows and calves differ physiologically in their response to *Campylobacter* infection (Giacoboni et al, 1993; Grau, 1988), and on-farm management of these two groups differs (types of housing, feeding practices, disease treatment, exposure to other animals, etc.). Taking this into consideration, and based on differences in the apparent prevalence of *Campylobacter* between cows and calves during analysis of these data

(Chapter 2), two separate models were developed: one for the cow population, one for calf population. A list of risk factors used for the multivariable analysis is provided in Table 3. Risk factors that were directly associated with the animal group being modeled (e.g., washing calf housing in the calf model, dry lot housing in the adult model) were included in the multivariable analysis. In addition, risk factors that could influence the levels of *Campylobacter* in the other animal group were examined in the analysis. Since both animal groups could serve as potential sources of *Campylobacter* for each other, the possibility that these 'indirect' risk factors could influence rates in the group of interest by affecting the quantity of *Campylobacter* present in the other animal group.

Multivariable logistic regression models with random effects were used to identify the major risk factors associated with each animal's *Campylobacter* status. All variables identified in the initial analyses were included in the multivariable model. Since herd size and state were confounders of several of the risk factors in the analysis, both were included in the multivariable model to control for confounding. A backward elimination procedure was used in order to find the best fitting model in each case: if removal of a potential confounder resulted in a 10% or more change in the odds ratio of the risk factors of interest (excluding state, herd size and season), the variable was retained in the model to control for confounding.

RESULTS

Study population

A total of 128 dairy herds from Michigan, Minnesota, New York, and Wisconsin were enrolled in the study (Table 1). The average number of milking cows per herd in Michigan, Minnesota, New York, and Wisconsin were 217, 169, 198, and 177, respectively. A total of 25,121 samples from dairy cattle were sampled, of which 20,380 (89%) were from healthy lactating cows, and 5% and 7% of the cows and calves, respectively, were sick (Table 2). The apparent prevalence of *Campylobacter* in this study was 12%, with 14.4% of calves and 11.4% of cows with samples positive for *Campylobacter* (Chapter 2).

The majority of lactating cows in this study lived in multiple housing (90%) in which cows were always in close proximity to one another. Of the lactating cows that lived in multiple housing, only 39% lived in dry lot housing and 35% had access to pasture. Less than half of the lactating cows had inorganic bedding provided for them (36%).

Calves were housed in areas with other calves (43%) or separately (57%). Approximately 50% of the calves did not have cleaned calf pens and the other 50% of calves had their calf pens cleaned with either water or disinfectant. Around 4% of the calves were exposed to inorganic bedding in the pens. And 85% of the calves were exposed to milk buckets that were not washed between feedings.

Cow population model

There were individual animal and herd level risk factors associated with increased risk for *Campylobacter* in adult cows. Individual animal factors associated with increasing risk included an animal's health status (being sick, a cull, or periparturient) and age. Herd level risk factors associated with increased *Campylobacter* risk included increased levels of calf scours on the farm, the use of inorganic bedding for lactating cows, pasture access by any cattle on the farm, pasture access specifically for dry cows, higher percentages of the milking herd coming from off-farm sources, protecting feed only from moisture, using a bucket loader for feed, and availability of sick animal housing on the farm (Table 4). Risk factors associated with the reduced risk for *Campylobacter* included high bacterial counts in bulk tank milk, the use of dry lot housing for lactating cows, washing calf housing between animals, and using the same loader bucket for feed and manure. Risk factors that were not significant, but retained in the model to control for confounding, included group housing for lactating cows and cattle access to surface water (ponds, creeks, etc).

Calf population model

In the model for pre-weaned calves, factors associated with increased *Campylobacter* risk included increased levels of calf scours on the farm, herd access to surface water (ponds, creeks, etc.), pasture access by any cattle on the farm, using the same loader bucket for feed and manure, the use of inorganic bedding for calves, and high bacterial counts in bulk tank milk (Table 5). Risk factors associated with the reduced *Campylobacter* risk in calves included access to dry lots by any cattle on the farm,

cleaning calf milk buckets between feedings, spreading slurry on fields for manure disposal, and possible contact with other animals on the farm. Confounders that were included in the model were calf health status, organic herd certification, and higher percentages of the milking herd coming from off-farm sources.

Comparison of cow and calf population models

Risk factors that were retained in both the cow and calf population models included increased levels of calf scours on the farm, access to surface water, washing calf housing, the use of inorganic bedding, pasture access by any cattle on the farm, higher percentages of the milking herd coming from off-farm sources, using the same loader bucket for feed and manure, and high bacterial counts in bulk tank milk (Table 6). Both the cow and calf population models showed an increase in the risk for *Campylobacter* when the level of calves scours was high, inorganic bedding was used, the herd had access to pasture, and higher percentages of the milking herd came from off-farm sources. A decrease in the risk of *Campylobacter* was observed for both the cow and calf population model when calf housing was washed. Risk factors associated with an increase in the risk of *Campylobacter* in the calf model but with a decrease in risk for the cow model were herd access to surface water, using the same loader bucket for feed and manure, and higher percentages of the milking herd coming from off-farm sources.

DISCUSSION

When examining risk factors associated with the individual animal *Campylobacter* status on dairy farms, it is helpful to view them in an epidemiological context, as being measures of host (cattle), agent (*Campylobacter spp.*), or environmental factors.

The Cow Population Model

Host-related Risk Factors - Host related factors are those related to the general health status of individual adult cattle. Factors in the multivariable analysis that were host-associated were animal health status and cattle age.

Being classified as a non-healthy cow (sick, cull, or periparturient) was associated with increased risk of *Campylobacter*. Sick cattle were considered sick because of clinical signs of illness, excluding any localized infections. For cattle in the sick/cull category, illness may weaken the immune system, creating opportunities for microorganisms to more readily establish themselves in an immune compromised animal (Carter and Chengappa, 1991), and the majority of cattle in this classification (79%) had some form of illness. Periparturient cows, cows within 14 days before or after calving, have increased stress levels associated with pregnancy, calving and early lactation, which would explain why *Campylobacter* was more commonly found in these animals (Jones et al., 1999).

The effects of age on the likelihood of *Campylobacter* shedding are probably due to the association between age and an individual animal's immune status. As cattle age, their exposure to a wide range of diseases increases, which, in turn, aids in the

development of immunity to a variety of agents. Consequently, younger cattle have an increased risk for *Campylobacter* infection because of the lack of this acquired immunity. As cattle mature, their immune responses become more effective than younger animals (particularly calves), and they are better able to fight infection regardless of their prior exposure. The overall result would be that their risk of *Campylobacter* colonization would decrease, and the likelihood that they would be shedding detectable levels of *Campylobacter* would also decrease (Benjaminin and Leskowitz, 1991; Weijtens, 1993).

Agent-related Risk Factors- Agent-related risk factors are those that are related to the agent in question, *Campylobacter*. A risk factors in the multivariable analysis that was agent-associated were the percentage of cattle from off-farm sources. In this study, increasing the percentage of cattle introduced from sources off the farm increased the likelihood for individual cattle to become *Campylobacter* positive. One basic cattle management practice known to reduce the risk of imported infection is to maintain a "closed" herd, in which no cattle are brought from outside the operation into the herd (Etgen et al, 1987). Imported cattle can introduce new strains of bacteria into a herd that was not previously exposed, allowing the bacteria to quickly spread within this naive population. Also, the stresses associated with transporting animals can cause increased shedding of bacteria (Stern et al, 1995), which would provide a greater possible source of infection for cattle already on the farm, and making it more likely to isolate *Campylobacter* from these transported animals.

Environment-related Risk Factors - Environment-related risk factors are those that are related to the increasing the likelihood of a host becoming exposed to the agent. This exposure can either be direct (animal to animal), or indirect (exposure through

contaminated feed, water, or housing). Environmental risk factors supporting direct exposure between cattle in this study included the use of dry lot housing for lactating cows. Environmental risk factors supporting indirect exposure included the use of inorganic bedding for lactating cows, the levels of diarrhea reported in calves on the farm, washing calf housing with water, feed storage that protected against moisture only, the use of loader buckets for feed, the combined use of a loader bucket for feed and manure, and high bulk milk bacteria counts. Factors that decreased risk were the use of dry lot housing for lactating cows, washing calf housing with water, and the combined use of a loader bucket for feed and manure. Risk factors that were associated with the increased risk of *Campylobacter* were the use of inorganic bedding for lactating cows, feed storage that protected against moisture only, and the use of loader bucket for feed.

