# A RETROSPECTIVE STUDY OF CAMPYLOBACTER JEJUNI ENTERITIS AND GUILLAIN-BARRE SYNDROME IN MICHIGAN: 1992 – 1999

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ABSTRACT

A RETROSPECTIVE STUDY OF *CAMPYLOBACTER JEJUNI* ENTERITIS AND GUILLAIN-BARRE SYNDROME IN MICHIGAN: 1992-1999

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The association between *Campylobacter jejuni* and Guillain-Barré Syndrome (GBS) has been extensively researched in recent years. Because *C. jejuni* enteritis is common and GBS is severe, understanding the distribution and risk factors for *C. jejuni* enteritis and how this infection leads to GBS has become a priority.

In this work, three major objectives were addressed in three studies. The first study was designed to determine if increased rate of *C. jejuni* infection was evident in areas of high poultry density. The second study assessed if GBS rates were also higher in these areas. The third study was designed to determine what animal species were associated with increased odds of infection in rural counties at the individual level.

In the first study, Michigan was divided into two regions of high and low poultry density and *C. jejuni* incidence rates were compared between the regions. The incidence of *C. jejuni* was 1.3 times higher in the high poultry density region. In the second study, the GBS rate was compared between the high and low *C. jejuni* enteritis regions determined by the first study. No significant differences were found. In the third study, poultry husbandry was found to significantly increase the odds for *C. jejuni* enteritis in rural areas.

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Special thanks go to my advisor, John Kaneene. I have learned a great many things from him and by his example. I feel very lucky to have had the opportunity to work with him. It has been a great experience and I look forward to working with him again in the future.

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# INTRODUCTION

## Purpose

*Campylobacter jejuni* is a gram-negative bacterium that can cause severe gastroenteritis in humans, but is a commensal in many warm-blooded animals. These animals are the reservoirs for the organism. *Campylobacter jejuni* infection is thought to be the most common cause of Guillain-Barré Syndrome (GBS), a post-infectious flaccid paralysis. Previous ecological studies have revealed that *C. jejuni* incidence rates are highest in farming areas, followed by rural areas, with urban areas having the lowest rates, but the risk factors underlying these rates have not been elucidated. There is a need to identify the risk factors in rural and farming communities so that control and prevention measures can be focused appropriately.

## Objectives

1. Describe the epidemiology of GBS and *C. jejuni* enteritis in Michigan.
2. Identify the risk factors for *C. jejuni* enteritis in rural counties.
3. Compare the distribution of GBS cases to that of *C. jejuni* cases.
4. Assess the usefulness of the Michigan Department of Community Health reporting system for future studies of GBS in Michigan.

## Hypotheses Tested

1. The odds of developing *C. jejuni* enteritis are higher in counties with high poultry density than those with low density (hypothesis tested in Chapter 3).
2. *Campylobacter jejuni* is strongly associated with Guillain-Barré Syndrome (Hypothesis tested in Chapter 4).

3. Exposure to food animals is a major risk factor for *C. jejuni* enteritis and the odds of infection changes with exposure to different food animal species (Hypothesis tested in Chapter 5).

**Overview**

Chapter 1 is a literature review of the risk factors for *C. jejuni* enteritis. Particular attention is paid to urban and rural disease determinants. Chapter 2 is a review of the association between *C. jejuni* and GBS. Gaps in the current understanding of this association are identified and areas where future research is needed are described. Chapter 3 is an ecological study of *C. jejuni* enteritis in Michigan in the eight-year period between 1992 and 1999. Chapter 4 describes the epidemiology of GBS in Michigan between 1992 and 1999. Chapter 5 is a case-control study of *C. jejuni* enteritis in rural Michigan. Each chapter is in a format for independent publication. The overall contribution of this work to the understanding of both diseases is presented in the conclusion.

CHAPTER 1

# RISK FACTORS FOR SPORADIC *C. JEJUNI* ENTERITIS IN DEVELOPED COUNTRIES: A REVIEW

## Introduction

*Campylobacter jejuni* is the leading cause of food-borne disease outbreaks in the United States. The yearly incidence rate was 15.7 / 100,000 person-years in 2000, according to the CDC FoodNet active surveillance system (CDC, 2001). *Campylobacter jejuni* is a gram-negative, microaerophilic, thermophilic organism. Morphologically, it is a spiral or curved rod that is highly motile due to a single polar flagellum. Illness occurs three to five days after ingestion of a dose as low as 500 organisms (Robinson, 1981). Clinical signs and symptoms include fever, profuse, often bloody, diarrhea, abdominal pain, which may be severe enough to mimic appendicitis, and nausea. Clinical signs may remain severe for 24 to 48 hours before resolving gradually over a period of one week or longer. Rarely, infection occurs at other sites causing cholecystitis, pancreatitis, cystitis, or septic abortion. Fatal bacteremia may occur in immunocompromised hosts (Manfredi et al., 1999). Sequelae to the infection include Guillain-Barre syndrome (GBS), rheumatic or reactive arthritis (Blaser, 1997; Bremell et al., 1991), and Hemolytic-uremic syndrome (HUS) (Sillero and Almirall, 1999).

GBS is a subacute polyneuropathy affecting motor, sensory, and autonomic nerves that supply the limbs and respiratory muscles. Cranial nerves may also become involved. The GBS mortality rate is approximately ten percent and recovery is often incomplete and / or delayed (Hughes and Rees, 1997). An annual incidence of 0.20 to 1.46 cases of GBS associated with *Campylobacter* per 100,000 population has been estimated (Buzby

et al., 1997). A retrospective cohort study in Sweden in revealed that *C. jejuni* cases experienced an incidence of GBS that was 100 times higher what was expected in the general population (McCarthy and Giesecke, 2001). Reactive arthritis may cause pain and incapacitation for several weeks to months in approximately one percent of *C. jejuni* patients (Skirrow and Blaser, 2000). HUS is characterized by microangiopathic anemia, thrombocytopenic purpura, and acute oliguric renal failure. It is only rarely reported in association with *Campylobacter jejuni* (Sillero and Almirall, 1999).

The main focus of this review will be the epidemiology of sporadic cases, which cause the burden of disease, in developed countries. Sources of infection will be discussed and areas where additional research is needed will be highlighted.

**Descriptive Epidemiology**

The epidemiologic features of *C. jejuni* enteritis are unique. Incidence rates vary by season, gender, age, and place.

*Season*

Sporadic campylobacteriosis in the U.S. overall shows a seasonal distribution characterized by a low number of reported cases from January through April, an increase in May and June, and a peak in July and August. After August, the rate falls off (CDC, 2000). Investigators in Scotland showed that the seasonal distribution of rural and urban cases differed, however. Isolations were more frequent in the third quarter of the year in cities, whereas in rural populations they were more predominant in the first quarter of the year. The authors noted that the first quarter peak in infection rates in rural areas coincided with spring calving (Sibbald and Sharp, 1985). This is plausible because it has

been found that calves have a higher prevalence of *C. jejuni* carriage before they are weaned than after (Busato et al., 1999).

*Gender and Age*

For the whole United States, age-specific incidence rates show a bimodal distribution. The highest isolation rate occurs in infancy (<1 year of age) followed by a later peak in infection among young adults. Males are affected more commonly than females. The reasons for the age and gender distribution are not currently known. The high rates in infants may be due to increased care seeking by parents, because they may rapidly become dehydrated, or because of the immaturity of their immune systems. The second peak occurring in young adults may be due to the so-called second weaning effect, in which self-reliance in food preparation is first learned.

The ratio of childhood to adult infections was significantly higher in rural areas than urban areas in one study (Sibbald and Sharp, 1985). A hypothesis generated from this finding was that children might have early exposure to *C. jejuni*, via raw milk consumption, and then go on to develop and maintain immunity that protects them from clinical infection later in life (Sibbald and Sharp, 1985). A case-control study of endemic campylobacteriosis in Dubuque, Iowa, a small city in a rural area had findings that supported the raw milk hypothesis generated by Sibbald and Sharp in 1985. Of 15 cases who drank raw milk, 12 were less than 10 years old (80%) and nine (60%) lived in rural areas, both significant differences from those that did not drink raw milk. Even more interestingly, the six cases who drank raw milk and were from urban areas had visited rural areas and consumed raw milk there (Schmid et al., 1987). It has also been shown

that regular raw milk consumption is protective against clinical signs of disease (Blaser et al., 1987; Jones et al., 1981).

The incidence of campylobacteriosis in HIV-infected patients is almost 39 times higher than the rate in the general population. In Los Angeles County between 1983 and 1987, the reported incidence of campylobacteriosis in AIDS patients was 519 cases per 100,000 population (Sorvillo, 1991).

*Place*

A few ecological studies have made comparisons between urban and rural disease rates in developed countries with mixed results. A study in Norway found a higher urban rate, which was explained by a higher proportion of imported cases in urban areas. Imported cases are those that are acquired during foreign travel. The isolation rates were almost the same when these imported cases were excluded (Kapperud and Aasen, 1992). In Yugoslavia, higher *Campylobacter* spp. isolation rates were found in rural areas, but this was due to the high proportion of *C. coli* isolates in rural areas; *C. jejuni* isolation rates were actually lower in rural areas than urban areas (Popovic-Uroic, 1989). A study in New Zealand (Briesman, 1990) found elevated rates in the rural population and a study in Canada (Thompson et al., 1986) found higher rates in the rural farm population.

**Analytical Epidemiology**

The risk factors for sporadic infections and outbreaks are different. Outbreaks are most commonly linked to the consumption of untreated water (Alary and Nadeau, 1990; CDC, 1999; Millson et al. 1991) or unpasteurized milk (Evans et al. 1996; Harris et al. 1987; Kornblatt et al., 1985; Orr et al., 1995; Potter et al., 1983; Wood et al., 1992). A continuous source outbreak of campylobacteriosis has been traced to one supplier of

poultry, however (Pearson et al., 2000). The majority of cases of *C. jejuni* are sporadic, i.e., not associated with an outbreak (Friedman et al., 2000a). The epidemiological features of outbreaks are beyond the scope of this review. The following discussion is limited to sporadic cases.

*Person-to-Person Transmission*

Asymptomatic carriage of *C. jejuni* does not typically occur in industrialized nations (Blaser et al., 1980). Therefore, person to person transmission is uncommon but there are a few reports in the literature. In an outbreak involving 35 nursery school students a father of one of the children developed enteritis (Itoh et al., 1980). In another study, spread to household contacts was documented in six of 24 families with children testing positive for *C. jejuni* (Pai et al., 1979). In another outbreak due to contaminated milk in school age children, 21% of the cases apparently became infected due to sibling contact (Jones et al., 1981). It is noteworthy that person-to-person transmission often involves children as index cases and may reflect poor hygiene in this age group

*Foodborne Transmission*

Case-control studies have shown repeatedly that the majority of sporadic infections are food-borne, usually associated with the consumption of undercooked or raw chicken (see Table 1-1). Acquiring campylobacteriosis from undercooked chicken is biologically plausible because it is known that chickens harbor the organism in their intestinal tract (Achen et al., 1998), that their carcasses can become infected during processing (Atabay and Corry, 1997, Rivoal, et al., 1999), and that the carcass is still contaminated when on sale in the retail market (Uyttendaele et al., 1999). Thorough cooking of chicken is required to kill the organism. The consumption of undercooked poultry may contribute

to the age-specific and seasonal features of disease. The age peak in young adults is thought to correspond with self-reliance in food preparation and probably undercooking chicken. This has been described as a "second weaning" effect (Altekruse et al., 1994). Additionally, fried chicken and barbecued chicken, which tend to be undercooked, are consumed more commonly in the summer months and this may explain the increased incidence in warmer months (Ikram et al., 1994). Other studies have shown an association with eating any chicken, undercooked or not.

**Table 1-1: Odds ratios for *C. jejuni* enteritis associated with undercooked chicken consumption**

| Author | Study Design | Sample Size | OR* | 95% CI† |
|---|---|---|---|---|
| Hopkins, et al. 1984 | Case-control | 40 cases<br>71 controls | 2.77 | 1.10, 12.7 |
| Harris, et al. 1986 | Case-control | 218 cases<br>526 controls | 7.6 | 2.1, 27.6 |
| Deming, et al. 1987 | Case-control | 45 cases<br>45 controls | 9.0 | 1.1, 71.0 |
| Ikram, et al. 1994 | Case-control | 100 cases<br>100 controls | 4.94 | 1.03, 23.62 |
| Eberhart-Philips, et al. 1997 | Case-control | 621 cases<br>621 controls | 3.71 | 2.24, 6.13 |
| Neal and Slack 1997 | Case-control | 531 cases<br>512 controls | 2.7 | 1.1, 7.2 |
| Friedman, et al. 2000 | Case-control | 1463 cases<br>1317 controls | 1.9 | 1.3, 2.9 |

OR* = Odds Ratio CI† = Confidence Interval

The finding that consuming any chicken at all would increase the odds of infection (Table 1-2) may reflect some unidentified undercooked chicken consumption or cross-

contamination in the kitchen. Foods that were cross-contaminated with chicken juices have been linked to cases of *C. jejuni* enteritis (CDC, 1998). Thus, improper handling of raw chicken may confound this exposure.

**Table 1-2: Odds ratios for *C. jejuni* enteritis associated with any chicken consumption**

| Author | Design | Sample Size | OR* | 95% CI† |
|---|---|---|---|---|
| Harris, et al. 1986 | Case-control | 218 cases<br>526 controls | 2.4 | 1.6, 3.6 |
| Deming, et al. 1987 | Case-control | 45 cases<br>45 controls | 4.7 | 1.3, 16.2 |
| Kapperud, et al. 1992 | Case-control | 52 cases<br>103 controls | 2.69 | 1.23, 5.87 |
| Neal and Slack, 1997 | Case-control | 531 cases<br>512 controls | 1.3 | 1.1, 1.7 |
| Studahl and Andersson, 2000 | Case-control | 101 cases<br>198 controls | 2.29 | 1.29, 4.23 |
| Effler, et al. 2001 | Case-control | 211 cases<br>211 controls | 1.8 | 1.1, 2.9 |

OR*= Odds Ratio; 95% CI† = 95% Confidence Interval

Other foods associated with campylobacteriosis include poultry liver (Schorr et al., 1994), barbecued sausages (Kapperud et al., 1992), pork chops or loin (Studahl and Andersson, 2000), raw seafood (Friedman et al., 2000b), and raw milk (Friedman et al., 2000b; Hopkins et al., 1984; Schmid et al., 1987; Eberhart-Philips et al., 1997). Any raw or undercooked meat, fish, or shellfish may also increase the odds for sporadic infection

(Eberhart-Philips et al., 1997; Abeyta et al., 1993). The consumption of milk from bottles with bird-damaged tops is also associated with illness (Neal and Slack, 2000; Lighton et al., 1991). These risk factors are also biologically plausible.