Dry lot housing is a form of multiple-animal housing for cattle. Cattle in this environment are in close contact with one another, which would cause rapid transmission of the bacteria, but no associated increase in risk for *Campylobacter* shedding was found in this study. Instead, cattle in dry lot housing had decreased risk for *Campylobacter.* Any environmental contamination by fecal material in a dry lot would be exposed to sunlight, wind, and other environmental factors that would reduce the survivability of *Campylobacter* outside the host, and consequently reduce the risk of infection. Also, this association could be due to the density of cattle in housing. While there may be direct contact between cattle in any group housing environment, the stocking density of cattle in a dry lot will be lower than in cattle group housed in free stalls in a building, and lowering the density of potential hosts will reduce disease transmission in the dry lot.

In this study, inorganic bedding was defined as bedding that consisted of inorganic material such as sand, rubber tires or mats, mattresses, crushed limestone, etc. These materials were washed or changed much less frequently than organic bedding (i.e., straw, sawdust, etc.) (Chapter 2), which would result in fecally-contaminated bedding being used for long periods of time. This would increase the likelihood for cattle to become exposed to manure contaminated with *Campylobacter*. On the other hand, washing calf housing decreased the risk of *Campylobacter*. Routinely washing calf housing would decrease levels of fecal contamination, which would, in turn, decrease the risks of infection with *Campylobacter*.

The use of pasture housing for dry cows and pasture availability were associated with an increased risk for *Campylobacter*. Pasture access provides a unique opportunity for fecal-oral contamination due to cows defecating in areas that may be grazed by their herd-mates. This finding agrees with observed increases in *Campylobacter* prevalence found when sheep grazed pastures in comparison to being fed hay and silage diets (Jones et al., 1999). Another possible exposure to cattle on pasture could be wildlife reservoirs of *Campylobacter*. While the parameters of this study did not attempt to measure wildlife contact with cattle, other authors have documented the carriage of *Campylobacter* by rodents (Annan-Prah and Janc, 1988), insects (Gregory et al, 1997; Refregier-Petton et al, 2001), and birds such as starlings and gulls (Stanley and Jones, 1998; Piddock et al., 2000; Craven et al. 2000). These same environmental risk factors may also be associated with the increased risk observed with feed storage that was only protected from moisture, but not pests.

Higher levels of scours in calves may be an indirect indicator of higher levels of pathogens on the farm. Calves, with their new and relatively weak immune systems, would show evidence of infection to pathogens that would not be observed in adult cattle that have better-developed immune systems, and may have already acquired immunity to those specific pathogens.

There were several environmental risk factors associated with the potential contamination of feed by *Campylobacter* through feed storage and handling. Feed storage that only protected feed from moisture (not from pests like rodents, insects, and wild birds) was associated with increased risk for *Campylobacter.* From previous studies, rodents (Annan-Prah and Janc, 1988; Kapperud et al, 1993; Evans and Sayers, 2000), insects (Gregory et al, 1997; Refregier-Petton et al, 2001), and birds (Whelan et al, 1988) have been identified as important sources of *Campylobacter* on the farm.

The use of loader buckets for both manure and feed transport were associated with a decrease risk of *Campylobacter.* The majority of farms that reported using the same loader bucket for feed and manure also reported washing the loader bucket between uses, which would improve the overall hygiene of the loader buckets and decrease opportunities for fecal contamination during feed handling. However, the use of a loader bucket for feed was associated with an increased risk of *Campylobacter.* The reason for this association is unclear, and further work is needed to understand the impact of the use of loaders for handling feed and/or manure on the farm. It is possible that, if loader buckets were used for feed handling alone and are not carefully washed, there would be increased chances for contamination of feed from the environment.

Another environment-related risk factor associated with increased risk of *Campylobacter* in this study was high levels of bacteria in bulk tank milk. The association between bulk tank milk bacteria counts and *Campylobacter* risk is not clear. Higher bacterial levels would be expected in herds with poorer overall herd health, but this association is not seen. It is possible that bacterial levels in bulk tank milk may be due to poor hygiene during milking. Additional research into the association between bulk tank milk bacteria counts and cattle risk for *Campylobacter* is needed.

The Calf Population Model

Host-related Risk Factors - Factors in the multivariable analysis that were host-associated were the levels of calf scouring on the farm, and calf health status. As described under the cow population model, higher percentages of calves scouring on a farm increased the risk of *Campylobacter* in calves. For adult animals, levels of calf scours could be an indirect measure of pathogen load on the farm, but in the calf model, the level of calf scours reported at the beginning of the study may be an indirect measure of overall calf health. When more calves are sick, the chance for the spread of disease increases, either through direct contact with sick calves in group housing, contact with contaminated environments, or from handling by farm workers that become contaminated while handling sick calves and do not use any precautions to prevent the spread of disease. Since the majority of farms in this study housed calves individually, exposure from contaminated environments or through poor hygiene practices are the more likely sources of infection for these calves.

Agent-related Risk Factors - In the calf model, agent-related risk factors were those associated with the introduction of novel *Campylobacter* to calves. The risk factor in the calf multivariable analysis that was agent-associated was the presence of other species known to be intestinal carriers of *Campylobacter* (Harvey et al, 1999; Engvall, et al, 1986; Penner and Hennessy, 1980). It was expected that *Campylobacter* prevalence would increase when these other animals were present on the farm, but this was not observed. It is difficult to conclude why the presence of these animals was associated with reduced *Campylobacter* prevalence. It is possible that the index used was not sufficiently sensitive to the risk that these other species posed to the cattle herd. A more likely explanation of this finding is that the presence of various other livestock species on a dairy farm may be associated with other styles of overall farm management that function to reduce the levels of *Campylobacter* on the farm. Farms that maintain a variety of different livestock species on their facilities may reflect a more diversified approach to livestock farming, and may not manage their dairy operation as intensely as a farm whose sole occupation is dairy production.

Environment-related Risk Factors - Environment-related risk factors associated with *Campylobacter* risk in calves can be divided into two groups: factors that were directly associated with calves and calf management; and factors that were not directly associated with calves, but did influence calf risk for *Campylobacter*. Calf-related environmental risk factors included the use of inorganic bedding for calf housing, washing calf milk buckets, and high levels of bacteria reported in bulk tank milk. Other environmental risk factors included herd access to surface water, pasture, and dry lot housing, the use of the

same loader bucket for both feed and manure, and manure disposal by spreading slurry on fields.

When examining calf-related environmental risk factors, washing calf milk buckets reduced risk for *Campylobacter*, while the use of inorganic bedding and high levels of bacteria in bulk tank milk were associated with increased calf risk. Washing milk buckets between feedings would reduce the chance of spreading infection through milk that may become contaminated from environmental sources while in the bucket.

As described in the Cow Population model, the effect of using inorganic bedding for calves probably increases risks for *Campylobacter* exposure due to increased fecal contamination, since the bedding is changed or washed less frequently than organic bedding.

If milk with high levels of bacteria is fed to calves, they are more likely to become infected with *Campylobacter* and any other bacteria in the milk. In this study, *Campylobacter* was isolated from both milk filters and bulk tank milk (Chapter 2), making this highly likely. Interestingly, no statistically significant associations were found between calf *Campylobacter* and the feeding of waste milk to calves.

Environmental risk factors that are not directly associated with calves or calf management would increase the risk for calves to become infected by increasing herd exposure to *Campylobacter* through fecal contamination. Risk factors associated with increased *Campylobacter* risk included herd access to surface water, herd access to pasture, and the use of the same loader bucket for feed and manure, while risk factors associated with reduced *Campylobacter* prevalence included herd access to dry lots, and spreading slurry on fields for manure disposal.

Contaminated water has been reported as a source of *Campylobacter* for man (Piddock et al 2000, Evans et al, 1996) and animals (Pearson et al, 1993; Gregory et al 1997). Since *C. jejuni* can be isolated from surface water for up to four days when temperatures exceed 20°C (Korhonen and Martikainen, 1991), contaminated surface water is a likely source of *Campylobacter* in cattle that have access to surface water. Any surface water that cattle have contact with may become contaminated with cattle feces, causing the spread of any *Campylobacter* in these feces. As greater numbers of adult cattle carry *Campylobacter*, the likelihood of bacterial shedding is increased, and the risk for calves acquiring the bacteria is increased. The associations between *Campylobacter* in adult cattle and surface water was evaluated, but was not significant in the multivariable analysis.

Pasture access was associated with the increase in *Campylobacter*. Pastured cattle will graze on grass, which can easily be contaminated by feces, thereby creating more opportunities for *Campylobacter* ingestion and spread. Loader buckets used for handling both feed and manure increases the risk of feed contamination with *Campylobacter* if the bucket is not thoroughly washed between uses.

As in the adult cow model, herd access to dry lots decreased in the risk of *Campylobacter*, presumably through the development of herd immunity in the cattle in dry lot housing. Additionally, spreading manure slurry on fields removes manure from animal housing areas, which would reduce chances of exposure to *Campylobacter*, and subsequently reduce prevalence in the calf population.

Comparison of Cow and Calf Population Models

There were five risk factors present in both models that had similar effects on the risk of *Campylobacter* in both models. These risk factors included the increased percentage of scouring calves in the 6 months prior to the beginning of the study, the use of inorganic bedding, herd access to pasture, the increased level of imported milking cows, and washing calf housing. The effect of the percentage of scouring calves was fairly consistent between both models (cow model O.R. = 1.02, calf O.R. = 1.04, Table 6). The effect of the level of imported cattle in the herd, and washing calf housing were similar in magnitude between both models (imported cows: cow O.R. = 1.05, calf O.R. = 1.03; washing calf housing: cow O.R. = .76, calf O.R. = .82; Table 6), even though these risk factors were significant in the cow model but not significant in the calf model. The effect of the use of inorganic bedding was slightly higher in the calf model (O.R. = 1.9) compared to the cow model (O.R. = 1.4), while the effect of herd pasture access was notably higher in cows (O.R. = 2.5) than in calves (O.R. = 1.5).

In risk factors where effects were consistent between cow and calf model, it is interesting to note how the magnitude of effect differed between the models. Risk factors directly associated with the cattle group being modeled (cow model: level of imported cattle and pasture access; calf model: percent of calves scouring, washing calf housing, use of inorganic bedding) had higher magnitudes of effect than factors not directly associated with the cattle group being modeled. These factors would directly affect the age group being evaluated in the model, so it would be expected that these direct effects would be stronger than any indirect effect of a risk factor on the other cattle group.

There were three risk factors present in the Cow Population and Calf Population models that had opposite effects on the individual animal risk of *Campylobacter*. Surface water access, the use of a loader bucket for manure and feed, and high bulk tank milk bacterial count increased the risk of *Campylobacter* in the Calf Population model, but decreased the risk of *Campylobacter* in the Cow Population model. Calves and cows are managed differently on the farm, so the effects of the management risk factors may function differently in these two groups, as described above. Differences in cow and calf immune status may also result in changes in the effect of different risk factors. Additional research is needed in this area to determine whether the effects of these and other potential risk factors for *Campylobacter* shedding are influenced by an animal's immune status.

CONCLUSIONS

As previously mentioned, this study is a subset of a larger study investigating the ecology and dynamics of shedding of *Campylobacter* on conventional and organic dairy farms. For this study, we were able to collect more than 25,000 fecal samples for bacterial isolation from 128 dairy farms in 4 states over a 12-month period, which allowed for the opportunity to look at the associations between various risk factors and *Campylobacter* shedding in dairy calves and milking cows. While there may be areas in which more detailed information about risk factors is needed, this study provided information that can be used to direct future research on specific herd management practices.

In summary, risk factors associated with reduced health of the herd were associated with increasing prevalence of *Campylobacter*. Herd management risk factors associated with increased *Campylobacter* prevalence were those which increased risk of fecal contamination and exposure for cattle, and increased calf exposure to infected animals. Management risk factors associated with decreased risk for *Campylobacter* were those which reduced fecal contamination risks, and increased the opportunities for adult animals to develop natural immunity to *Campylobacter*. The findings of this study were by no means exhaustive, but the specific risk factors identified in this study can be used to develop programs aimed at reducing the risk of infection on farms. Additional targeted research on management strategies to reduce *Campylobacter* shedding on dairy farms will provide the dairy industry with the tools necessary to provide a safer milk supply to the food chain.

Table 1. Description of farms by herd size and state

Herd Size[a]	Michigan	Minnesota	New York	Wisconsin
30 – 49	0	6	4	6
50 – 99	10	9	9	9
100 – 199	11	8	9	8
200 +	11	9	10	9
Total	32	32	32	32

a – Number of cows in milking herd

Table 2. Cattle population in the analysis

Age Group	Health Status	Total number	*Campylobacter* +
Calves	Healthy	4398	14.6
	Sick	343	12.2
Cows	Healthy	19303	11.3
	Sick or Cull	1227	14.1
	Periparturient	2638	15.4

Table 3. Risk Factors used in Cow and Calf Population Multivariable Analyses

Risk Factor	Coding Used in Analysis
General Herd Management	
Herd certified Organic	1 = yes; 0 = no
Cattle-related Factors	
Health Status	1 = cull / sick; 2 = Within 14 days before or after calving; 3 = healthy
Age	1 = bred heifers; 2 = 1st lactation; 3 = 2nd lactation; 4 = 3rd – 4th lactation; 5 = 5th lactation and above
Percent of herd calves scouring	Continuous (0 - 100%)
High somatic cell count (> 30,000)	1 = yes; 0 = no
High bacteria counts in milk (> 300,000)	1 = yes; 0 = no
General Cattle Housing	
Lactating cows in dry lot housing	1 = yes; 0 = no
Any cattle kept in dry lot housing	1 = yes; 0 = no
All age groups with access to dry lots	1 = yes; 0 = no
Lactating cows in multiple housing	1 = yes; 0 = no
Calves housed on inorganic bedding	1 = yes; 0 = no
Lactating cows on inorganic bedding	1 = yes; 0 = no
Herd has pasture access	1 = yes; 0 = no
Dry cows on pasture	1 = yes; 0 = no

Table 3. Risk Factors used in Cow and Calf Population Multivariable Analyses (cont.)

Risk Factor	Coding Used in Analysis
Biosecurity and Sanitation	
% milking herd imported	Continuous (0 - 100%)
Feed protected from moisture only	1 = yes; 0 = no
Calf housing washed with water	1 = yes; 0 = no
Calf milk buckets washed	1 = yes; 0 = no
Loader used for feed	1 = yes; 0 = no
Wash feed loader buckets	1 = yes; 0 = no
Contact with other animals	Categorical index (0 – 3)
Manure pack used	1 = yes; 0 = no
Slurry spread on fields	1 = yes; 0 = no
Sick animal housing available	1 = yes; 0 = no
Herd has access to surface water	1 = yes; 0 = no

Table 4. Final multivariable logistic regression model with random effects for prevalence of *Campylobacter*, for cows (n = 20,380), controlling for herd size, state, and season

Risk Factor	Risk Ratio	95% C.I.
Cattle-related factors		
Cattle health status (baseline: healthy)	-	-
Sick or culls	1.25**	1.08 - 1.45
Within 14 days before or after calving	1.40***	1.27 - 1.55
Age group (baseline: 5+ lactations)	-	-
Bred heifers	2.52***	1.79 - 3.54
1st lactation	1.88***	1.60 - 2.22
2nd lactation	1.56***	1.32 - 1.84
3rd - 4th lactation	1.17	.99 - 1.39
Percent of herd calves scouring (for 10% change)	1.02*	1.00 - 1.03
High bulk tank milk bacteria count	.82**	.70 - .95
Cattle housing		
Lactating cows in multiple housing	1.19	.99 - 1.43
Lactating cows in dry lot housing	.89*	.80 - .99
Lactating cows on inorganic bedding	1.38***	1.24 - 1.53
Any pasture availability	2.52***	1.98 - 3.21
Dry cows on pasture	1.52***	1.20 - 1.94
Access to surface water	.91	.82 - 1.02
Biosecurity and Sanitation		
% Milking herd imported (for 10% change)	1.05***	1.04 - 1.07
Feed protected from moisture only	1.36***	1.16 - 1.60
Calf housing washed/w water	.76***	.68 - .85
Loader used for feed	1.18*	1.02 - 1.36
Same bucket for feed, manure	.84**	.75 - .93
Sick animal housing available	1.50***	1.35 - 1.66

Model -2 log L = 99216.4

* - $p \leq 0.05$; ** - $p \leq 0.01$; *** - $p \leq 0.001$

Table 5. Final multivariable Logistic regression model with random effects for prevalence of *Campylobacter*, for calves (n = 4,741), controlling for herd size, state, and season

Risk Factor	Risk Ratio	95% C.I.
Cattle-related factors		
Percent of herd calves scouring (10%)	1.04***	1.02 - 1.06
Sick calf	.78	.58 - 1.04
Cattle housing		
Herd has access to surface water	1.28**	1.06 - 1.53
All age groups with access to dry lots	.77*	.61 - .97
Calf housing washed/w water	.82	.67 - 1.00
Herd has pasture access	1.45***	1.18 - 1.78
Biosecurity and Sanitation		
Organic farm	1.19	.95 - 1.47
% Milking herd imported (10%)	1.03	1.00 - 1.06
Wash milk buckets	.76**	.64 - .90
Slurry spread on fields	.82*	.69 - .98
Same loader bucket used for feed & manure	1.19*	1.00 - 1.42
Inorganic bedding used for calves	1.87***	1.32 - 2.64
High bulk tank milk bacteria count	1.56***	1.24 - 1.97
Other animals (swine, poultry) present on farm	.78**	.68 - .91