Pigs were once thought to carry primarily *C. coli*, but they are now known to carry *C. jejuni* as well. In the Netherlands, 79% of pigs were intestinal carriers of *C. jejuni* (Oosterom et al., 1985). and the cecal contents of 31% of Texas pigs tested positive in another study (Harvey et al., 1999). *Campylobacter* is not commonly isolated from pork for retail sale, however (Oosterom et al., 1985; Duffy et al., 2001).

Milk may become contaminated with *Campylobacter* either by direct excretion of the organism from a mastitic udder or, more commonly, by fecal contamination of milk. *Campylobacter jejuni* mastitis has been induced experimentally and *C. jejuni* has been reisolated from the milk (Lander and Gill, 1979). Contamination of milk due to *C. jejuni* mastitis is thought to be rare, but direct milk excretion leading to human cases has been reported (Orr et al., 1995). Surveys of raw milk from bulk tanks have revealed variable rates of infection. Only 1.4% of raw milk samples from 27 farms were positive for *C. jejuni*, in one survey, but these farms were chosen specifically because they were known to produce high quality milk (Desmasures et al., 1997). Another survey of 256 raw milk samples in Manitoba found only 1.2% of the samples were contaminated (Davidson et al., 1989) while 12.3% of the milk shipped to an east Tennessee processing plant tested positive (Rohrbach et al., 1992). Only 500 organisms in milk are needed to induce enteritis in humans (Robinson, 1981). *Campylobacter* enters milk through improper hygiene when milking (Dilworth et al., 1988) or when instruments come in contact with the milking parlor floor (Humphrey and Beckett, 1986). Though *Campylobacter* cannot

multiply in milk, it can survive for up to 21 days at refrigerator temperatures (Doyle and Roman, 1982). Pasteurized milk can become contaminated after processing when bottle tops are pecked by birds (Lighton et al., 1991; Southern et al., 1990).

*Animal Contact*

*Campylobacter jejuni* is known to be a normal enteric commensal in the digestive tract of many warm-blooded animals. Zoo animals, including the llama, cape hydrax, chimpanzee, flamingo, common peafowl, and fantail pigeon have been found to carry the organism without exhibiting symptoms of infection (Misawa et al., 2000). Dogs and cats harbor the organism (Hald and Madsen, 1997; Baker et al., 1999; Fox et al., 1985), as do many bird species. Wild birds such as crows, gulls, and domestic pigeons can also harbor and transmit the organism (Kapperud and Rosef, 1983, Riordan et al., 1993). Investigators in Georgia collected fecal droppings and intestinal and cloacal samples of wild birds within a 200 foot radius of broiler chicken houses. Ten percent of these total samples were positive for *C. jejuni*, indicating that they carry the organism and could transmit it (Craven et al., 2000). *Campylobacter jejuni* has also been isolated from the bill and cloaca of birds (Hudson et al., 1991). Food animals, including chickens (Uyttendaele et al., 1999; Atabay and Corry, 1997; Achen et al., 1998; Rice et al., 1997), turkeys (Wallace et al., 1998), goats (Harris et al., 1987), sheep (Jones et al., 1999), cows (Busato et al., 1999), and swine (Harvey et al., 1999) carry *C. jejuni* in their digestive tracts. It is not surprising, then, that direct contact with animals is associated with *C. jejuni* enteritis. Examples of the kind of odds associated with animal contact are presented in Table 1-3.

**Table 1-3: Odds ratios for *C. jejuni* enteritis associated with animal contact**

| Author | Study Design | Sample Size | Animal | OR* | 95% CI† |
|---|---|---|---|---|---|
| Hopkins et al.1984 | Case-control | 40 cases<br>71 controls | Cat | 3.21 | 1.25, 8.3 |
| Deming et al. 1987 | Case-control | 45 cases<br>45 controls | Cat | 9.0 | 1.1, 71.0 |
| Kapperud et al. 1992 | Case-control | 52 cases<br>103 controls | Dog | 5.04 | 1.81, 14.02 |
| Saeed et al. 1993 | Case-control | 218 cases<br>526 controls | Adult Dog‡<br>Puppy‡ | 11.5<br>8.6 | 2.0, 65.9<br>1.3, 57 |
| Adak et al. 1995 | Case-control | 598 cases<br>738 controls | Household pet‡<br>Livestock and feces (occupational) | 2.39<br>0.44 | 1.09, 5.25<br>0.21, 0.92 |
| Eberhart – Philips et al. 1997 | Case-control | 621 cases<br>621 controls | Puppy<br>Calf feces | 3.94<br>4.40 | 1.57, 9.88<br>1.34, 14.39 |
| Neal and Slack, 1997 | Case-control | 313 cases<br>512 controls | Puppy | 11.3 | 1.2, 105 |
| Friedman et al. 2000 | Case-control | 1463 cases<br>1317 controls | Farm animals<br>Puppy | 2.2<br>2.0 | 1.5, 3.1<br>1.5, 2.8 |
| Studahl and Andersson 2000 | Case-control | 101 cases<br>198 controls | Chicken | 11.83 | 3.41, 62.03 |

OR* = Odds Ratio; 95% CI† = 95% Confidence Interval; ‡ = diarrheic

Companion Animals. Some studies have investigated the association between pets and *C. jejuni* infection, but have not found an association (Engleberg et al., 1984; Lighton et al., 1991). In at least one of these studies (Engleberg et al., 1984), however, the sample size was too small to detect a risk to pet ownership.

The age and health status of companion animals in the aforementioned studies is worth noting. In a study of household pets and kennel dogs and cats, the highest isolation

rates were in puppies and kittens (Blaser et al., 1980). *Campylobacter jejuni* has been isolated from cats and dogs with and without gastroenteritis (Gondrosen et al., 1985), but dogs that are less than 12 months old are almost three times as likely to be shedding *C. jejuni* if they have diarrhea, compared to those that do not (Burnens et al., 1992). A randomly selected sample of 11-17 week old puppies found that of 72 puppies, 29% were positive by culture for *Campylopbacter spp.* and 76% of those isolates were *C. jejuni* (Hald and Madsen, 1997). Other studies have also shown an inverse relationship between shedding of the organism and age of the dog, with the highest rates in dogs less than six months of age (Torre and Tello, 1993). The same study also showed that shedding was heaviest during summer and autumn, compared to winter. This increased shedding, in addition to the habit of acquiring new puppies in the summer months, may be another factor contributing to the seasonal distribution of disease in humans. It has been shown at the ecological level that the months of peak canine births correspond with the peak months of campylobacteriosis in humans (Evans, 1993). Similarly, the age distribution may be explained by puppy exposure, since children often have more intimate contact with puppies than adults do. A small study found a significant association between *Campylobacter* infection and presence of puppy in the household in the zero to five-year old age group (Salfield and Pugh, 1987).

Pets have been directly linked to individual cases. A case report of *C. jejuni* enteritis traced the source of infection back to a pet cat. The cat was shown to be excreting *C. jejuni* and the patient's serum titer to the isolate from the cat was high (Blaser et al., 1982). In a case series, five patients are described; all of which had contact with diarrheic puppies. *Campylobacter jejuni* was isolated from these dogs or their littermates (Blaser

et al., 1978). Another pet that is gaining in popularity and is known to harbor the organism is the ferret (Taylor et al., 1989). To date, there is no epidemiological evidence that the ferret is associated with sporadic cases of campylobacteriosis. Future case-control studies should include an analysis of this pet exposure.

Food Animals. A study in New Zealand revealed the increased odds with several food animal contacts. These included handling of calf or bovine feces, any contact with cattle, with new or aborted calves, or with cattle or calf carcasses in the ten days prior to illness or at work (Eberhart-Philips et al., 1997). Another study of *Campylobacter* enteritis on Hopi and Navajo reservations found that case households were more likely than control households to own farm animals, including poultry, cattle, sheep, goats, horses, or rabbits. Direct contact with poultry is associated with campylobacteriosis.

While case-control studies have shown that daily contact with poultry increases the odds of infection (Studahl and Andersson, 2000) there is evidence that long-term occupational contact with chickens is protective. That poultry workers are exposed to *C. jejuni* is clear. *Campylobacter jejuni* organisms have been isolated from the hands of processing-line poultry workers (Oosterom et al., 1983) and antibody response has been demonstrated (Jones and Robinson, 1981). A study of chicken abattoir workers in Sweden found evidence of higher mean IgG antibody levels in long term workers than short term workers and blood donors and, of eight workers with positive fecal cultures only one, a new employee, had enteritis (Cawthraw et al., 2000). This has been supported by decreased risk for occupational contact with livestock in a case-control study (Adak et al., 1995). Future studies on farm animal contact should assess the specific type of contact, if the contact is occupational, how frequently the contact occurs, and its duration.

*Waterborne*

Contaminated treated or well water and untreated surface water are both associated with *C. jejuni* enteritis. In a case-control study in Colorado, drinking untreated water was strongly associated with *Campylobacter jejuni*, increasing the odds for infection more than 10-fold (Hopkins et al., 1984). Investigators in England found that drinking untreated water from lakes, rivers, or streams also increased the odds of infection significantly (Adak et al., 1995). Non-urban water supplies were associated with illness in New Zealand (Ikram et al., 1994). Isolation rates for *C. jejuni* was significantly higher in rural areas than urban areas in a study carried out in Finland (Martikainen et al., 1990). The source of this contamination was thought to be grazing animals and pig or cow manure applied as fertilizer. Campylobacter contamination of a river system was found to increase after heavy rains increased surface run-off in rural areas (Bolton et al., 1987). *Campylobacter jejuni* was isolated from groundwater after a leaking slurry pit on a dairy farm contaminated it (Stanley et al., 1998). These findings may explain the increased incidence noted in rural areas.

**Conclusion**

Though much is known about how *Campylobacter jejuni* infection is acquired, much remains to be learned. The reasons for the unique age, gender, and seasonal distribution are not yet fully understood, and the reasons may be different in urban and rural populations. Future research should be geared towards understanding the risk factors for disease in these groups because prevention should be targeted to be most effective.

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# CHAPTER 2

## *CAMPYLOBACTER JEJUNI*, GUILLAIN-BARRE SYNDROME, AND THE CAUSAL CRITERIA


### Abstract

Guillain-Barré Syndrome (GBS) is the most common cause of acute generalized paralysis since the decline of polio. Current estimates suggest that approximately 30% of these cases are triggered by *Campylobacter jejuni*, the most frequently isolated cause of bacterial diarrhea in the United States. Because the costs of *C. jejuni* associated GBS are very high due to treatment, loss of productivity and life, and because of the moderate potential for exposure to *C. jejuni*, research investigating the association between *C. jejuni* and GBS has become a priority. In this paper Hill's criteria are used to evaluate the evidence for a causal relationship between *C. jejuni* and GBS and to identify gaps in current understanding of the association. While there is increasing evidence for an association, we conclude that additional research needs to be done before *C. jejuni* is considered a cause of GBS.


### Introduction

Guillain-Barré Syndrome (GBS) is the most common cause of acute, generalized paralysis since the decline of polio (Blaser et al., 1997). It is a subacute polyneuropathy that affects the motor, sensory and autonomic nerves supplying the limbs and may extend to involve the respiratory muscles and cranial nerves. Currently, between 2,628 to 9,575 new cases of GBS occur every year in the United States (Buzby and Roberts, 1997). About 10% of these patients will die, while another 10% will not completely recover (Hughes and Rees, 1997).

Since Guillain, Barré, and Strohl described the syndrome in 1916, it has been known as a post-infectious condition. If *C. jejuni* enteritis is a cause of GBS, it will be the most common antecedent infection, accounting for between 532 and 3,830 cases per year (Buzby and Roberts, 1997). The risk of GBS following *C. jejuni* infection has been estimated to be between one in 1,058 (Allos, 1997) and one in 3,285 (McCarthy and Giesecke, 2001). The cost of *C. jejuni* associated GBS is estimated to be between $0.2 to $1.8 billion each year in the United States alone (Buzby et al., 1997). When one considers the cost of GBS, the ubiquitous nature of *C. jejuni* in the environment and that it is the most common cause of bacterial gastroenteritis in the United States (CDC, 2001), it is apparent that determining if *C. jejuni* is a true cause of GBS should be a priority.

Epidemiologists use established criteria to help determine if an association is causal (Hill, 1965). These include strength of the association, consistency, specificity, temporality, biologic gradient, plausibility, coherence, and analogy. Evaluating the association with these criteria allows one to determine if the putative association between *C. jejuni* and GBS is causal and, if it is not, to direct targeted research. Therefore, the objective of this chapter is to evaluate the published literature to determine whether there is sufficient evidence for a causal association between *C. jejuni* and GBS.

**Strength of the Association**

In 1982, the first case of GBS that was preceded by a gastrointestinal illness caused by *C. jejuni* was reported. *Campylobacter jejuni* had been cultured from the patient's stool and a serum antibody response to the organism was also demonstrated (Rhodes and Tattersfield, 1982). Numerous case reports and case series from other investigators followed (Molnar et al., 1982; Sovilla et al., 1988).

Serosurveys of GBS patients also showed that *C. jejuni* antibody titers were higher than would be expected in the general population (Speed et al., 1987; Kuroki et al., 1991). Though these studies were not comparing cases and controls, they suggested a potentially strong association between *C. jejuni* and GBS because it appeared that *C. jejuni* enteritis would be one of the most, if not the most, common antecedent illnesses in GBS patients. Strength of association cannot be assessed by case series, however. It must be measured in an analytical epidemiologic study.

In 1993, Gregson, et al. compared GBS patients with healthy controls (Gregson et al., 1993). Elevated antibody titers to *C. jejuni* were detected in 15 of the 42 cases (35%) and none of the 41 controls. Another 1993 study also found that GBS cases were more than five times as likely (95% CI 2.4, 12.5) to have *C. jejuni* seropositivity compared to controls (Mishu et al., 1993). In a later study, *C. jejuni* antibody titers in GBS patients were compared to those in healthy controls, multiple sclerosis patients, myasthenia gravis patients, and neuroborreliosis patients. The GBS patients were significantly more likely ($p<0.05$) to be *C. jejuni* specific IgA positive than all other control groups, except for the myasthenia gravis group (Enders et al., 1993). Kaldor and Speed found evidence of *C.jejuni* in 21 of 56 GBS patients and none of 30 healthy controls (Kaldor and Speed, 1984). In 1995, Rees, et al., found 26% of GBS patients were diagnosed as *C. jejuni* positive compared to two percent of household controls and one percent of hospital controls, a highly significant difference (Rees et al., 1995). Ho et al. also found a large odds ratio for evidence of preceding *C. jejuni* infections in GBS

patients when compared with controls. Their point estimate was 10.2 with 95% confidence interval 3.8 to 27.8 (Ho et al., 1995). In a study by Jacobs, et al. (Jacobs et al., 1996), *C. jejuni* was found to be the most common antecedent infection among 154 GBS patients. It was more common than infection with the other common antecedent infections; cytomegalovirus, Epstein-Barr virus, *Mycoplasma pneumoniae*, *Haemophilus influenzae*, parainfuluenza 1 virus, influenza A, influenza B, adenovirus, herpes simplex virus, or varicella zoster virus. The odds ratio for infection with *C. jejuni* was 3.1 (95% CI 1.7, 5.9) between GBS cases and other neurological disease controls and 5.4 (95% CI 1.9, 15.1) between cases and healthy subjects controls.

Numerous studies have shown that the association between *C. jejuni* and GBS is strong and this finding enhances our suspicion that the relationship between the two may be causal. The reason for this is that it is hard to imagine a potential confounding biological or environmental variable that is so strongly associated with both *C. jejuni* infection and GBS that it could drive this association.

**Consistency**

Consistency refers to the repeated observation of an association by different investigators in different populations, places, circumstances, and times. This means that for a given association to be considered causal, the association should be found in a wide variety of situations and analytical designs. Consistency does not imply repeatability *per se*, because a flaw in study design may also be repeated.

A review of the strength criterion above will show that the association has been found repeatedly by different investigators. We are unaware of any investigations that have failed to find an association between GBS and *C. jejuni*, but this may only reflect the under-publishing of negative results. The association has also been found in different populations including those in Italy, China, Japan, the United States, the United Kingdom, and South Africa.

The analytical epidemiologic investigations to date, however, have been limited to retrospective or prospective case-control study designs with hospitalized cases. The consistency of the association has, therefore, not been established. The case-control design is well suited to the study of GBS because the disease is rare and because multiple etiologic factors can be assessed. Additionally, it is relatively quick and inexpensive when compared to cohort designs. The limitations of this study design, however, are several. First, it is inefficient for the study of rare exposures, such as some *C. jejuni* serotypes, unless the attributable risk is high. Second, as will be discussed later, the temporal relationship between exposure and outcome is difficult to establish. Third, case-control studies are particularly prone to biases, particularly selection and recall bias.

Selection bias is defined as an error due to systematic differences in the characteristics of those who are selected for study and those who are not. Selection of study participants may be biased due to the exposure, the disease, or a confounder. One possible example of selection bias that could be occurring on exposure in the hospital based case-control studies is Berkson's bias. Berkson's

bias occurs when the combination of exposure and disease under study increase the risk of admission to a hospital which leads to a systematically higher exposure rate among the hospital cases than the hospital controls. Evidence for this is that *C. jejuni* seems to lead to a more severe form of GBS, acute motor axonal neuropathy (AMAN), and may have a more rapid onset and delayed recovery (Hadden et al., 2001). Between 4.7 and 28% of GBS patients have a milder disease, retaining their ability to walk throughout their illness (Green et al. 2001) and these mildly affected patients are less likely to have evidence of *C. jejuni* and other antecedent infections (VanKoningsveld et al., 2000). Thus, GBS patients with antecedent *C. jejuni* infection may be more likely to be admitted to a referral center or be included in a clinical trial and become a study participant than GBS patients without prior *C. jejuni* infection. Several studies have been limited to cases in referral centers, infectious disease hospitals, or clinical trials (Enders et al., 1993; Kaldor and Speed, 1984; Jacobs et al., 1998). Admitted hospital patients with other neurological diseases have often been used as controls. Prior *C. jejuni* infection would play no role in the risk of their admission to the hospital. If this bias were operating, it would lead to an over-estimation of the odds ratio. Additional studies should be done to determine the effect of this bias and how many, if any, GBS patients never come to the attention of a neurologist.

Recall bias is a systematic error due to differences in accuracy of recall of past events between cases and controls. Cases are more likely to remember antecedent events than are healthy controls. When *C. jejuni* exposure is defined by elevated titers, recall bias is not a problem, but recall of diarrheal illness should not be used

as a proxy for *C. jejuni* infection because it would lead to an overestimate of the association.

Another difficulty with case-control studies is choosing an appropriate control group. Controls should represent the exposure experience of the source population. Because *C. jejuni* enteritis has a strong gender, age, and seasonal distribution, controls should be matched to GBS patients on these criteria. A failure to do so could bias study results in any direction. Failure to adhere to these principles leads not only to the selection bias discussed above, but it also decreases the internal and external validity of the study results.

Though the association has been observed by different investigators in different places and circumstances, the exclusive use of the case-control design with hospitalized cases means that the causal criterion of consistency has not been satisfied. Consistency is not repeatability.

**Specificity**

The criterion of specificity may be reviewed in two different ways. It may be looked at in terms of the specificity of the *relationship* between the exposure and outcome or in terms of the specificity of the *definition* of exposure and outcome.

The first way is not very useful because diseases often have more than one cause. GBS itself is a syndrome of multiple causes. It is possible to get GBS without ever having had *C. jejuni*, just as it is possible to have *C. jejuni* without ever developing GBS. Indeed, many possible causes of GBS have been identified including Epstein-Barr virus, *Mycoplasma pneumoniae*, and cytomegalovirus infections. There are case-control studies to support these associations (Jacbos et

al., 1998, Winer et al., 1988). Other infectious agents, such *as Toxoplasma gondii* (Bossi et al., 1998), *Helicobacter pylori* (Chiba et al., 1998), hepatitis A (Ono et al., 1994), Rocky Mountain Spotted Fever (Toerner et al., 1996), *Cyclospora* (Richardson et al., 1998), and West Nile Virus (Ahmed et al., 2000) have been incriminated in GBS case reports. These findings do not mean that the relationship is not causal; it only means that *C. jejuni* is neither a necessary nor a sufficient cause of GBS and that is an indication to apply the causal criteria.

A more useful application of this criterion is to the definition of exposure and outcome. If both exposure and outcome are very specifically defined and the relationship holds, causality is enhanced. In terms of GBS and *C. jejuni*, this means specificity of serotype of the organism and form of GBS.

*Campylobacter jejuni* must be cultured in order for it to be typed. During the two week period between *C. jejuni* infection and GBS the organism is often cleared from the GI tract or is shed in such low numbers that repeated cultures are needed to detect it (Nachamkin, 1997). When *C. jejuni* has been cultured from the stools of GBS patients and typed, serotypes O:2, O:4, O:11, O:19, and O:41 are predominantly found (Allos et al., 1998, Goddard et al., 1997). In retrospective studies, O:19 (Kuroki et al., 1993) and O:41 (Lastovica et al., 1997) is particularly predominant in isolates from GBS patients compared to uncomplicated *C. jejuni* enteritis isolates. Perhaps there may be other serotypes that also precede GBS, but these serotypes may tend to resist clearance long enough for the patient to develop GBS and for the organism to be cultured. Strains that induce GBS but are rapidly cleared would not be available for culture.

GBS may manifest itself in one of several ways. It may be the demyelinating form (AIDP), or cause axonal degeneration of exclusively motor nerves (AMAN) or both motor and sensory nerves (AMSAN). The AMAN and AMSAN forms are associated with poor or delayed recoveries. In the early 1990s, case reports started to suggest that *C. jejuni* was associated with a specific, severe form of GBS. Case reports have revealed evidence of antecedent *C. jejuni* in AMAN patients (Lugaresi et al., 1997). Case-comparison studies have also been done. One retrospective study of GBS patients found that poor recovery was significantly more frequent in 10 patients with high anti-*C. jejuni* titers (3 of 10) than in patients without this infection (0 of 48) (Kaldor and Speed, 1984; Vriesendorp et al., 1995). Nerve conduction studies of these patients were initially consistent with the AIDP form of the disease but as their disease progressed, axonal degeneration was evident. In 1996, Jacobs, et al., found that patients who were *C. jejuni* and anti-GM1 antibody positive were clinically distinct from patients without this antibody profile. They more often had the AMAN form of GBS (Jacobs et al., 1996). Additionally, *C. jejuni* positive Chinese patients with GBS were more likely to exhibit the AMAN form of the disease than *C. jejuni* negative patients (Nevo and Pestronk, 1997). Another study of 55 GBS patients in Japan, however, found no association between the type of GBS and the presence of specific anti-ganglioside antibodies (Yuki et al., 1999). Additionally, *C. jejuni* is not the only organism associated with AMAN; *Haemophilus influenzae* is also capable of eliciting this pathophysiologic response (Mori et al., 2000).

At this time, the authors are not aware of any study that attempts to directly link a specific *C. jejuni* serotype with severity or form of GBS. It has been indirectly investigated, however, and this will be discussed as evidence for the biological plausibility criterion. If future studies find that a specific serotype of *C. jejuni* causes an elevation of some specific anti-ganglioside antibodies and produces a specific form of GBS, then that would be strong evidence for causality.

**Temporality**

Because the *C. jejuni* and GBS association has been primarily investigated through case-control studies that measure exposure and outcome simultaneously, the temporal relationship between the two has not been well established. Exposure has been measured by means of antibody titers, culture, and recall of gastrointestinal illness. Elevated antibody titers have been very commonly used to determine exposure and are most troublesome for this criterion.

Serum antibody levels are typically elevated for several weeks and even months after infection, so anti-*C. jejuni* antibody in the serum of a GBS patient may reflect an exposure that occurred several months prior, and may have no relevance to the onset of GBS. It could also represent an exposure that occurred after the disease process had begun. Specifically, IgA titers are known to decline rapidly about one week after infection, while IgG levels peak about two weeks after onset of symptoms and decline after three or more weeks. IgG antibody may persist at high levels for several months in some patients (Kaldor et al., 1983, Blaser and Duncan, 1984). Studies that rely on recall of symptoms of gastroenteritis two to three weeks before onset of GBS are trying to establish

time-order, but they are prone to recall bias, and some *C. jejuni* cases may be mild or subclinical. In other case-control studies, exposure is measured by culture and, though this method is less sensitive than serology, it is a better measure for very recent antecedent infection than antibody titers because the organism is typically cleared from the GI tract two to three weeks after infection.

Similarly, the role of anti-ganglioside antibodies has not been established. They, too, have been measured after onset of GBS but does their presence indicate a response to the disease or a cause of it? The best way to establish time order for this disease is through a prospective cohort study or with an animal model.

**Biologic Gradient**

This criterion refers to a dose-response relationship. In this case, it could be interpreted either as seeing an increased incidence of GBS in locations with higher incidence of *C. jejuni*, or as an increasing risk for GBS with increasing dose of *C. jejuni*. The descriptive epidemiology of the disease in China argues more strongly for an infectious cause then does the descriptive epidemiology of the disease in developed countries. In China, where *C. jejuni* is a common enteric pathogen and significant numbers of children are asymptomatic carriers (Young et al., 1986), epidemics of the AMAN form of Guillain-Barré syndrome occur in the rural areas of northern China every summer. The primary involvement of children from rural areas with peak incidence in the summer is especially intriguing. Rural Chinese have been reported to drink unboiled well water and have contact with a variety of farm animals, including chickens, pigs, goats, and

dogs - all known risk factors for *C. jejuni* (McKhann et al., 1993). In a case-control study of GBS patients from these rural areas, significantly more had elevated titers to *C. jejuni* (66%) than did village controls (16%). The authors calculated that GBS patients were 10.2 (95% CI 3.8, 27.8) times more likely to have elevated anti-*C. jejuni* antibody titers than village controls (Ho et al., 1995). In an area of the world where *C. jejuni* infection is common, there is a pattern of GBS that is more severe (AMAN) and has the epidemiologic features of an infectious disease.

It is very difficult for an epidemiologic study to estimate the dose of pathogen ingested by cases, so traditional information on dose-response will have to wait for an appropriate animal model.

**Plausibility**

Fulfillment of this criterion requires that the hypothesized causal relationship between *C. jejuni* and GBS is biologically sound. There is circumstantial evidence to support the role of *C. jejuni* in GBS because secretory antibody is one of the primary barriers to adherence of the organism and the timing of peak antibody response to infection corresponds with GBS onset. This criterion has been extensively investigated in the literature.

It is believed that *C. jejuni* infection stimulates an autoimmune response through the process of molecular mimicry. In molecular mimicry, a host acquires an infection with an agent that has antigens that are immunologically similar to a host antigen, but differs sufficiently to induce an immune response. As a result, the tolerance to autoantigens breaks down and the pathogen-specific immune

response cross-reacts with host structures to cause tissue damage and disease (Albert and Inman, 1999). If this describes the pathogenesis of GBS following *C. jejuni* infection, then *C. jejuni* antigen must be similar to neurological host antigen, antibodies to host antigen should be produced in the immune response to infection, and these autoantibodies should cross-react with *C. jejuni* antigen.