Model -2 log L = 22,358.4

* - $p \leq 0.05$; ** - $p \leq 0.01$; *** - $p \leq 0.001$

Table 6. Comparison of risk factors between Cow and Calf Logistic regression models for *Campylobacter* status, controlling for herd size, state, and season

	Cows (n = 20,380)		Calves (n = 4,741)	
Risk Factor	**R.R.**	**95% C.I.**	**R.R.**	**95% C.I.**
Cattle-related factors				
Percent of herd calves scouring (10%)	1.02*	1.00 - 1.03	1.04***	1.02 - 1.06
Cattle housing				
Herd has access to surface water	.91	.82 - 1.02	1.28**	1.06 - 1.53
Calf housing washed/w water	.76***	.68 - .85	.82	.67 - 1.00
Use of inorganic bedding	1.38***	1.24 - 1.53	1.87***	1.32 - 2.64
Herd has pasture access	2.52***	1.98 - 3.21	1.45***	1.18 - 1.78
Biosecurity and Sanitation				
% Milking herd imported (10%)	1.05***	1.4 - 1.07	1.03	1.00 - 1.06
Same bucket used for feed & manure	.84**	.75 - .93	1.19*	1.00 - 1.42
High bulk tank milk bacteria count	.82**	.70 - .95	1.56***	1.24 - 1.97

* - $p \leq 0.05$; ** - $p \leq 0.01$; *** - $p \leq 0.001$

OVERALL CONCLUSIONS

A sample of 25,155 cattle from 128 randomly selected dairy farms from Michigan, Minnesota, New York and Wisconsin were used to assess the associations between the apparent period prevalence (APP) of *Campylobacter* at the farm level, and the risk for shedding *Campylobacter* at the individual animal level. The overall APP of *Campylobacter* was 12%, and ranged from 5% to 15% per herd. Calves had higher *Campylobacter* APP than adult cattle, sick adults had higher APP than healthy adults, and APPs were highest in the winter and lowest in the summer.

In general, factors associated with poor farm hygiene and biosecurity were associated with increasing prevalence and risk of *Campylobacter*, in both the herd-level and individual-level analyses.

Herd management risk factors associated with higher APPs were those that increased risk of fecal contamination and increased calf exposure to infected animals. Factors associated with higher APPs include the use of infrequently-changed inorganic cattle bedding, the percentage of cows in the milking herd from off-farm sources, cattle access to surface water, and milk with high bacteria counts. Inorganic bedding was changed less frequently than other types of bedding, which would allow contaminated feces to accumulate in the housing environment. Introducing a high percentage of the milking herd from outside sources would increase the chances of introducing foreign organisms into the herd, which could rapidly spread through the naïve herd population. Surface water is commonly contaminated with feces, which allows the opportunity for cattle to ingest contaminated water.

Herd risk factors associated with decreased APPs were those that decreased opportunities for *Campylobacter* contamination and survival in the environment. Factors associated with lower APP include washing calf housing, washing feed loader buckets, housing lactating cows on dry lots, and spreading manure slurry on fields. Basic hygiene, such as washing calf housing and washing feed loader buckets, decreases the risk of fecal contamination through direct exposure of calves to manure, and avoiding feed contamination in the loader bucket. Washing is particularly important when feed loader buckets are also used to transport manure. Proper manure handling, such as removing manure from cattle housing and spreading slurry on fields, decreases the amount of feces in the areas with high cow traffic, thereby decreasing the chances for cattle exposure to fecal contamination. The use of dry lot housing influences *Campylobacter* prevalence in several ways. Dry lot housing would decrease animal stress by stocking cattle at lower densities than in barns, provide opportunities for improved herd immunity through direct contact between animals, and the physical environmental conditions in dry lots do not support the survival of *Campylobacter* outside the host.

In the individual level analyses, factors associated with animal health and factors identified in the herd level analyses were associated with *Campylobacter* risk for the individual animal. The individual animal level analysis also identified risk factors that were associated with reduced odds of a cow or calf acquiring *Campylobacter,* and all of these risk factors were similar to those identified under the herd level analysis. Since significant differences were seen in the APP between calves and adult animals, separate analyses were conducted for calves and adults. New factors associated with a greater risk of *Campylobacter* included poor or stressed health status, and reported levels of calf

scouring at the beginning of the study. Poor health (disease) or stress (periparturient cows) can cause the animal to have a weakened immune system, which would increase the likelihood of *Campylobacter* shedding. High levels of calf scours may be an indicator of a large volume of microorganisms on the farm, since calves are more likely than adults to show signs of illness because of their immature immune systems.

This study was able to utilize a longitudinal approach to investigating the associations between *Campylobacter* and herd management practices on conventional and organic dairy farms. The sample size achieved (25,155 cattle from 128 farms) and the time period in which sampling occurred (a 10-month period) was an improvement over many studies in the current literature. The study design and data collected allowed us the flexibility to look at *Campylobacter* from both the herd level and individual animal level. The herd level analysis offered a considerable amount of information on the risk factors associated with *Campylobacter* prevalence within a herd, while the individual level analysis provided additional risk factors that applied to an individual animal. We were also able to conduct individual animal analyses by cattle age class, which targets the identification of risk factors for each age class more specifically.

In summary, farm hygiene, to reduce on-farm contamination, and herd biosecurity, to avoid the introduction of potentially harmful microbes to the herd, are two extremely important areas that affect the prevalence of *Campylobacter*. This information can be used to develop specific interventions to reduce herd levels of *Campylobacter*, such as in controlling fecal contamination, which will not only reduce levels of this non-pathogenic organism, but can also reduce levels of harmful bacteria (e.g. *Salmonella* spp.) on the farm. Reductions in overall bacterial loads in cattle on the farm will also

help in reducing the levels of any bacteria that have developed resistance to antimicrobial agents, a known animal and human health problem. Minimizing levels *Campylobacter* on farms will reduce the reservoir of *Campylobacter* for introduction into the human food chain, and result in a safer food supply.

APPENDIX A

Initial Questionnaire

Risk Factors for Salmonella and Campylobacter Infections and Drug Resistance in Dairy Cattle

This in-person questionnaire is to be given once for each producer e.g., at the initial herd visit. A much shorter questionnaire will be used to collect data that changes frequently.

Producer Information:

Farm name:_____

Owner(s) name:_____

Contact person or herdsman
(if different from owner):_____

Farm Address:_____

_____

Business Address (if different from above)_____

_____

Home Phone:( )_____ Fax:( )_____

Barn Phone:( ) _____

E-mail:_____

Herd Veterinarian:_____

DHIA Number (if applicable):_____

Directions to farm:_____

_____

Person to whom survey is administered_____

Survey administrator_____

Date of next visit_____

A. Inventory—Herd Size

1. As of today, what is your inventory of the following groups of **dairy cattle**?

	Lactation 1*	Lactation 2 & up*	Total
A. Milking cows	1	2	3
B. Dry cows	4	5	6
C. Total cows (add totals of A. and B. above)			7
D. Preweaned (milk-fed) heifer calves			8
E. Weaned replacement calves and heifers**			9
F. Other youngstock***			10
G. Bulls ****			11
H. Total cattle (Add C-G above)			12

* Lactation numbers here refer to the current lactation in the case of milking cows and to the lactation just completed for dry cows.

** "Weaned replacement calves and heifers" here means all female animals that will be kept as replacement cows, have not yet calved, and are no longer receiving milk or milk replacer as part of the diet.

*** "Other youngstock" here means **all animals that will not be kept as replacements** that are weaned or will be kept up to or past weaning (e.g., steers and heifers raised for beef—exclude calves that are only kept for a short period after birth)

**** Include only bulls kept for breeding purposes (e.g., breeding age bulls or younger bulls being saved for breeding purposes)

2. As of today, how many of the total **milk cows (both milking and dry)** were:

(NOTE: Add up the total cows in 1.C. and compare to 2.D. as a check before moving on to next page. These numbers should be the same—if not, investigate to see where the problem is)

A. Born and raised on this operation? (refers to all sites managed by this operation) ..... [13] _____ head

B. Born here but raised elsewhere? (refers to contract rearing: in case they have done this in past but are not now) ..... [14] _____ head

C. Not born on this operation? ..... [15] _____ head

D. Total of A. + C. (Should equal 1.C. above.) ..... [16] _____ head

3. This question refers to animals **other than dairy cattle** on this operation.

Within the last 12 months, have any of the following types of animals been present on this operation? If so, please indicate whether these animals had physical contact* with any of this operation's dairy cows or heifers, or their feed, minerals, or water supply.