Lipopolysaccharides (LPS), also known as endotoxins, are parts of the outer membrane of most gram-negative bacteria. Structurally, the LPS is composed of a polysaccharide or oligosaccharide covalently bound to a lipid component. Gangliosides are glycospingolipids that tend to occur in regional patterns in the nervous system. Gangliosides are referred to by an alphanumeric abbreviation. G stands for ganglio and the M, D, T, or Q following indicate the number of sialic acid residues. The numbers and lowercase letters following refer to the sequence of migration that is determined by thin-layer chromatography (Willison and O'Hanlon, 2000). The ganglioside GM1 is widely distributed in the nervous system. It has been found on axonal membranes at the node of Ranvier and the neuromuscular junction and on myelin, especially around the nodes (Feasby and Hughes, 1998). It has been shown that the core oligosaccharide (OS) of *C. jejuni* O:19 LPS contains tetra- and pentasaccharide moieties identical to those of GM1 and GD1a gangliosides, respectively (Moran et al.,1996). The LPS of O:41 isolates bear a GM1-like epitope and the core OS of O:4 mimics GD1a and GM1 epitopes (Yuki et al., 1997). These findings indicate that there are structural similarities between *C. jejuni* antigen and neurological host antigen.

It has also been shown that there is strong cross-reactivity between anti-GM1 IgM and the LPS from *C. jejuni* serotypes O:4 and O:19 (Wirguin et al., 1994). Additionally, cholera toxin, which specifically recognizes the GM1 oligosaccharide has shown reactivity with the LPS fraction of *C. jejuni* O:19 (Yuki et al., 1990).

Case control studies have found anti-GM1 antibodies in *C. jejuni*-preceded GBS patients, but not in controls (Walsh et al., 1991). In a study of GBS patients with anti-GM1 and / or anti-GD1b antibodies, these patients were more likely to have serological evidence of antecedent *C. jejuni* enteritis than GBS patients without these antibodies (Schwartz et al., 1982). In contrast, other studies have found no correlation between GM1 binding and *C. jejuni*, but did find the infection was associated with poor recovery and severe disease (Mishu et al., 1993). The association between *C. jejuni* and antiganglioside antibodies has not consistently been shown.

An alternate explanation of the role of antiganglioside antibodies is that they block neuronal sodium channels causing AMAN-like clinical signs, but with rapid resolution. There is some experimental evidence that GM1 can do this (Weber et al., 2000). It has not been determined how commonly this occurs, and what host factors, *C. jejuni* strains, or dose determines when this occurs.

The timing of the occurrence of antiganglioside antibodies is significant because, if they are induced by *C. jejuni*, they should precede the onset of GBS clinical signs. The implication here is that degenerating and damaged nerves may be the source of the anti-ganglioside response in GBS. It has been shown that

anti-ganglioside antibodies are produced following nerve injury (Winer et al., 1988). Future prospective studies should measure these antibodies in *C. jejuni* patients before they develop GBS. The presence of these antibodies before nerve damage was evident would strongly enhance the plausibility of *C. jejuni*-induced GBS.

The plausibility of a causal relationship between *C. jejuni* and GBS would be further enhanced if the putative host-environment interaction were better understood. The major histocompatibility complex (MHC), or human leukocyte antigen (HLA), has been implicated in the susceptibility to disease and to the development of autoimmunity because HLA molecules function as antigen-presenting structures. GBS is a heterogenous syndrome characterized by multiple etiologies and pathologies and it has therefore been difficult for investigators to identify a single HLA type associated with the syndrome (Ma et al., 1998; Rees et al., 1995). Studies that have been restricted to or have divided the GBS group into those with common antecedent infections or common outcomes (AIDP, AMAN) have more often found associations (Monos et al., 1997; Koga et al., 1998)

From an epidemiologic standpoint, it is not clear what kind of variable HLA type may be in the causal pathway between *C. jejuni* and GBS. It may function as an antecedent variable that causally precedes the *C. jejuni* - GBS association. This type of variable does not affect the strength of the association. Alternatively, HLA type may be a marker for an immune response to *C. jejuni* infection, in which case it would play no role in the causal chain. HLA may also be a modifying variable causing a more severe type of GBS when in combination

with *Campylobacter jejuni*. Further studies are needed to elucidate the role of HLA as a third variable in the causal pathway.

**Analogy**

This criterion is satisfied when the proposed causal relationship is comparable to some other established causal relationship. Two diseases and a few animal models have been proposed to satisfy this criterion.

In humans, acute disseminated encephalomyelitis (ADEM) and acute hemorrhagic leukoencephalitis (AHLE) are other post-infectious demyelinative diseases, but the demyelination occurs in the central nervous system rather than the peripheral nervous system. These diseases represent an immune-mediated complication often related to *Mycoplasma pneumoniae* infection, which parallels the hypothesized mechanism for *C. jejuni* induced GBS.

A disease of dogs, acute polyradiculoneuritis, or Coonhound Paralysis, is similar to GBS both clinically and pathologically. It usually occurs shortly after a raccoon bite, but has also occurred after rabies vaccination. The condition has been reproduced experimentally by injection of raccoon saliva into a dog that had recovered from two earlier spontaneous attacks.

Experimental allergic neuritis (EAN) in the Lewis rat is considered the *in vivo* model of GBS. EAN is quite different from the AMAN form of GBS. Unfortunately, EAN cannot be induced in the rat by *C. jejuni* or gangliosides (Vriesendorp et al., 1997). It can, however, provide some insight into the process of immune-mediated peripheral nerve demyelination.

Based on the examples from other human diseases and animal models, it is apparent that the criterion of analogy has been satisfied.

**Conclusion**

Whether or not these criteria should be used to determine if a relationship can be elevated from association to causation is a debate that is beyond the scope of this paper. These criteria can be used, however, to look at an association in a new way and to reveal the gaps in the current understanding of that association. We conclude that the criteria of temporality, consistency, and plausibility have not yet been satisfied, nor can they be with retrospective epidemiological studies. A prospective study or animal model is required to determine when in the course of the disease antiganglioside antibodies are developed, the role of HLA, what *C. jejuni* strains are associated with the disease, and the environmental source of these strains.

If, indeed, *C. jejuni* does cause GBS, then public health measures aimed at reducing the incidence of *C. jejuni* exposure could decrease the number of GBS cases by almost one third.

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CHAPTER 3

# CAMPYLOBACTER JEJUNI ENTERITIS IN MICHIGAN 1992-1999: A COMPARISON OF INCIDENCE RATES IN AREAS OF HIGH AND LOW POULTRY DENSITY


## Abstract

*Objectives.* To compare the incidence of *Campylobacter jejuni* enteritis in high and low poultry density counties in Michigan between the years 1992 and 1999.

*Methods.* An ecological study was conducted in the state of Michigan to evaluate the incidence of *Campylobacter jejuni* during an eight-year period in regions of high and low poultry density. A loglinear model was fit with Poisson regression methods to determine the association between incidence rate and poultry density controlling for age, gender, season, and year.

*Findings.* The overall incidence of *C. jejuni* enteritis was higher in counties with a high poultry density than in those without, particularly among children and young adults.

*Conclusions.* The findings suggest that high poultry density is associated with high rates of *C. jejuni* enteritis. Excess cases may be due to elevated risk among poultry workers or manure management and transport practices. Future studies should be conducted to address these issues.


## Introduction

Previous descriptive studies have compared the incidence rates for *C. jejuni* enteritis between urban, rural, and farming communities. In general, these studies have found that rates are higher in rural areas (Sibbald and Sharp, 1985), particularly rural farm areas (Thompson et al. 1986). Case-control studies have revealed that living on or visiting a farm (Friedman, 2000), having contact with farm animals (Friedman, 2000; Engleberg,

1984), and poultry contact (Studahl and Andersson, 2000; Potter, et al. submitted 2002) are major risk factors in rural areas. Rural is usually defined by human population density. If exposure to animals the cause of higher rates in rural areas, then rates should be higher in areas with high animal density than in those with low animal density. We hypothesized that the odds of developing *C. jejuni* enteritis would be higher in counties with high poultry density than in those with low density. Poultry were selected because very high isolation rates have been obtained from chickens. Using data collected from the National Agricultural Statistics Service (NASS), the counties of the state of Michigan were considered either high or low poultry density. Because there is a large variation in poultry density between counties, an ecological study design was considered.

**Methods**

*Study Design*

Ecological studies may be conducted when a group-level variable is of interest, when data on individuals is not available, or when convenience and cost are a concern. Ecological studies are most appropriate, however, when the role of a group property that cannot be measured at the individual level is being investigated. In this study, poultry density per county, a proxy for both occupational exposure to *C. jejuni* and the environmental burden of the organism, was a group-level variable hypothesized to be the antecedent cause of disease and not measurable in individuals.

*Source of Data*

Incidence rates for *Campylobacter* enteritis were obtained from cases reported to the Michigan Department of Community Health (MDCH) for the eight-year period between 1992 and 1999 and population counts from the 1990 census. Incidence rate data were

then categorized into strata for the covariates age (19 strata), gender (2 strata), year (8 strata), and season (4 strata) for each region. It was assumed that the age distribution of the population remained constant throughout the eight-year period and that disease occurrence was independent.

Poultry population estimates were obtained from the National Agricultural Statistics Service (NASS), which conducts a complete count of livestock inventory every five years. Additional data, collected yearly, are based on sample surveys. Data are not always published for all counties. If a county has less than 500 head or if birds are concentrated in only one operation within a county, data are not published for that county but provided as a summary number for the district. For its poultry count, Michigan only collects data on layers 20 weeks of age and over. Counties with less than one percent of the layers in the state were grouped into the low-density region. High-density counties had between one percent and 27% of the yearly inventory. In all, the high-density region had between 71% and 97% of the layers in the state between 1992 and 1999. Because of the way data is reported counties in which more than one percent of the state total chicken inventory was concentrated in one operation were misclassified as unexposed. This misclassification may bias results to the null because of the mixing of the exposure effect among the unexposed. High and low density counties by year are shown in table 1.

*Statistical Analyses*

Estimates of the incidence rate were calculated by dividing the number of new cases by population counts from the 1990 census.

A loglinear model was fit with Poisson regression methods to estimate incidence rate ratios (IRR). The model assumes independence and homogeneity (Flanders and

Kleinbaum, 1995). Independence was assumed because person-to-person transmission of *C. jejuni* is rare. If one person has *C. jejuni* enteritis, it does not change the risk of others in the group of developing *C. jejuni* enteritis. Homogeneity means that disease risks are the same across people and time. We assumed that the dependent variable, the case count, followed a Poisson distribution. Independent variables included in the model were five-year age group, gender, season, year, and poultry density. Epi Info, Microsoft Excel and SAS Version 8 were used for data entry and analysis.

**Results**

The risk (estimated by the rate ratio) for *C. jejuni* enteritis was 1.31 (95% CI 1.04, 1.42) times higher in the high-density region than the low-density region after controlling for age, gender, season, and year. The incidence rates for each region with 95% confidence intervals (95% CI) are 12.01 cases / 100,000 population (95% CI; 11.09, 12.98) in high poultry density counties and 8.60 cases / 100,000 population (95% CI; 8.38, 8.82) in low-density counties (Table 2). Counties with a high poultry density had a significantly higher incidence every year during the eight-year study period except 1997 and 1998. In unexposed counties and the state as a whole, the incidence of *Campylobacter* enteritis has steadily decreased between 1992 and 1999. In counties with a large poultry inventory, however, the rate was lowest in 1997 but rose again in 1998 and 1999 (Figure 1).

Age-specific and gender-specific rates varied by region. Rates were significantly higher for one to nine year-olds, 25 to 29 year-olds, 35 to 39 year-olds and 45 to 49 year-olds in counties with high poultry density compared to the referent (Figure 2). Males were at higher risk in the state as a whole and in the referent region, but there was no

significant difference in gender-specific rates in the region with high poultry inventory (Figure 3).

The high poultry prevalence region had the highest rate every season of the year, but the rate ratio was largest in winter. Incidence rates were 1.36 times higher (95% CI; 1.18, 1.79) in winter in counties with a large poultry inventory than those with a low inventory (Figure 4).

**Discussion**

Ecological studies are necessary for describing group-level properties but are criticized for not being applicable to individuals. Group-level variables do, however, cause disease (Schwartz, 1994). We hypothesized that poultry density was a variable particular to a group, much like "contagion" (Susser, 1994). A high prevalence of *Campylobacter*-excreting chickens in a county may increase the risk of disease for everyone in that county whether they have direct contact with poultry or not because *C. jejuni* may be highly prevalent in the environment and in other animal species. It may modify the effect of individual-level risk factors.

There may be at least two mechanisms functioning to increase *C. jejuni* enteritis rates in the high-density region. First, poultry workers may be responsible for the excess cases. Second, poultry may be shedding *Campylobacters* into the environment causing other animals, including sheep, swine, cattle, dogs, and cats, to become colonized. This would increase the risk for campylobacteriosis associated not only with direct contact with these animals but also with drinking unpasteurized milk and untreated water. In this case, the immediate cause of campylobacteriosis may be contact with a dog, cow, cat or

consumption of untreated water or raw milk, but the ultimate cause is the large environmental pool of *Campylobacters* in the area.