	Present on operation?	Physical contact*?
A. Beef cattle?	☐ Yes ☐ No [17]	☐ Yes ☐ No
B. Chickens, turkeys, domestic geese, or other poultry?	☐ Yes ☐ No	☐ Yes ☐ No
C. Horses or other equines (such as ponies, donkeys, mules, burros, etc.)?	☐ Yes ☐ No	☐ Yes ☐ No
D. Pigs?	☐ Yes ☐ No	☐ Yes ☐ No
E. Sheep?	☐ Yes ☐ No	☐ Yes ☐ No
F. Goats?	☐ Yes ☐ No	☐ Yes ☐ No
G. Farmed (confined to a pen) exotic animals (such as deer, llamas, ostriches, etc.)? Specify:_____ ____	☐ Yes ☐ No	☐ Yes ☐ No
H. Dogs?	☐ Yes ☐ No	☐ Yes ☐ No
I. Cats?	☐ Yes ☐ No	☐ Yes ☐ No
J. Wild geese?	☐ Yes ☐ No	☐ Yes ☐ No
K. Other animals? Specify:_____ ____ **Include Nuisance Birds**_____	☐ Yes ☐ No	☐ Yes ☐ No

* As used here, "physical contact" means nose-to-nose contact or sniffing/touching/licking each other, including through a fence.

B. Herd Expansion Status

4. Were any of the following groups of animals brought onto this operation from outside sources **during the last 12 months**?

	Brought onto operation?	IF YES, How many?	IF YES, How many of these animals were isolated* upon arrival?	IF YES, On average, how long were they isolated* (in days)?
A. Preweaned (milk-fed) calves?	☐ Yes ☐ No			Days
B. Weaned dairy	☐ Yes ☐ No			Days
C. Dairy cows?	☐ Yes ☐ No			Days
D. Bulls?	☐ Yes ☐ No			Days
E. Other cattle,	☐ Yes ☐ No			Days
E. Total.				

* "Isolated" here means that the animal(s) is held for a period of time in a separate pen or other facility where nose-to-nose contact with cattle in the existing herd is prevented.

5. In the last 12 months, what is the largest number of dairy cows or weaned heifers that were introduced to the herd from outside sources **within a period of one week.**
..... _____ head

C. Housing

6. Which one of the following types of milking facilities did this operation primarily use during the past 12 months? (Circle the appropriate letter A-D)

   A. Pit parlor?

   B. Flat parlor or step-up milking facility?

   C. Tie Stall or stanchion barn milking facilities?

   D. Any other type of milking facility? (specify)_____

7. What housing facilities did this operation use during the past 12 months for the following (check all that apply):

	Hutch (for single animal only)	Freestall	Tie Stall or Stanchion	Calf is tied in stanchion or tie stall barn	Individual animal area**	Multiple animal area***
A. Preweaned (milk-fed) dairy calves?						
B. Weaned dairy calves & heifers?						
C. Lactating dairy cows?						
D. Maternity housing*?						

* "Maternity housing" here refers to where cows normally calve.

** "Individual animal area" here refers to a pen housing only one animal (e.g., individual calf pen) that is not covered by one of the previous options (e.g., if "hutch" has been selected, do not also mark "individual animal area" to refer to hutches).

*** "Multiple animal area" here refers to a pen housing multiple animal (including "super hutches") that is not covered by one of the previous options (e.g., if "freestall" has been selected, do not also mark "multiple animal area" to refer to freestalls).

8. During the past 12 months, approximately how many months of **daily access** to outside areas did the following groups of dairy animals have? (Enter "0" if no access)

	Drylot	Pasture	
		Does not provide at least 90% of roughage in ration)	Provides ≥ 90% of roughage in ration
A. Weaned dairy heifers?	Months	months	months
B. Lactating dairy cows?	Months	months	months
C. Dry cows?	Months	months	months
D. Maternity, close-up, or recently fresh cow housing?	Months	months	months

9. Is maternity housing* in a separate pen or facility from other lactating cows?..... ☐ Yes ☐ No

* "Maternity housing" here refers to where cows normally calve.

10. Which of the following bedding types are typically used for the following groups of animals? Mark bedding types for each group of cattle using letters A-F corresponding to how often the bedding is changed. (e.g., if inorganic bedding for lactating cows is changed monthly, but organic bedding for lactating cows is changed every 2-3 days, put "B" in "other organic bedding" column and "E" in "inorganic bedding" column for lactating cows.)

	For each bedding type, put a letter A-F (select from list below) corresponding to how often the bedding is changed or added to		
	Dried manure	Other organic bedding*	Inorganic bedding**
Lactating cows			
Maternity, close-up, or recently fresh cows			
Sick cows			
Preweaned (milk-fed) calves			

A. Daily.
B. Every 2-3 days.
C. Weekly (more than 3 days, less than 8 days)
D. 2-3 times per month
E. Monthly
F. Greater than monthly

* "Organic bedding" here includes any organic materials used for bedding, such as straw, sawdust, newspaper, corn cobs or stalks, excluding dried manure.

** "Inorganic bedding" here includes any inorganic materials such as sand, rubber tires or mats, mattresses, crushed limestone, etc.

D. Feed and Water System

11. Do you feed a total mixed ration (TMR) to lactating dairy cows? ..... ☐ Yes ☐ No

12. In the last 60 days, which of the following feeds have been used in the following groups of dairy animals? Include only **purchased feeds or feeds obtained from off-farm sources.** Check all that apply

Type of Feed	High-Producing Cows *	Other Milking Cows*	Dry Cows
A. Whole cottonseed/hulls			
B. Cottonseed meal			
C. Whole soybeans or soybean meal			
D. Bakery by-products			
E. Brewers by-products (includes distillers' grains)			
F. Blood meal			
G. Meat & bone meal (e.g., porcine-only or equine-only)			
G. Milk products (e.g., whey)			
H. Tallow/animal fat			
I. Other protein meal (e.g., meal from fish or poultry) Pleasespecify_____ __			

* If high-producing cows are not fed differently from other cows, put N/A in "Other Milking Cows" column.

13. The following questions refer to the storage areas used for protein and concentrates fed to dairy cattle.

	Is storage area for this feed type in an enclosed building or other enclosed structure?	Does storage area for this feed type provide protection against moisture?	Does storage area for this feed provide protection Against birds or rodents?
A. Protein feeds	Yes ☐ No ☐	Yes ☐ No ☐	Yes ☐ No ☐
B. Concentrates	Yes ☐ No ☐	Yes ☐ No ☐	Yes ☐ No ☐

14. Which of the following coccidiostats or ionophores, if any, do you normally use for the following groups of animals? Include products used in feed, water, or milk replacer.

	Preweaned (milk-fed) calves	Weaned calves up to breeding	Heifers after breeding
Deccox (or other decoquinate product)			
Rumensin (or other monensin product)			
Bovatec (or other lasalocid product)			
Corid (or other amprolium product)			
Sulfaquinoxaline (many oral products)			
Other (Please specify)_____			

15. During the last 12 months, did cows drink from the following (check all that apply):

	Milk cows	Dry cows	Frequency **cleaned*** (times per year)	Frequency **disinfected**** (times per year)	List disinfectant
A. Automatic waterer—for individual cows (each has own cup or one cup shared by two cows)			_____Times/year	_____Times/year	
B. Automatic waterer—cows drink individually, but waterer shared by group			_____Times/year	_____Times/year	
C. Water tank—multiple cows can drink at once			_____Times/year	_____Times/year	
D. Lake, pond, stream, river, etc.—occasional use only					
E. Lake, pond, stream, river, etc.—seasonal main source (e.g., if primary source of water in summer is lake, pond, river, etc)					
F. Other: Please specify_____			_____Times/year	_____Times/year	

* "Cleaned" here refers to removal of water from waterer and removal of scum or feed accumulation—regardless of whether a disinfectant is used.

** "Disinfected" means that after cleaning, a chemical disinfectant is used to sanitize waterer.

16. Is the water that dairy cattle drink usually chlorinated? ☐ Yes ☐ No

17. What is the source of drinking water for cows? (Check all that apply)

A. Well
B. Municipal water
C. Surface water (stream, lake, spring, etc.)
D. Other (Please specify)_____

18. Is the ration for close-up dry cows different from the ration for far-off dry cows (i.e., does this operation have a transition/close up ration)? ☐ Yes ☐ No

19. Does this operation normally feed anionic salts in transition cow diets (e.g., during the last 2 to 3 weeks of gestation) Common anionic salts are the sulfates or chlorides of magnesium, calcium, or ammonium?. ☐ Yes ☐ No

E. Calf Management and Feeding

20. Which **one** of the following methods **is used most frequently** for the first feeding of colostrum to newborn dairy heifer calves? (Colostrum is the first milk produced after a calf is born.) (Circle the appropriate letter A-D)

A. Calf is left with cow to nurse for a period of time (e.g., for 2-4 hours)
B. Hand feeding from bucket or bottle
C. Hand feeding using esophageal feeder
D. Do not get colostrum

Answer #21 only if B or C is circled.

21. How much colostrum is normally fed during the first 24 hours? (A calf bottle is typically 2 quarts) (Circle the appropriate letter A-C)

A. Two quarts or less
B. More than 2, but less than 4 quarts
C. Four quarts or more

22. During the past 60 days, what types of <u>milk</u> have **usually** been fed to preweaned calves **that are kept up to weaning**, after they have received colostrum? Do not include calves (e.g. bulls) that are kept for only a few days, and do not include diets that are not fed as a usual practice (e.g., if waste milk is always fed to calves whenever available, mark "yes" for "B" regardless of the number of times it was fed in the past two months. On the other hand, if waste milk was discarded more often than it was fed, mark "no" for "B").

	Included in diet? (Check all that apply)	If A or B is YES, Is the milk pasteurized?
A. Whole milk from untreated* cows	Yes ☐ No ☐	Yes ☐ No ☐
B. Whole milk from treated* cows (waste milk)	Yes ☐ No ☐	Yes ☐ No ☐
C. Milk replacer without antibiotics	Yes ☐ No ☐	
D. Milk replacer containing antibiotics	Yes ☐ No ☐	
E. Calf starter without antibiotics	Yes ☐ No ☐	
F. Calf starter containing antibiotics	Yes ☐ No ☐	
G. Other (specify)_____	Yes ☐ No ☐	

* "Treated cows" refers to cows that have been given antibiotics and are still within the milk withholding period. (A cow given Naxcel/Excenel is **not** considered a "treated cow" here).

Answer question #23 only if D. or F. is YES,

23. List the types of antibiotics used below. If unknown, ask to look at tag of bag/container. Include only antibiotics here.

_____

24. How often is maternity housing used as a hospital area for sick* cows? (Circle appropriate letter A-C)

A. More than once a month

B. Less than once a month

C. Never

* "Sick" as used here refers to cattle designated as sick by personnel on your farm or by a veterinarian. <u>Include all illnesses that would result in cattle being segregated (e.g., placed in sick pen) and/or treated with systemic antibiotics</u>. This would include, but is not limited to lameness, respiratory disorders, and diarrhea.

25. After removal from the dam, at what age do heifers first have direct contact with adult cows in the herd? _____months

26. Which of the following best represents your normal practice regarding the cleaning of calf **milk** buckets or containers between feedings? (Circle the appropriate letter A-C)

A. Between each feeding, all calf milk buckets or containers **washed with water only**.

B. Between each feeding, all calf milk buckets or containers **washed and disinfected** . → List disinfectant_____

C. Buckets or containers not washed or disinfected between feedings on a routine basis.

27. Are preweaned (milk-fed) calves fed milk or calf starter on an individual basis (e.g., individual bucket in hutch or individual calf pen, as opposed to group feeding where a common trough is used)? ☐ Yes ☐ No

28. Are individual calf pens or hutches washed and/or disinfected on a regular basis? (Circle the appropriate letter A-D.)

A. Washed with water only. → _____times per year

B. Washed and disinfected. → _____times per year → List disinfectant_____

C. Not washed or disinfected.

D. Calf pen or hutch is not used.

29. How often are individual hutches moved to a new location? (Choose the appropriate letter A-D)

A. Every time a calf is weaned. (Before introducing each new calf.)

B. Not after every weaning, but on a regular basis → _____times per year

C. Calf hutches are not relocated.

D. Calf hutches are not used.

30. Do personnel on your farm use any of the following precautionary practices when handling calves? (Check all that apply)

	After handling each calf	When finished with all calves (e.g., before entering a different area of the farm)	Do not routinely use this practice when handling calves
A. Wash boots or use boot dip			
B. Wash hands after handling calf or use disposable gloves			

31. Is unpasteurized milk that is produced on this operation consumed by family members, farm workers, or others?

A. Unpasteurized milk from this operation is consumed.

B. Home pasteurizer is used for milk produced on this operation.

C. Unpasteurized milk is not consumed. All milk consumed is purchased.

G. Production and Health

32. During the last six months, which of the following best describes the average bulk tank somatic cell count for milk shipped? (Circle the appropriate letter A-F below)

A. <100,000

B. 100,000-199,000

C. 200,000-299,000

D. 300,000-399,000

E. 400,000-499,000

F. 500,000+

33. During the last six months, which of the following best describes the average bacterial count (aka: standard plate count, plate loop count) for milk shipped? (Circle the appropriate letter A-E)

Colony forming units per millimeter (cfu/ml)

A. 0-24,999

B. 25,000-49,999

C. 50,000-74,999

D. 75,000-99,999

E. 100,000+

34. Do you use DHIA or other computerized records? .... Yes ☐ No ☐

If YES, answer #35

If NO, go to #36

35. What is your current rolling herd average for milk production? ..... _____ Annual

36. What is your **average** pounds of milk produced per day? (This question is to be asked for purposes of approximating a rolling herd average if one is not available by DHIA or other records. Thus, try to get an average pounds per day for as long a time as possible—not just over the past few days) ..... _____ Daily

37. Are sick* cattle placed in a pen or facility separate from lactating cows? ..... Yes ☐ No ☐

* "Sick" as used here refers to cattle designated as sick by personnel on your farm or by a veterinarian. Include all illnesses that would result in cattle being segregated (e.g., placed in sick pen), and/or treated with systemic antibiotics. This would include, but is not limited to lameness, respiratory disorders, and diarrhea.

38. Within the past two years, have any of your dairy cattle been positively diagnosed (i.e., by evidence of positive fecal culture or other laboratory test) with any of the following diseases? (Circle all that apply)

A. Salmonella

B. Johne's disease

C. Bovine Viral Diarrhea (BVD)

D. No cattle have been diagnosed with any of the diseases above.

39. Do you normally vaccinate cows with any of the following vaccines? (Circle all that apply)

A. J5 (Enviracor by Upjohn or J. Vac J5 by Rhone Merieux)

B. Endovac Bovi

C. Salmonella bacterin vaccine → Please specify which one, (e.g., Colorado Serum Companies *S. dublin/S.typhimurium* bacterin)

_____

40. Within the last 60 days, how many dairy cattle within the following groups had diarrhea or died?

	Number of animals with diarrhea lasting at least 24 hours?	Number of deaths among animals with diarrhea lasting at least 24 hours	Number of total animals that have died
Preweaned calves			
Weaned heifers			
Milk cows (milking or dry)			

41. Are any of the following methods of rodent control routinely used on this operation? (Circle all letters A-D that apply.)

A. Chemicals/bait?

B. Traps?

C. Cats?

D. Other methods? (specify)_____

H. Manure Management

42. Do you use any of the following to remove manure from cow housing areas? (Circle all letters A-E that apply)

A. Gutter cleaner

B. Tractor (bucket loader or skid steer)

C. Hand fork or shovel

D. Alley scraper--mechanical

E. Alley flushed with water → If so, is the water recycled? ☐ Yes ☐ No

F. Other (specify)_____

43. Are any of the following waste **storage** systems used on this operation? (Circle all letters A-K that apply)

A. Below floor or deep pit
B. Anaerobic lagoon with cover
C. Slurry storage in earth-basin
D. Anaerobic lagoon without cover
E. Slurry storage in Slurrystore® (or similar storage structure)
F. Aerated lagoon
G. Manure pack (inside barn)
H. Outside storage within dry lot or pens
I. Outside storage for solid manure not in dry lot or pen
J. Storage of solid manure in a building without cattle access
K. Other storage system used or no storage system used (specify)_____

44. You may respond to this question in miles or feet. What is the distance between the manure storage area and the nearest:

A. Well?..... _____miles or_____feet

B. Waterway or body of water?..... _____miles or_____feet

45. Which of the following methods are used to dispose of manure on owned or rented land? (Circle all letters A-E that apply)

A. Irrigation
B. Slurry (surface application)
C. Broadcast/solid spreader
D. Slurry (subsurface application)
E. Other method (specify)_____
F. Do not apply manure on owned or rented land.