Exposure of poultry workers to *C. jejuni* has been established. *Campylobacter jejuni* organisms were isolated from the hands of processing-line poultry workers in a study in The Netherlands (Oosterom et al., 1983) and antibody to *C. jejuni* was higher in poultry processing workers than in antenatal patients in Great Britain (Jones and Robinson, 1981). A study of chicken abattoir workers in Sweden found evidence of higher mean IgG antibody levels in long term workers than short term workers and blood donors (Cawthraw et al., 2000). Evidence for a direct relationship between antibody titers and length of employment was suggested from the data, but it was not statistically significant. Interestingly, fecal cultures were positive for eight workers in the study, but only one, a short-term worker, had symptoms of enteritis (Cawthraw et al., 2000). These findings and others (Blaser et al., 1987) suggest that the low antibody titers are probably protective against symptomatic *C. jejuni* enteritis. Infection may occur among new employees. This has been supported by a protective effect for occupational contact with livestock in a case-control study (Adak et al., 1995). In contrast, daily contact with poultry has been associated with increased odds for enteritis (Studahl and Andersson, 2000), as has poultry husbandry (Potter et al. submitted, 2002) in case-control studies. The increased risk among young adults in high-density areas would suggest that workers are responsible for at least some of the excess cases. The three poultry processing plants in Michigan are not located in high poultry density counties. They are located in Kent, Barry, and Livingston Counties. Kent and Barry counties are adjacent counties to the east of the high poultry density counties of Allegan and Ottawa. This may contribute to

the non-differential misclassification of poultry density in the study. Additional study should be undertaken to elucidate the epidemiology of *C. jejuni* infection in farm and abattoir workers.

Warm-blooded animals are good reservoirs for *C. jejuni* because they rapidly become colonized by the organism but do not develop illness. Animals may acquire the infection in one of several ways. They may become infected by direct contact with other members of their flock or herd who are excreting the organism. Fomites, such as boots, may transmit the organism from infected animals to non-infected animals. Water can carry the organism to susceptible animals (Kapperud et al., 1993, Stanley et al., 1998). Vectors, such as flies (Khalil et al., 1994) or wild birds (Riordan et al., 1993, Craven et al., 2000) may also introduce the organism into a flock or herd. Companion animals may also carry the organism (Gondrosen et al., 1985) so they might also act as vectors, moving the organism between farm animal species and humans.

These findings may also be due to biases. If there are differences in reporting in the high and low density region or if there is a high percentage of uninsured in the low density counties, for example in the urban centers, then the incidence rate differences we found may be due to these biases. Future studies should investigate the potential influence of these biases on the study results. One method would be to compare the distribution of another reportable disease, not associated with chicken exposure, in the high and low poultry density counties.

Additional work is needed to discover the source of the excess cases in the high-density region. The measurement of chicken density by county is a crude way to determine exposure but we were limited by the data that were available. Future studies

may look at poultry per person per area as a better measure of exposure. Because only data on layers per county was available, we did not measure exposure in this way. Additionally, the exposure to chickens within counties may vary considerably. For example, Monroe County was considered high density but is an urban county near Detroit, the largest city in Michigan, and has a very large population. However, the poultry houses must have been in a sparsely populated area within that county. The epidemiology of *C. jejuni* infection in poultry workers should be investigated to understand if they are experiencing higher or lower than expected rates of infection. Investigations of the way manure is managed and its impact on the environment may also reveal the cause of the excess cases. A comparison of *C. jejuni* colonization in farm, wild, and companion animals in the two regions would also be of interest. Changes in manure management practices or hygiene in poultry workers may be needed to decrease *C. jejuni* enteritis in the high poultry density counties. These interventions should be implemented at the ecological level.

**Table 3-1: High and low density counties by year 1992 – 1999**

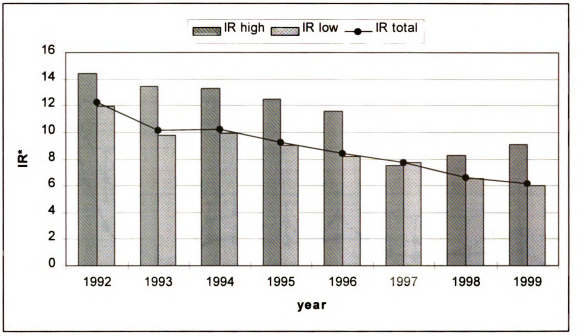
| 1992 | 1993 | 1994 | 1995 | 1996 | 1997 | 1998 | 1999 |
|---|---|---|---|---|---|---|---|
| Ottawa | Ottawa | Ottawa | Ottawa | Ottawa | Ottawa | Ottawa | Ottawa |
| Allegan | Allegan | Allegan | Allegan | Allegan | Allegan | Allegan | Allegan |
| Ionia | Ionia | Ionia | | | | | |
| Huron | Huron | Huron | Huron | Huron | Huron | Huron | Huron |
| K-zoo* | K-zoo | K-zoo | | | | | K-zoo |
| Isabella | Isabella | Isabella | Isabella | | | | |
| Tuscola | Tuscola | Tuscola | Tuscola | Tuscola | Tuscola | Tuscola | Tuscola |
| VanBuren | VanBuren | VanBuren | | | | | |
| Newaygo | Newaygo | Newaygo | Newaygo | Newaygo | | | |
| Hillsdale | Hillsdale | Hillsdale | Hillsdale | Hillsdale | Hillsdale | Hillsdale | |
| Gratiot | Gratiot | | | | | | |
| Monroe | Monroe | Monroe | | | | | |

K-zoo* = Kalamazoo county

**Table 3-2: Distribution of cases between areas of high and low poultry density 1992 – 1999.**

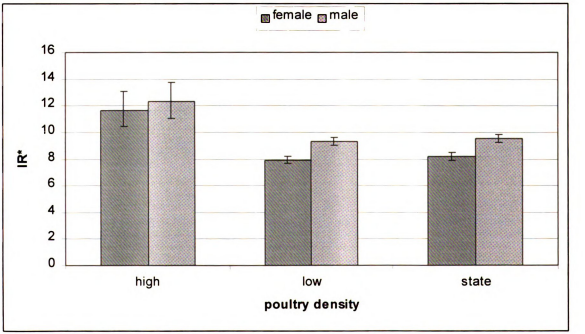
| Region | Number of cases | Population | Incidence Rate per 100,000 population |
|---|---|---|---|
| High Density | 639 | 665,297 | 12.01 |
| Low Density | 5935 | 8,630,000 | 8.37 |
| Total | 6574 | 9,295,297 | 8.63 |

**Figure 3-1: Yearly incidence rates in high and low poultry density regions: Michigan 1992-1999**



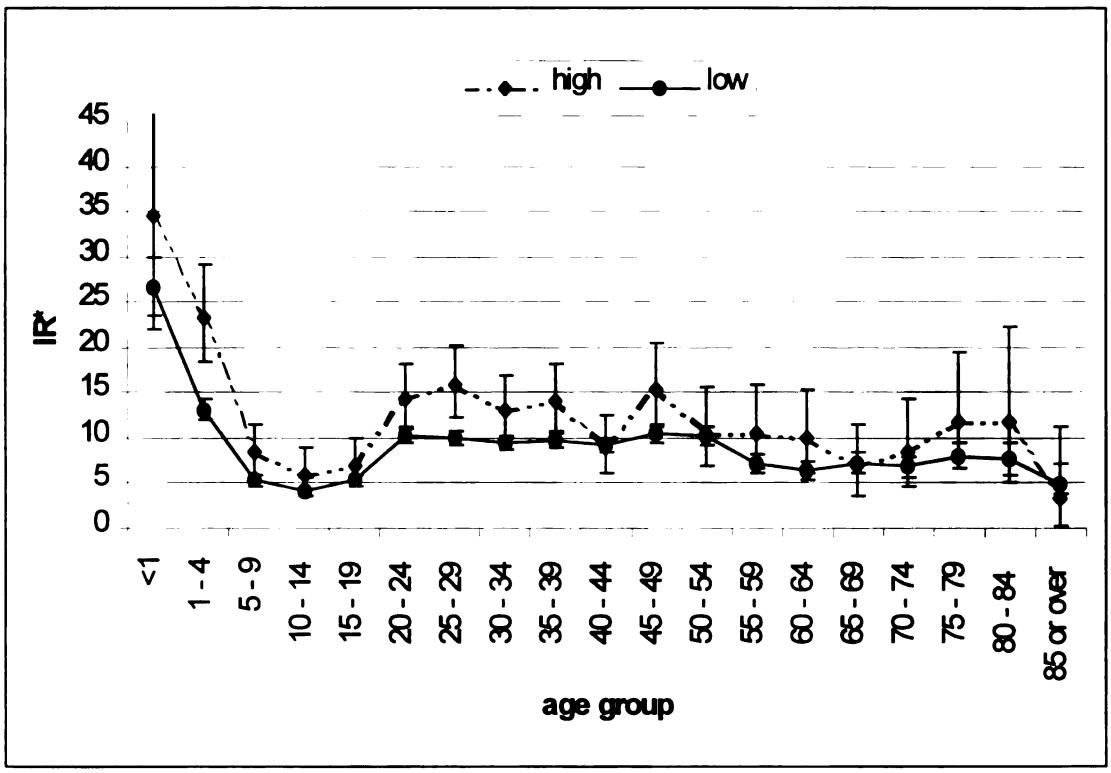

IR* = incidence rate / 100,000 population

**Figure 3-2: Gender-specific incidence rates in high and low poultry density regions: Michigan 1992-1999**



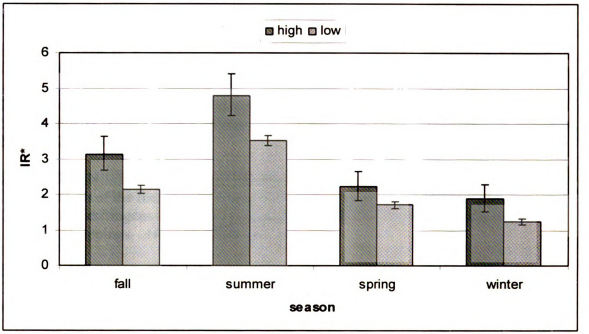

IR* = incidence rate / 100,000 population

**Figure 3-3: Age-specific incidence rates in high and low poultry density regions: Michigan 1992-1999**




IR* = incidence rate / 100,000 population

**Figure 3-4: Seasonal incidence rates in high and low poultry density regions: Michigan 1992-1999**




IR* = Incidence rate / 100,000 population

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CHAPTER 4

# DESCRIPTIVE EPIDEMIOLOGY OF GUILLAIN-BARRE SYNDROME IN MICHIGAN: 1992 - 1999

## Introduction

Guillain-Barré Syndrome (GBS) is an acute inflammatory demyelinating polyneuropathy that is usually post-infectious. *Campylobacter jejuni* is the most common cause of bacterial gastroenteritis in the United States (CDC, 2001). A previous ecological study of the distribution of reported *C. jejuni* enteritis cases in the state of Michigan between 1992 and 1999 revealed significantly elevated incidence rates in counties with high poultry density (Potter, et al. submitted 2002). A recent *C. jejuni* infection has been estimated to increase the risk of developing GBS approximately 100-fold (McCarthy and Giesecke, 2001). We hypothesized that because *C. jejuni* is strongly associated with GBS (Enders et al., 1993, Mishu et al., 1993, Rees et al., 1995), the distribution of GBS cases would follow that of *C. jejuni* cases. Reported cases were used to test this hypothesis

## Methods

### *Study Design*

Ecological studies may be conducted when a group-level variable is of interest, when data on individuals is not available, or when convenience and cost are a concern. Ecological studies are most appropriate, however, when the role of a group property that cannot be measured at the individual level is being investigated. In this study, an area of high *C. jejuni* incidence was compared with a low incidence region. These regions have

been described (Potter et al., submitted 2002). The level of contagion in a community is a group level variable.

*Sources of Data*

Reported cases of GBS were collected the Michigan Department of Community Health (MDCH). Population denominator data were obtained from 1990 census data.

*Statistical Analyses*

The number of new cases per 1,000,000 population was used to estimate incidence rates. Exact 95% Poisson confidence intervals were calculated around the point estimate of the rate.

**Results**

Overall, 479 cases of GBS were reported to MDCH between 1992 and 1999. The records for eight of these cases were incomplete, so they were dropped, leaving 471 cases for analysis. This gives a crude annual incidence rate of 6.3 / 1,000,000 population. This rate is lower than has been reported in other descriptive studies (Hughes and Rees, 1997), and may indicate under-reporting.

Men were affected more often than women. The overall incidence rate for men was 7.4 cases per 1,000,000 population (95% Confidence Interval; 6.6, 8.4) and 5.3 cases per 1,000,000 population (95% CI; 4.6, 6.1) for women. There was no significant difference in the incidence rates of GBS in high and low *Campylobacter jejuni* incidence regions. In the high incidence region the crude rate was 5.8/1,000,000 population (95% CI: 3.9, 8.3) compared to 6.4/1.000,000 population (95% CI: 5.8, 7.0) in the low incidence region.

For all years and both sexes, the age-specific incidence rate showed a bimodal distribution with a low peak in the 10-14 year old age group and a higher peak in the 70-74 year old group (Table 4-1).

**Table 4-1: Age-specific incidence rates for GBS in Michigan: 1992-1999**

| Age | Cases | Population (1992 – 1999) | Incidence Rate /1,000,000 population |
|---|---|---|---|
| < 1 | 1 | 129255 | 1.0 |
| 1-4 | 17 | 573299 | 3.7 |
| 5-9 | 17 | 692247 | 3.1 |
| 10-14 | 29 | 666370 | 5.4 |
| 15-19 | 24 | 696803 | 4.3 |
| 20-24 | 23 | 705318 | 4.1 |
| 25-29 | 19 | 764262 | 3.1 |
| 30-34 | 27 | 810291 | 4.2 |
| 35-39 | 31 | 749062 | 5.2 |
| 40-44 | 24 | 657087 | 4.6 |
| 45-49 | 24 | 523730 | 5.7 |
| 50-54 | 24 | 424389 | 7.1 |
| 55-59 | 44 | 392787 | 14.0 |
| 60-64 | 39 | 401936 | 12.1 |
| 65-69 | 44 | 369111 | 14.9 |
| 70-74 | 44 | 286727 | 19.2 |
| 75-79 | 23 | 212494 | 13.5 |
| 80-84 | 14 | 133222 | 13.1 |
| 85 and over | 3 | 106907 | 3.5 |

The incidence of GBS decreased between 1992 and 1999. In 1992 the incidence rate per 1,000,00 population was 8.8. It decreased to 5.5 in 1992 (Figure 4-1).

There were no significant differences in the age-specific incidence rates between the two regions, but point estimates were unusually elevated in 5-14 year-olds and 75-79 year olds in the high *C. jejuni* incidence region (Figure 4-2). These estimates are unstable due to low numbers, however.