46. In this question, the term "roughage" means hay, fresh chop forage, or pasture that dairy animals may eat or graze. Do cows eat or graze on roughage obtained from fields where manure in solid or liquid form was applied to the surface but not plowed under during the same growing season?..... ☐ Yes ☐ No

If YES, answer #47

47. How many days do you wait after applying manure to a field before cows are allowed to eat or graze the roughage from that field?_____days

48. Do you use a loader bucket on a tractor or skid steer to move feed? ☐ Yes ☐ No

If YES, answer #49

If NO, go to Section I

49. Do you use separate loader buckets for moving feed and for handling manure? (Circle the appropriate letter A-C).....

A. Yes, use separate buckets.

B. No, do not use separate buckets.

C. Do not use this equipment for handling manure.

If B. is circled, answer #50

50. After you have used the loader bucket for handling manure, do you do any of the following before using it for feed?: (Circle the appropriate letter A-D)?

A. Rinse bucket with water only.

B. Power wash bucket with high pressure water.

C. Wash and disinfect bucket. → List disinfectant_____

D. Do not wash or disinfect bucket

I. Antimicrobial Use

51. Which of the following best describes the use of dry cow tubes (intramammary infusions) used to treat your cows at final milk out? (Circle one of the following letters A-C)

A. Dry treat all 4 quarters on all or almost all the cows

B. Dry treat selected cows only, 1 or more quarters

C. Do not dry treat any cows

52. Does this operation routinely record antibiotic treatment for the following groups of cattle in some way?

		If YES, what types of records are kept? (Check all that apply)			
	Antibiotic treatment recorded?	Computer	Barn sheet, log, or notebook	Calendar	Other (specify)
A. Lactating cows	☐ Yes ☐ No				
B. Non-lactating cows	☐ Yes ☐ No				
C. Calves and heifers	☐ Yes ☐ No				

53. Where do you get recommendations on the following aspects of antibiotic use? (Check all that apply)

	Veterinarian	Pharmaceutical Representative	Personal Experience	Product label-Manufacturer label only—not labels from your veterinarian	Other farmers	Other-Please specify
Recommeded use i.e., what drugs to use for certain diseases)						
Dosage						
Withdrawal Time						

54. When you treat **respiratory disease in adult cows** with antibiotics, what antibiotics do you normally use? (Circle all that apply)

A. Naxcel/Excenel (ceftiofur)

B. Tetracyclines (e.g., Liquamycin--LA-200)

C. Penicillin

D. Ampicillin (e.g., Polyflex)

E. Albon (sulfadimethoxine)

F. Others (please specify)_____

55. When you treat **respiratory disease in calves and heifers** with antibiotics, what antibiotics do you normally use? (Circle all that apply)

A. Naxcel/Excenel (ceftiofur)

B. Nuflor (florfenicol)

C. Penicillin

D. Tetracyclines (e.g., Liquamycin--LA-200, Oxy-Tet-100)

E. Ampicillin (e.g., Polyflex)

F. Micotil (tilmicosin)

G. Others (please specify)_____

56. When you treat **calf scours** with systemic antibiotics, what antibiotics do you normally use (oral or injectable)? (Circle all that apply)

A. Panmycin boluses (tetracycline) B. Spectam (spectinomycin)

C. Nuflor (florfenicol) D. Trimethoprim-Sulfa

E. Others (please specify)_____

F. Do not use systemic antibiotics for calf scours.

57. When you treat **mastitis** with systemic (oral or injectable) antibiotics, what antibiotics do you normally use? Do not include intramammary antibiotics. (Circle all that apply)

A. Polyflex (ampicillin) B. Amoxi-Inject (amoxicillin)

C. Penicillin D. Erythromycin (e.g., Gallimycin)

E. Others (please specify)_____

F. Do not use systemic antibiotics for mastitis.

58. When you treat **metritis or retained placenta (RP)** with systemic (oral or injectable) antibiotics, what antibiotics do you normally use? (Circle all that apply)

A. Naxcel/Excenel

B. Penicillin

C. Ampicillin (e.g., Polyflex)

D. Others (please specify)_____

E. Do not use systemic antibiotics for metritis/retained placenta.

59. When you treat **foot problems in adult cows** with systemic antibiotics (oral or injectable), what antibiotics do you normally use Do not include topical treatments such as in foot wraps. (Circle all that apply)

A. Ampicillin (e.g., Polyflex)

B. Penicillin

C. Albon (sulfadimethoxine)

D. Naxcel/Excenel (ceftiofur)

E. Tetracyclines (e.g., Liquamycin--LA-200)

F. Ampicillin (Polyflex)

G. Others (please specify)_____

H. Do not use systemic antibiotics for foot problems.

60. Do you routinely use **antibiotics in footbaths** to control or treat lameness? ..... ☐ Yes ☐ No

A. **If YES**, do you use the antibiotics in footbaths on a continuous basis (i.e., all year long)? ..... ☐ Yes ☐ No

B. Please list what antibiotics are used, if any:_____

_____

61. Do you routinely **use any medications in feed or water in weaned calves or heifers** (other than coccidiostats)? ..... ☐ Yes ☐ No

A. **If YES**, do you use the additives on a continuous basis?..... ☐ Yes ☐ No

B. Please list what feed or water additives are used, if any:_____

_____

62. Approximately what percent of the following groups of cattle have received at least one antibiotic injection (or oral dose of antibiotics) within the past two months? Include treatments given by personnel on your farm or by your veterinarian. Do not include intramammary or topical administration of antibiotics. (Make only **one check per column**)

	Milk cows (milking or dry)	Bred heifers	Heifer calves (weaned or preweaned)
0 %			
1-10 %			
11-25 %			
26-50 %			
51-75 %			
76-100 %			

63. Within the past two months, approximately how much of the following antibiotics have you used? **Fill in only one column per row in the table below.**

	Approximate number of bottles used, including bottle size (put "0" if do not use or if used less than one bottle in past two months)	Approximate number of doses*, if less than one bottle was used.
Penicillin-type Includes penicillin, amoxicillin (Amoxi-inject), ampicillin (Polyflex)	_____bottles of size_____ml or g	_____doses
Cephalosporin-type Includes ceftiofur (Naxcel, Excenel)	_____bottles of size_____ml or g	_____doses
Tetracycline-type (includes LA-200, Oxy-Tet-100)	_____bottles of size_____ml or g	_____doses
Sulfonamides Includes sulfadimethoxine (Albon)	_____bottles of size_____ml or g	_____doses
Florfenicol (NuFlor)	_____bottles of size_____ml or g	_____doses
Other antibiotics Includes tilmicosin (Micotil), Erythromycin (Gallimycin), and any others not covered in the groups above.	_____bottles of size_____ml or g	_____doses

* A "dose" here means one administration of antibiotic. E.g., if you give 20 ml of Naxcel to a cow, that is one dose. If you give another 20 ml the next day to the same cow, that is another dose.

Glossary of Terms

The terms listed below are defined according to how they are meant to be used in this survey.

Calving Interval: the time from one calving to the next calving

Colostrum: The first milk produced after a calf is born

Heifer: Non-lactating weaned female animal that has not yet calved.

Inorganic bedding includes any inorganic materials such as sand, rubber tires or mats, mattresses, crushed limestone, etc.

Isolated/Isolation: A newly acquired animal(s) is held for a period of time in a separate pen or other facility where nose-to-nose contact with cattle in the existing herd is prevented

Maternity housing refers to where cows normally calve.

Organic bedding includes any organic materials used for bedding, such as straw, wood products such as sawdust or newspaper, corn cobs or stalks, excluding dried manure.

Physical Contact: means nose-to-nose contact or sniffing/touching/licking each other, including through a fence

Sick as used here refers to cattle designated as sick by personnel on your farm or by a veterinarian. Include all illnesses that would result in cattle being segregated, and/or treated with systemic antibiotics. This would include, but is not limited to lameness, respiratory disorders, and diarrhea.

Treated cows means cows that have been given antibiotics.

Youngstock: means all animals that are past weaning age and will not be kept as replacements (e.g., steers and heifers raised for beef)

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[1] As of date of survey completion, number of milking cows in first lactation

[2] As of date of survey completion, number of milking cows, lactation 2 & up

[3] As of date of survey completion, total number of milking cows (lactation 1 and up)

[4] As of date of survey completion, number of dry cows finished with first lactation, but before start of second lactation

[5] As of date of survey completion, number of dry cows, lactation 2 & up.