Similarly, the twelve months of the year were divided into four three-month seasons. The highest rate occurred in winter (November, December, and January) but the difference was not significant.

**Discussion**

The age, gender, and seasonal distributions of GBS in Michigan are similar to what has been found in other developed countries. Men are affected more often than females, most cases occur in the elderly, and there is no change in incidence by season. The epidemiology of *Campylobacter jejuni* enteritis is only similar to that of GBS in its gender distribution. Possible explanations for this lie in the multiple causes of GBS. Many infections can precede GBS and their relative preponderance in different seasons of the year, e.g., *Campylobacter* in summer and flu in winter, may contribute to the lack of seasonality. Host factors, such as a diminished ability to distinguish self from non-self may explain why most cases of GBS occur in the elderly, while its most common antecedent *C. jejuni* usually occurs in infants and young adults, but the reasons are not clear. Reported cases declined between 1992 and 1999, but the reasons for this decline are not known. The reported incidence of *C. jejuni* also declined during this period (Potter et al., submitted 2002). The incidence of GBS did not vary between the previously identified high and low *C. jejuni* incidence regions. Indeed, the point estimate of the incidence rate was higher in counties with a lower *C. jejuni* incidence rate. This may be due to the multiple causality of GBS. Other infectious diseases antecedent to GBS may be more common in the urban areas within the low *C. jejuni* incidence region. *Campylobacter jejuni* serotype and human leukocyte antigen (HLA) type of the host are also thought to play an important role in the pathogenesis of *C. jejuni*-associated GBS.

We could not reject the null hypothesis of no difference in GBS rates between high and low *C. jejuni* incidence regions. A more robust method of data analysis may have been able to detect small differences in rates between regions, while controlling for potentially confounding variables but the data was too sparse for both Poisson regression models and zero-inflated Poisson regression models. If, in the future, validated hospital discharge data are available, these models may be used for the analysis.

**Figure 4-1: Crude annual incidence rates for GBS in Michigan 1992-1999**

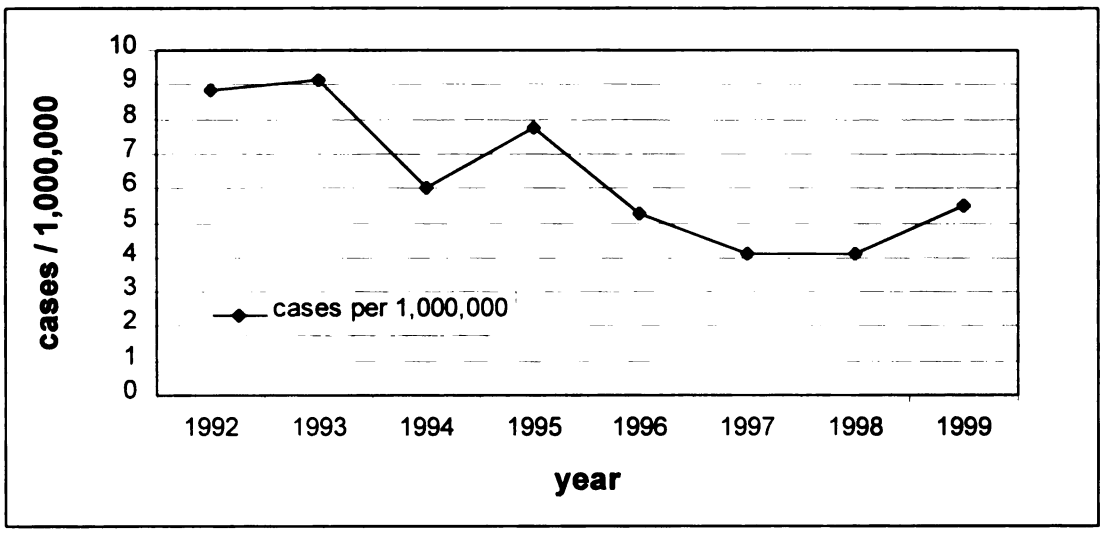
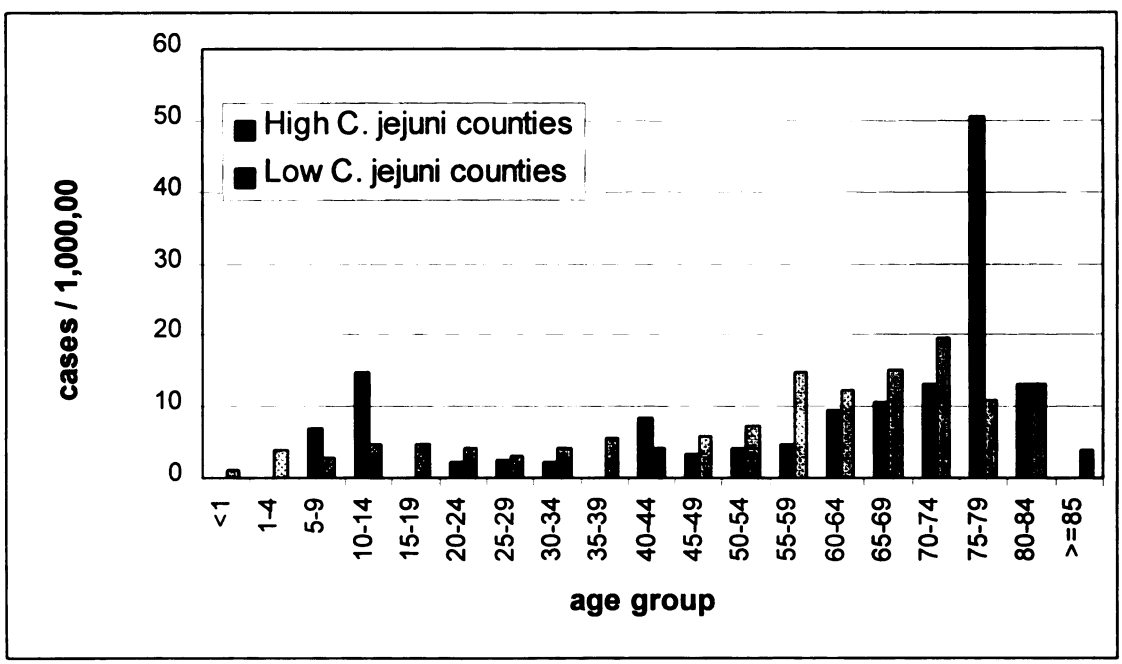



**Figure 4-2: Age-specific incidence rates for GBS in high and low *C. jejuni* enteritis regions in Michigan 1992-1999**



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CHAPTER 5

# RISK FACTORS FOR *CAMPYLOBACTER JEJUNI* INFECTIONS IN RURAL MICHIGAN: A PROSPECTIVE CASE-CONTROL STUDY


## Abstract

*Objectives.* To investigate the risk factors for campylobacteriosis in a rural population. Exposure to live farm animals was hypothesized to increase the risk for *Campylobacter jejuni* enteritis.

*Methods.* In this prospective case-control study, incident cases from rural counties reported to the Michigan Department of Community Health and matched controls were asked to complete a self-administered postal questionnaire.

*Results.* Persons engaged in activities related to the care and raising of poultry, husbandry, had an increased odds of campylobacteriosis, odds Ratio (OR) 6.884, 95% confidence interval (CI) 1.438, 32.954. There was evidence for dose effect between the number of types of poultry contact and campylobacteriosis.

*Conclusions.* In rural populations, contact with live poultry is a significant risk factor for campylobacteriosis.


## Introduction

*Campylobacter jejuni* is the most common cause of bacterial gastroenteritis in the United States (CDC, 2001). Including undiagnosed and unreported cases it is estimated to affect over two million people every year. The annual cost of campylobacteriosis has been estimated to be between $1.3 to $6.2 billion dollars (Buzby et al., 1997). This cost increases when sequelae, such as Guillain-Barré Syndrome and reactive arthritis are

considered. Of additional concern, *Campylobacter jejuni* and *C. coli* are becoming increasingly resistant to some antimicrobials (Engberg et al., 2001).

Risk factors for outbreak cases and sporadic cases differ. Outbreaks are typically due to raw milk (Kornblatt et al., 1985; Harris et al., 1987; Evans et al., 1996) or contaminated water consumption (St. Louis, 1988; CDC, 1999) but the vast majority of cases are sporadic. Identified risk factors for sporadic cases include consumption of undercooked chicken (Hopkins et al., 1984; Deming et al., 1987; Friedman et al., 2000), contact with pets, especially puppies and kittens (Adak et al., 1995; Neal and Slack, 1997), and contact with diarrheic animals (Adak et al., 1995; Saeed et al., 1993). Some ecological descriptive studies have shown that rates of infection are higher in rural areas than urban areas and that, among rural areas, farming regions have the highest rates (Thompson et al., 1986). Living on or visiting a farm has been shown to increase the odds of infection (Friendman et al., 2000), but a multicenter study in England and Wales showed a decreased odds associated with occupational contact with livestock or their feces (Adak et al., 1995). A study of campylobacteriosis on Hopi and Navajo Indian reservations, rural areas, showed an increased risk with ownership of farm animals (Engleberg et al., 1984).

We conducted a study to determine the risk factors for *C. jejuni* enteritis in rural communities. We hypothesized that exposure to food animals is a major risk factor and that the odds of infection changes with exposure to different species.

## Methods

*Design*

A prospective matched case-control study design was implemented. The study duration was one year, from October 2000 to October 2001. Incident cases reported to the Michigan Department of Community Health (MDCH) were identified weekly. Cases were contacted and invited to participate if they met the following inclusion criteria. First, the cases must have been residents of a rural county. Rural counties were defined as those having a population less than 70,000 by the 1990 census and not adjacent to a major metropolitan center. Fifty-eight of Michigan's 84 counties met this criterion. Second, the cases must have been reported to MDCH within 30 days of the onset of symptoms. This was to avoid potential problems with recall bias. Third, the cases must not have been part of an identified outbreak and fourth, must have been the first in a household. Only first cases in a household were considered because the effect of person to person transmission (though uncommon) was not of interest. If these criteria were satisfied, the case was contacted by phone about the study and if they expressed an interest in participating, a postal questionnaire was sent. Two controls were matched to each case by age group, gender, and county of residence. These variables were chosen for matching to eliminate them as potential confounders. Age groups were divided into those less than one year old, 1 to 2 year-olds, 3 to 4 year-olds, 5 to 12 year-olds, 13 to 19 year-olds, then by 20 year age groups, and finally those 60 years and over. After a case was contacted by phone, two controls were identified by random digit dialing (RDD) within the same area code and three-digit prefix as the case. If the potential control was

of the same age group and gender as the case and had not had any gastrointestinal symptoms during the two weeks prior to contact, they were sent a postal questionnaire.

*Questionnaire*

The questionnaires sent to cases and controls were identical except that where cases were asked about behaviors two weeks prior to onset of symptoms, controls were asked about behaviors two weeks prior to contact by the investigators. After an informed consent form was signed, information was collected on demographic characteristics, foreign travel, antibiotic and antacid use, animal contact and husbandry behaviors, and food consumption habits. If the case or control was a child, the parent or guardian was asked to complete the questionnaire. A case or control was considered to engage in animal husbandry if that person participated in feeding, cleaning, or raising an animal for milk, eggs, or meat, or had housed an animal in their home.

*Data Analysis*

Microsoft Access and Excel were used to enter and organize data. Statistical analysis was performed using SAS version 8. A conditional logistic regression model was used for univariable and multivariable analyses. Independent variables associated with the outcome ($p<0.15$) were then tested for association using a chi-square test. If two or more independent variables were significantly associated with each other ($p<0.05$) the more biologically important variable was included in the model and the other discarded. Variables that showed an association at the $p<0.15$ level were then entered into a conditional logistic regression model for the multivariable analysis. The final model was obtained through hierarchical backward elimination of variables using statistical and epidemiological criteria for assessment of interaction and confounding. Interaction terms

were considered significant if the p-value for the term was less than 0.05 by the Wald test. Confounding was assessed by the impact of the potential confounder on the parameter estimate for the main effects, i.e. poultry husbandry. If removal of a confounding variable caused a 10% or greater change in the value of the parameter estimate, that variable was considered a confounder and left in the model.

*Modeling*

The questionnaire asked about contact with a particular species of animal and then specific questions about the type of contact. These questions included feeding, cleaning, raising an animal for meat, eggs, or milk, keeping an animal in the home or garage, and having clothing contaminated with fecal material. (Table 5-1) When analyzed separately, these variables were highly associated with each other, as expected. Raising animals for eggs, milk, or meat almost always involves feeding, cleaning, and fecal contact. As a result, these variables were combined into one dichotomous summary variable, husbandry, for each species. Cases and controls were considered to be positive for the husbandry exposure if they indicated exposure to any of the independent variables described above. This modeling of independent variables is biologically plausible because husbandry, or the care and raising of livestock, is a special kind of contact. It involves repeated, at least daily, direct contact with the animals including contact with the fecal material of species that have the potential to carry *Campylobacter*.

**Results**

Of the 193 *C. jejuni* enteritis cases reported to the MDCH from rural counties during the year of our study, 48 did not meet the inclusion criteria described above, leaving 143 cases. Forty-three of the 48 who did not meet the inclusion criteria did not do so because

they were reported more than 30 days after the onset of their illness. Twenty-one of the cases reported too late to participate were from very rural counties with a population of 35,000 or less by 1990 census counts. We were not able to contact 31 of the eligible cases. There was contact with 110 cases, among which there were six refusals and 21 who initially agreed to participate in the study but later changed their minds, were not sure they could remember the period prior to their illness, or did not return their postal questionnaire. Using the method described by Slattery, et al., we calculated a cooperation rate of 75% (Slattery et al., 1995). The cooperation rate is the percentage of people interviewed of those who were contacted. All cases and controls were contacted two weeks after the questionnaire had been sent to ensure that it had been received and to answer any questions that had arisen. If the case or control could not be contacted by phone, a second copy of the questionnaire was sent. Questionnaires were completed for 83 cases and 122 controls. We calculated a response rate of 58% for cases. Response rate is defined as the percentage of people interviewed of those who were selected for and eligible for study. Similarly, the response rate for controls was calculated at 48%. There was no significant difference in age, county of origin, or week of report between those cases included in the study and the baseline available rural case population of 193 cases (Wilcoxon rank sum test). Males were under-represented in our study group, however (Chi Square p=0.0237).

*Univariable Analyses*

Contact with any food producing animal (bovines, swine, or poultry) was significantly associated with illness (OR, 4.722; 95% CI, 1.737, 12.833). Of factors considered significant in the univariable analysis ($p < 0.15$), there were increased odds of illness if

there was contact with adult domestic poultry (OR, 3.216; 95% CI, 0.811, 12.763) and participation in the care and raising of poultry (OR, 8.454; 95% CI, 1.877, 38.081). The care and raising of cattle was also associated with illness (OR, 3.058; 95% CI, 0.907, 10.307) as was the care and raising of swine (OR, 7.358; 95% CI, 0.845, 64.079) and horses (OR 3.380; 95% CI, 0.860, 13.294). Contact with foals also conferred increased odds for infection (OR, 6.275; 95% CI, 0.689, 57.165).

We inquired about consumption and preparation of poultry, ground beef, and pork. Of these, only the consumption of undercooked pork and poultry were significant in the univariable analysis, but their effect was protective (OR, 0.333; 95% CI, 0.110, 1.013 and OR, 0.180, 95% CI, 0.052, 0.622, respectively). Raw milk was not associated with illness. Eleven percent of controls and ten percent of cases reported raw milk consumption in the two weeks prior to contact or illness.

Of the other exposures, including foreign travel, living on a farm, taking antibiotics or antacids, and having problems with rodents or houseflies in the home, only living on a farm was associated with illness (OR, 2.484; 95% CI, 1.041, 5.930). (Table 5-1)

We considered the interactions of antacid use, antibiotic use, and poultry exposure with the other significant animal contact and food consumption terms. None were statistically significant.

*Assessment of associations between independent variables*

Equine husbandry and exposure to foals were strongly associated with poultry husbandry and bovine husbandry. Because chickens and cows are known to be important reservoirs of *C. jejuni*, while horses are not (Prescott and Bruin-Mosch, 1981) the horse exposure variables were dropped from the model. Similarly, farm exposure was very

highly associated with animal husbandry but of the two, the specific animal exposure is more biologically important. Farm exposure was, therefore, dropped from the model. Farm exposure was not as strongly associated with outcome as the animal husbandry variables.

*Multivariable model*

Factors significant in the univariable model were entered into the multivariable model. These included: poultry husbandry; cattle husbandry; swine husbandry; consuming poultry that was pink at the center, had red juices running from the meat, or was raw; and consuming pork that was pink at the center, had red juices running from the meat, or was raw. The consumption of undercooked poultry and pork were not statistically significant nor was there any evidence of confounding, so they were removed from the model. In the final model, only poultry husbandry (OR, 6.884; 95% CI, 1.438, 32.954) was associated with *C. jejuni* enteritis. Swine and cattle husbandry showed increased risk but it was not significant over and above poultry husbandry (Table 5-2).

*Dose-Effect*

Because we found a strong association with poultry, swine, and bovine husbandry exposures, we further investigated these variables to look for a dose-effect relationship. From the seven questions asked about husbandry exposure, three categories were created and modeled as an ordinal variable. The lowest husbandry category was one or two exposures, the middle category was three to five exposures, and the highest was six or seven. A dose-response relationship was observed for poultry and bovine husbandry, but no cases had six or seven of the exposure variables for these species. Because the variable was modeled as an ordinal one, the odds for the second and third category were

calculated from the parameter estimate for the first category. The results are shown in Figure 5-1.

*Analysis of Non-Responders*

The response rate for cases was 58%. We compared the age distribution, week of onset, and gender of responders and non-responders. There was no significant difference between the two groups. Gastrointestinal illness case investigation reports were available for 17 of the 28 non-responders. These reports are the results of a telephone interview between a public health nurse at the local health department and the case. Of the 17 reports, two were blank and one had declined to give information, leaving 14 for analysis. Two of the 14 reported food-animal contact. One case had contact with poultry and one had contact with sheep. There was no significant difference between responders and non-responders in contact with farm animals (Fisher's exact test, $p = 0.1280$). Non-responders were less likely to have contact with companion animals, however, than were responders (Fishers exact test, $p = 0.0014$). These findings suggest that there was not a response bias on food animals, but we may have been under-powered to find such a bias if it does exist. The response rate for controls was 48%. There was no significant difference in age or gender for those controls who responded and those who did not. Data were not collected on farm or companion animal exposure for controls that did not respond.

**Discussion**

We found that contact with farm animals was a significant risk factor for *C. jejuni* enteritis in rural areas. Specifically, the care and raising of poultry increased the risk for disease by seven-fold over and above husbandry for other species known to be reservoirs

for *Campylobacter jejuni*. Additionally, we found evidence for a dose-effect relationship for increased odds of infection with increasing kinds of food animal contact.

Ecological studies have indicated that the incidence rate for *C. jejuni* infection is higher in rural areas than urban areas (Brieseman, 1990), especially farming communities (Thomopson et al., 1986). Raw milk consumption (Thompson et al., 1986; Schmid et al., 1987) contact with farm animals (Engleberg et al., 1984), and daily contact with chickens or hens (Studahl and Andersson, 2000) have been identified as potential risk factors in rural populations. This study confirms the findings of previous research implicating farm animal contact and further defines that contact as activities related to the care and rearing of poultry.

We did not find that raw milk consumption was a risk factor in our study, however. This may be due to over-matching on location or because raw milk consumption was a more common practice among controls than anticipated in power calculations. In a survey of milk producers, 35% reported drinking raw milk (Rohrbach et al., 1992). Additionally, regular raw milk consumption has been shown to cause an elevated anti-*C. jejuni* antibody titer that protects against symptomatic infection (Blaser et al., 1987).

We did not find any association with cat or kitten exposure. This is in contradiction with other studies that have found exposure to cats and kittens to be significantly positively associated with illness (Hopkins et al., 1984; Deming et al., 1987). This may be explained by the fact that the previous studies took place in urban environments and that, although cats are fairly ubiquitous on farms, their presence is discouraged around the chicken coop and there may be little actual contact with them.

This study may have been under-powered to find other significant associations with livestock. Many of the cases from very rural counties were excluded because they were not reported within 30 days of onset of disease. A study with a two-year duration or active case ascertainment from local health departments may enroll enough cases to find these associations. Additionally, our questionnaire was long so a postal questionnaire was used. This method has the disadvantage of a lower response rate. The findings of this study, however, confirm previous investigations and provide a basis for additional research.

Cases who responded to the questionnaire were more likely to have association with companion animals than those who did not respond to the questionnaire. Although questions were asked about a variety of known risk factors for *C. jejuni*, most of the questions were on animal contact. It is possible that cases who felt the questionnaire did not apply to them, i.e., they had no animal contact, did not return it. We did not collect the data to measure this bias in controls. A large number of controls had contact with pets (88%), and this finding may indicate that controls were also motivated to return their questionnaire if they had companion animal contact.

This study illustrates that, in rural areas, the care and raising of farm animals, particularly poultry, confers an increased risk for *C. jejuni* enteritis. Public health measures aimed at prevention should be population-specific.

**Table 5-1: Univariable analyses of covariates included in the summary husbandry variables.**

| Variable | No. Exposed | | No. Unexposed | | m OR* | 95% CI† |
|---|---|---|---|---|---|---|
| | Cases | Controls | Cases | Controls | | |
| Poultry housed in home or garage | 5 | 2 | 78 | 120 | 3.812 | 0.726, 20.014 |
| Poultry hatched on property | 4 | 2 | 79 | 120 | 5.767 | 0.621, 53.603 |
| Poultry raised for meat | 7 | 2 | 76 | 120 | 7.922 | 0.930, 67.472 |
| Poultry raised for eggs | 13 | 4 | 70 | 118 | 10.902 | 1.339, 88.744 |
| Feeding poultry | 9 | 8 | 74 | 114 | 2.169 | 0.619, 7.592 |
| Poultry associated cleaning | 6 | 3 | 77 | 119 | 3.412 | 0.672, 17.339 |
| Clothing contaminated with poultry feces | 6 | 4 | 77 | 118 | 2.234 | 0.516, 9.678 |
| Calves born on property | 1 | 1 | 82 | 121 | 0 | 0 |
| Bovines housed in home or garage | 0 | 0 | 83 | 122 | 0 | 0 |
| Bovines kept for milk | 1 | 0 | 82 | 122 | 0 | 0 |
| Bovines kept for beef | 6 | 2 | 77 | 120 | 2.637 | 0.434, 16.013 |
| Feeding bovines | 6 | 3 | 77 | 119 | 2.189 | 0.513, 9.342 |
| Bovine associated cleaning | 7 | 1 | 76 | 121 | 8.421 | 0.998, 71.041 |
| Clothing contaminated with bovine feces | 7 | 4 | 76 | 118 | 2.610 | 0.613, 11.119 |
| Pigs housed in home or garage | 0 | 0 | 83 | 122 | 0 | 0 |
| Pigs born on property | 3 | 0 | 80 | 122 | 0 | 0 |
| Pig kept as pet | 2 | 0 | 81 | 122 | 0 | 0 |
| Pigs raised for meat | 1 | 0 | 82 | 122 | 0 | 0 |
| Feeding Pigs | 2 | 1 | 81 | 121 | 2.561 | 0.225, 29.120 |
| Pig associated cleaning | 1 | 0 | 82 | 122 | 0 | 0 |
| Clothing contaminated with pig feces | 4 | 1 | 79 | 121 | 6.275 | 0.689, 57.165 |

*mOR = matched odds ratio †CI = confidence interval

**Table 5-2: Matched odds ratios for animal contact, demographic characteristics, and food consumption habits for cases of *C. jejuni* enteritis: exposures significant in univariable analysis ($p < 0.15$)**

| Exposure | No. exposed | | No. unexposed | | mOR* | 95% CI† |
|---|---|---|---|---|---|---|
| | Cases | Controls | Cases | Controls | | |
| Adult Poultry | 9 | 4 | 74 | 118 | 3.216 | 0.811, 12.763 |
| Poultry Husbandry | 18 | 8 | 65 | 114 | 8.454 | 1.877, 38.081 |
| Bovine Husbandry | 12 | 5 | 71 | 117 | 3.058 | 0.907, 10.307 |
| Swine Husbandry | 5 | 1 | 78 | 121 | 7.358 | 0.845, 64.079 |
| Equine Husbandry | 9 | 5 | 74 | 117 | 3.380 | 0.860, 13.294 |
| Foal | 4 | 2 | 79 | 120 | 6.275 | 0.689, 57.165 |
| Farm | 21 | 12 | 62 | 110 | 2.484 | 1.041, 5.930 |
| Undercooked Poultry | 6 | 26 | 77 | 96 | 0.180 | 0.052, 0.622 |
| Undercooked Pork | 4 | 18 | 79 | 103 | 0.333 | 0.110, 1.013 |

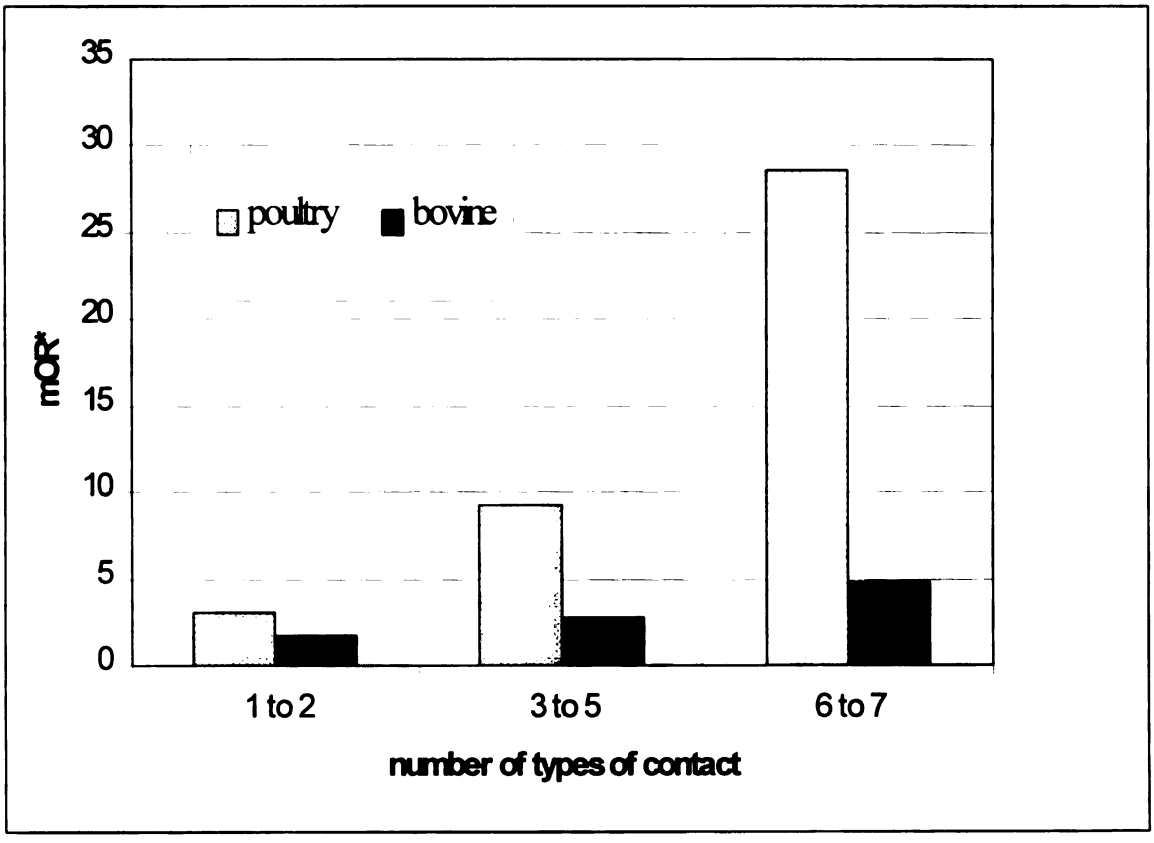
*mOR = matched Odds ratio †CI = Confidence interval

**Table 5-3: Final multivariable model**

| Exposure | mOR* | 95% CI† | p Value |
|---|---|---|---|
| Poultry Husbandry | 6.884 | 1.438, 32.954 | 0.0158 |
| Bovine Husbandry | 2.447 | 0.657, 9.114 | 0.1822 |
| Swine Husbandry | 2.149 | 0.178, 25.995 | 0.5477 |

*mOR = matched Odds ratio †CI = Confidence interval

**Figure 5-1: Demonstration of a linear dose-response in number of types of contact with poultry or bovines and odds ratios**




*mOR = matched odds ratio

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# SUMMARY AND CONCLUSIONS

In the first chapter, the current knowledge on risk factors for *C. jejuni* enteritis was reviewed. *Campylobacter jejuni* enteritis is known to be more common in rural communities than urban communities, but the reasons for this are not clear. Some studies had suggested that raw milk or untreated water consumption and animal contact may be the causes. Additional information on species of animal, age of animal, and type of animal contact was needed to create appropriate prevention strategies. The case-control study in Chapter 5 was designed to address this gap in the current understanding of the epidemiology of *C. jejuni* in rural areas.