[6] As of date of survey completion, total number of dry cows (lactation 1 & up)

[7] As of date of survey completion, total cows (milking and dry, lactation 1 & up)

[8] As of date of survey completion, number of preweaned (milk-fed) heifer calves

[9] As of date of survey completion, number of weaned replacement calves and heifers

[10] As of date of survey completion, number of "other youngstock"

[11] As of date of survey completion, number of bulls kept for breeding purposes

[12] As of date of survey completion, total number of dairy cattle present on operation

[13] Number of total milk cows (milking and dry) born and raised on this operation

[14] Number of total milk cows (milking and dry) born here but raised elsewhere.

[15] Number of total milk cows (milking and dry) not born on this operation

[16] Total number of total milk cows (milking and dry) (should agree with #7 above)

[17] Beef cattle present on operation 1=yes, 2=no

Herd Visit Questionnaire

Risk Factors for Salmonella and Campylobacter Infections and Drug Resistance in Dairy Cattle

This short questionnaire is to be given every two months (at each sampling visit) in order to capture management and inventory changes that may have occurred since the initial questionnaire was given.

IMPORTANT: Note that on questions 4 and 5, the questionnaire administrator should pencil in answers from the last administration of the questionnaire and note any changes between previous answers and what is being fed today. Ask questions in the format "Are you still feeding blood meal to high-producing cows?" for feeds that were previously fed. For feeds that weren't fed in the past, make sure they are not now feeding them, such as by asking "Are you feeding any blood meal to any cows now?" and, if so, ask further which groups are being fed blood meal.

Date:_____

Study ID number:_____

Person to whom herd visit questionnaire is administered_____

Herd visit questionnaire administrator_____

Date of next visit_____

1. As of today, what is your inventory of the following groups of **dairy cattle**?

	Total
A. Total cows (milking and dry)	1
B. Preweaned (milk-fed) heifer calves	2
C. Weaned replacement calves and heifers*	3

* "Weaned replacement calves and heifers" here means all female animals that will be kept as replacement cows, have not yet calved, and are no longer receiving milk or milk replacer as part of the diet.

2. Were any of the following groups of animals brought onto this operation from outside sources **during the last 60 days**?

	Brought onto operation? 1 = YES 2 = NO	IF YES, How many were brought onto operation?
A. Preweaned (milk-fed) calves?	4 ☐ Yes ☐ No	5
B. Weaned dairy calves or heifers?	6 ☐ Yes ☐ No	7
C. Dairy cows?	8 ☐ Yes ☐ No	9
D. Bulls?	10 ☐ Yes ☐ No	11
E. Other cattle, including beef?	12 ☐ Yes ☐ No	13
E. Total.		14

* "Isolated" here means that the animal(s) is held for a period of time in a separate pen or other facility where nose-to-nose contact with cattle in the existing herd is prevented.

3. Within the last 60 days, how many dairy cattle within the following groups had diarrhea or died?

Coding instructions: 1 = checked; 2 = unchecked	Number of animals with diarrhea lasting at least 24 hours?	Number of deaths among animals with diarrhea lasting at least 24 hours	Number of total animals that have died
Preweaned calves	15	16	17
Weaned heifers	18	19	20
Milk cows (milking or dry)	21	22	23

4. Have the ration ingredients for milking and dry cows changed since the last time our questionnaire was given? Compare answers from the previous questionnaire with what is now being fed and note any changes in the table below. Include only **purchased feeds or feeds obtained from off-farm sources**. (Check all that apply).

Type of Feed	High-Producing Cows*	Other Milking Cows*	Dry Cows
A. Whole cottonseed/hulls	24	25	26
B. Cottonseed meal	27	28	29
C. Whole soybeans or soybean meal	30	31	32
D. Bakery by-products	33	34	35
E. Brewers by-products (includes distillers' grains)	36	37	38
F. Blood meal	39	40	41
G. Meat & bone meal (e.g., porcine-only or equine-only)	42	43	44
G. Milk products (e.g., whey)	45	46	47
H. Tallow/animal fat	48	49	50
I. Other protein meal (e.g., meal from fish or poultry) Please specify_____ 51	52	53	54

* If high-producing cows are not fed differently from other milking cows, put N/A in the "Other Milking Cows" column.

5. Have the types of **milk or calf starter** fed to preweaned calves changed since the last time our questionnaire was given? Compare answers from the previous questionnaire with what is now being fed and note any changes in the table below. Include only calves **that are kept up to weaning**, after they have received colostrum.

	Included in diet?
A. Whole milk from untreated* cows	55 ☐ Yes ☐ No
B. Whole milk from treated* cows (waste milk)	56 ☐ Yes ☐ No
C. Milk replacer without antibiotics	57 ☐ Yes ☐ No
D. Milk replacer containing antibiotics	58 ☐ Yes ☐ No
E. Calf starter without antibiotics	59 ☐ Yes ☐ No
F. Calf starter containing antibiotics	60 ☐ Yes ☐ No
G. Other (specify)_____61	62 ☐ Yes ☐ No

Answer question #6 only if C, D, E, or F. is YES

* "Treated cows" refers to cows that have been given antibiotics and are still within the milk withholding period. (A cow given Naxcel/Excenel is **not** considered a "treated cow" here).

6. List the types of antibiotics used and the brand names of the milk replacer or calf starter below. If unknown, ask to look at tag of bag/container.

Antibiotics used, if any
_____63

Brand name of milk replacer_____64

Brand name of calf starter_____65

1 = YES 2 = NO

7. Within the past 60 days, have you **used any medications in feed or water in weaned calves or heifers** (other than coccidiostats)? .....66..... Yes ☐ No ☐

A. **IF YES,** Please list the feed or water medications used. Include brand name of additive, medication name, and duration of use:

_____67

8. Within the past 60 days, have you **used any medications in feed or water in adult cows?** ..... 68 ☐ Yes ☐ No

A.**If YES,** Please list the feed or water medications used. Include brand name of additive, medication name, and duration of use:

_____69

9. Within the past 60 days, approximately how much of the following antibiotics have you used? **Fill in only one column per row in the table below.**

		Approximate number of bottles used, including bottle size (put "0" if do not use or if used less than one bottle in past two months)			Approximate number of doses*, if less than one bottle was used.
		# bottles	size of bottle (# ml or g)	units (ml or g) coding: (1 = ml; 2 = g)	
Penicillin-type	Pencillin	94_____	95_____	96_____	97___doses
	Amoxicillin (e.g., Amoxi-inject)	98_____	99_____	100_____	101___doses
	Ampicillin (e.g., Polyflex)	102_____	103_____	104_____	105___doses
Cephalosporin-type Includes ceftiofur (Naxcel, Excenel)		106_____	107_____	108_____	109___doses
Tetracycline-type (includes LA-200, Oxy-Tet-100)		110_____	111_____	112_____	113___doses
Sulfonamides	Albon or other sulfas	114_____	115_____	116_____	117___doses
	Trimethoprim-sulfa type (e.g., Tribrissen, SMZ-TMP, Primor)	118_____	119_____	120_____	121___doses
Florfenicol (NuFlor)		122_____	123_____	124_____	125___doses
Tilmicosin (Micotil)		126_____	127_____	128_____	129___doses
LS-50 (Spectinomycin/Lincomycin soluble powder)		130_____	131_____	132_____	133___doses
Other antibiotics(e.g., Spectam, Gentocin, Erythromycin, etc. Please specify)					

* A "dose" here means one administration of antibiotic. e.g., if you give 20 ml of Naxcel to a cow, that is one dose. If you give another 20 ml the next day to the same cow, that is another dose

Glossary of Terms

The terms listed below are defined according to how they are meant to be used in this survey.

Dose: as used here means one administration of antibiotic. e.g., if you give 20 ml of Naxcel to a cow, that is one dose. If you give another 20 ml the next day to the same cow, that is another dose.

Heifer: Non-lactating weaned female animal that has not yet calved.

Isolated/Isolation: A newly acquired animal(s) is held for a period of time in a separate pen or other facility where nose-to-nose contact with cattle in the existing herd is prevented

Medications: as used here refers specifically to antibiotics—it does not refer to probiotics, anthelmintics and other non-antibiotic medications.

Physical Contact: means nose-to-nose contact or sniffing/touching/licking each other, including through a fence

Preweaned calves: as used here means calves that are still receiving milk or milk replacer.

Treated cows: means cows that have been given antibiotics and are still within the milk withholding period. (A cow given Naxcel/Excenel is **not** considered a "treated cow" here).

Weaned: refers to animals that are no longer receiving milk or milk replacer.

Weaned replacement calves and heifers: here means all female animals that will be kept as replacement cows, have not yet calved, and are no longer receiving milk or milk replacer as part of the diet

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