The second chapter reviewed the association between *C. jejuni* and GBS in the framework of the causal criteria. Through this review it became clear that future studies on the association should be prospective. A prospective or cohort study is necessary to satisfy the criterion of time order (the *sine qua non* of causality) and to elucidate the pathophysiological events that lead from *C. jejuni* infection to GBS. It was also noted that mild cases of GBS may be more common than previously thought and that these cases have not been investigated because they may not go to the hospital and may not be reported. For these reasons, therefore, GBS cases reported to the MDCH will need to be supplemented in order to have usage in studying the relationship between GBS and *C. jejuni*.

The third chapter was an ecological study of *C. jejuni* in high and low poultry density regions of Michigan between 1992 and 1999. It was found that the incidence of *C. jejuni* enteritis was significantly higher in high poultry density counties than in counties with less than one percent of the poultry in the state. Children and young adults had higher

rates in the high-density region than the low-density region. The reasons for these findings are not known, but several hypotheses were generated. Transport or manure management practices may be causing environmental contamination, which is a risk factor unto itself, and may also cause other species of animals in the region to become carriers and excreters of the organism. Workers in the poultry industry may account for the increased incidence. Additional studies to investigate manure management practices, environmental contamination with *C. jejuni,* and disease in poultry workers in the high poultry density region are recommended.

In the fourth chapter, the rates of GBS in the high and low *C. jejuni* incidence regions identified in chapter three were compared. Data on GBS reported to the MDCH were too sparse to conduct any meaningful analytical evaluation so descriptive methods were used. We found no significant difference in the rates between the two regions. The limited data that were available to us from the hospital discharge database indicated that GBS is probably under-reported in Michigan. Because it was not possible to verify the diagnoses in this database we were not able to quantify the magnitude of under-reporting. This further underlines the fact that MDCH reported cases will need to be supplemented with additional information in future studies.

The case-control study in chapter five showed that poultry husbandry was a significant risk factor in rural areas. Additionally, we showed an increasing number of types of poultry husbandry increased the odds of *C. jejuni* enteritis. This information can be used to educate poultry producers and hobby farmers in rural areas and prevent future cases. It also indicates that poultry workers may be the cause of excess cases in the high-poultry density region identified in Chapter 3.

# APPENDIX

## *Campylobacter jejuni* Consent Form

*Campylobacter jejuni* is a type of bacteria that can cause illness characterized by diarrhea, fever, nausea, and abdominal pain. Most people recover from the illness in a few days, but a very small number (less than 1%) will go on to develop severe secondary diseases such as arthritis or paralysis shortly after their illness. Because of the severity of this disease and the potential for complications, all persons in Michigan who are diagnosed with *Campylobacter jejuni* must be reported to the Michigan Department of Community Health by the physician or laboratory that made the diagnosis. Your name has been identified from this list of reported cases. We hope that you will join us in a research study of people who have had *Campylobacter jejuni* infections.

The purpose of this study is to investigate the events that occur prior to infection with the goal of determining why some people get sick and others do not. This research is important because *Campylobacter* infections are becoming more common in the United States and in Michigan. Your participation in this study will help us to learn more about this disease. Dr. John B. Kaneene and Dr. Rachel C. Potter of Michigan State University, East Lansing, Michigan are conducting this study in cooperation with the Michigan Department of Community Health.

As a member of the study you are asked only to participate in the completion of a detailed questionnaire. This questionnaire, which is included, asks for information on your health history, occupational history, food preparation habits, and other demographic characteristics. The questionnaire takes approximately 15 to 20 minutes to complete. A self-addressed, stamped envelope has been provided for you to return the questionnaire.

All information collected will be kept confidential and your privacy will be protected to the maximum extent allowable by law. As a study participant, you will not be identified by name on any of the information you provide, but will be assigned a study number. At no time will individuals be identified in the study results or summaries. Information identified by your study number will be kept separate from your name in a secure, locked file.

If you have any particular questions about the study, your participation, or if, after having volunteered, you wish to discontinue with the study, you may call Dr. John B. Kaneene at (517) 355-2269 or Dr. Rachel C. Potter at (517) 355-1745. If you have any questions or concerns regarding your rights as a study participant, or are dissatisfied at any time with any aspect of this study, you may contact – anonymously if you wish – Ashir Kumar, Chair of the University Committee on Research Involving Human Subjects (UCRIHS) by phone: (517) 432-2222, fax: (517) 353-2976, e-mail: ucrihs@msu.edu, or regular mail: 246 Administration Bldg, East Lansing, MI 48824.

To take part in the study, you need to sign the form on the following page and complete the questionnaire. If you choose not to take part in this study, or if you withdraw after you have started, you will not be penalized in any way.

I have had an opportunity to ask questions about the study and was given sufficient time to consider my decision to participate. I agree to be in the study.

Name (please print) _____

Signature_____ Date _____

If the participant is under the age of 18 years, the signature of a parent or guardian is required.

Name (please print) _____

Signature _____ Date _____

Once you have read and signed this consent form, **please place it in the postage paid envelope and mail it to us with the questionnaire**. We have enclosed a copy of the consent form for you to keep.

Thanks!

## *CAMPYLOBACTER JEJUNI* QUESTIONNAIRE

**ALL OF THE FOLLOWING QUESTIONS PERTAIN TO YOUR DIARRHEAL ILLNESS THAT OCCURRED AROUND _____.**

**Travel History:** The following questions pertain only to travel in the *two weeks before* your diarrheal illness:

1. Did you travel outside the continental United States and Canada? *(circle only one response)*

   Yes No

2. If you answered yes to question 1., where did you travel?

   _____

   _____

**Occupational History:** The following question pertains only to your job in the *two weeks before* your diarrheal illness:

3. Briefly describe your job:

   _____

**Residence:** The following questions pertain to the area where you lived during the *two weeks* prior to your illness:

4. Did you live on a farm?

   Yes No

5. Did you consider houseflies to be a problem at that time?

   Yes No

6. Did you have a problem with rodents in your home at that time?

   Yes No

**Medications:** The following questions pertain to any medications you were taking during the two weeks prior to your illness:

7. Were you taking any antibiotics?

   Yes No

8. Were you taking any antacids?

   Yes No

**History of Contact with Animals:** the following questions pertain only to any contact that you had with live animals *two weeks before* your illness.

9. In the two weeks before you became ill did you have <u>any</u> exposure to chickens, ducks, geese, turkeys, or other domestic poultry (not wild ducks, geese, or other wild poultry or pet birds) of any age?

   Yes No

10. If you answered **yes** to question 9, indicate the kind of exposure in the two weeks before you became ill by circling each that applies:

| | |
|---|---|
| 1 | direct physical contact with adult poultry |
| 2 | direct physical contact with young poultry or chicks |
| 3 | no physical contact but poultry are on your property |
| 4 | poultry have been inside your house or garage |
| 5 | poultry were hatched on your property |
| 6 | poultry are raised for meat on your property |
| 7 | poultry are kept for eggs on your property |
| 8 | you fed poultry |
| 9 | you cleaned-up after poultry |
| 10 | clothing contaminated with fresh poultry droppings/manure |
| 11 | other, specify_____ |

11. In the two weeks before your illness did you have <u>any</u> exposure to cows or cattle of any age?

Yes No

12. If you answered **yes** to question 11, indicate the kind of exposure in the two weeks before you became ill by circling each that applies:

1 direct physical contact with adult cattle or cow(s)

2 direct physical contact with young cattle or calves

3 no physical contact but cattle or cow(s) are on your property

4 cattle or cow(s) were born on your property

5 cattle or cow(s) have been inside your house or garage (inc. calf)

6 cows are kept for milking on your property

7 cattle are raised for beef on your property

8 you fed cattle or cow(s)

9 you cleaned-up after cattle or cows(s)

10 clothing contaminated with fresh cattle or cow manure

11 Other, specify_____

13. In the two weeks before you became ill did you have <u>any</u> exposure to any pigs / swine of any age?

Yes No

14. If you answered **yes** to question 13, indicate the kind of exposure in the two weeks before you became ill by circling each that applies:

1 direct physical contact with adult pig(s)

2 direct physical contact with young or juvenile pig(s)

3 no physical contact but pig(s) is on your property

4 pig or baby pig was in your house or garage

5 pig(s) was born on your property

6 pig kept as a pet on your property

7 raised for meat on your property

8 you fed pig(s)

9 you cleaned-up after pig(s)

10 clothing contaminated with fresh pig manure

11 other, specify_____

15. Did you have <u>any</u> exposure to horses of any age in the two weeks before you became ill?

Yes No

16. If you answered **yes** to question 15, indicate the kind of exposure in the two weeks before you became ill by circling each that applies:

1 direct physical contact with adult horse(s)

2 direct physical contact with young horses or foal(s)

3 no physical contact but horse(s) is on your property

4 horse(s) was born on your property

5 horse (or foal) was in your house or garage

6 you fed horse(s)

7 you cleaned-up after horse(s)

8 clothing contaminated with fresh horse manure

9 other, specify_____

17. Did you have <u>any</u> exposure to a dog(s) of any age in the two weeks before you were ill?

Yes No

18. If you answered **yes** to question 17, indicate the kind of exposure in the two weeks before you became ill by circling each that applies:

1 direct physical contact with an adult dog

2 direct physical contact with a juvenile dog or a puppy

3 no physical contact but dog(s) was on your property

4 a dog(s) was in you house or garage

5 a puppy(ies) was born on your property

6 you fed dog(s)

7 you cleaned-up after dog(s)

8 clothing contaminated with dog droppings

9 other, specify_____

19. Did you have any exposure to a cat(s) of any age in the two weeks before you became ill?

Yes No

20. If you answered **yes** to question 19, indicate the kind of exposure in the two weeks before you became ill by circling each that applies:

1 direct physical contact with adult cats

2 direct physical contact with kittens

3 no physical contact but cats are on your property

4 cats in house or garage

5 a kitten(s) was born on your property

6 you fed cat(s)

7 you cleaned-up after cat(s) or emptied a "cat box"

8 clothing contaminated with cat droppings

9 other, specify_____

21. Did you have any exposure to pet birds (not chicken, ducks, turkeys, other poultry, or wild birds) of any age in the two weeks before you became ill?

Yes No

22. If you answered **yes** to question 21, indicate the kind of exposure in the two weeks before you became ill by circling each that applies:

1 Direct physical contact with an adult pet bird(s)

2 physical contact with young or juvenile pet bird(s)

3 no physical contact but a pet bird(s) is on your property

4 a pet bird(s) in house

5 a pet bird(s) was hatched on your property

6 you fed a pet bird(s)

7 you cleaned-up after a pet bird(s) or cleaned a bird cage

8 clothing contaminated with fresh pet bird droppings

9 other, specify_____

**Food consumption habits before illness:** The following questions pertain only to your food consumption habits for the time period *two weeks before* your diarrheal illness.

23. How did you usually eat ground beef (hamburger)? *(circle only one response)*

| | |
|---|---|
| Well-done (cooked throughout / no pink showing) | 1 |
| Medium (pink at the center) | 2 |
| Rare (red juices running from the meat) | 3 |
| Raw | 4 |

24. Did you ever eat ground beef that was medium (pink at the center), rare (red juices running from the meat) or raw? *(circle only one response)*

Yes No

25. How did you usually eat poultry (chicken or turkey)? *(circle only one response)*

| | |
|---|---|
| Well-done (cooked throughout / no pink showing) | 1 |
| Cooked, pink at the center or on the bone | 2 |
| Cooked, with red juices running | 3 |
| Raw | 4 |

26. Did you consume any poultry (chicken or turkey) that was prepared in the following ways? *(circle all that apply)*

| | |
|---|---|
| Fried | 1 |
| Barbecued | 2 |
| Roasted | 3 |
| Baked | 4 |

**Food consumption habits** (continued):

27. Did you ever eat poultry (chicken or turkey) that was cooked and pink at the center or on the bone, or cooked with red juices running, or raw? *(circle only one response)*

Yes No

28. How did you usually eat pork? *(circle only one response)*

| | |
|---|---|
| Well-done (cooked throughout / no pink showing) | 1 |
| Cooked, pink at the center or on the bone | 2 |
| Cooked, with red juices running | 3 |
| Raw | 4 |

29. Did you ever eat pork that was cooked and pink at the center or on the bone, or cooked with red juices running, or raw? (circle only one response)

Yes No

30. Did you ever drink untreated water in the two weeks prior to illness (water that was not chlorinated or boiled)? *(circle only one response)*

Yes No

31. Did you ever drink raw (unpasteurized) milk? (circle only one response)

Yes No

32. Are you the person in your household that usually prepares meals?

Yes No

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