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OUTBREAK OF *CAMPYLOBACTER* INFECTION AMONG FARM WORKERS: AN OCCUPATIONAL HAZARD

Background

On 26 July, 1994, the local agricultural employment office notified the Wellington-Dufferin-Guelph Health Unit that a member of a work crew had suffered diarrheal illness following employment at a turkey farm on 18 July. The employment office also reported that two members from the same crew had been unable to work because of illness. Given this information, the Health Unit requested a list of all employees who had worked on the farm in order to investigate a possible outbreak.

Investigation

A public health inspector and field epidemiologist conducted phone interviews with each of the nine-member crew to determine if they were ill, their symptoms, medical interventions, and activities on the turkey farm. Stool samples had been submitted to a private laboratory from two of the workers. Upon request, the private laboratory forwarded the fecal samples to the Central Public Health Laboratory in Toronto for *Campylobacter* culturing and species identification. Isolates were forwarded for serotyping to the National Laboratory for Enteric Pathogens, Laboratory Centre for Disease Control (LCDC), Ottawa. The farm owner and manager were informed of the outbreak and questioned about current farm practices and any recent history of illness among other farm workers.

Agriculture and Agri-Food Canada was contacted to assist with the farm investigation. On 23 August, investigators visited the farm to interview the farm manager and collect environmental samples. Using sterile tongue depressors 20 (50 gram), cecal dropping samples were collected from the implicated barn, which was now being used to house 12-week-old turkey hens. Samples were placed into whirl pak bags and transported to the laboratory in an insulated shipping container with ice packs. On receipt at the laboratory, samples were immediately processed in accordance with official protocol[(1)]. Isolates were forwarded to the LCDC for serotyping.

Results

Age, gender, clinical history and duration of illness of the crew members are summarized in Table 1. Medical attention was sought by five of the seven people who became ill. Stool samples obtained from two patients were positive for *Campylobacter jejuni* ss. *jejuni* serotype 82. The person with the most severe illness (ID No. 3 in Table 1) had a history of chronic colitis.

Table 1
Clinical information for members of farm work crew

ID No.	Age	Gender	Date of Onset	Recovery Date	Clinical Symptoms
1	22	M	07/19/94	07/25/94	D,C
2	22	M	07/19/94	07/25/94	D,V,C,F
3	32	M	07/19/94	07/23/94	D,C,F, CH, FA
4	20	M	07/19/94	07/23/94	D,C,DZ
5	18	M	07/19/94	07/23/94	D,C,DZ
6	21	M	07/22/94	07/25/94	D,C
7	16	M	NOT ILL		
8	32	M	07/21/94	07/26/94	D,C,F
9	14	F	NOT ILL		

Key to symptoms: D = diarrhea, C = cramps, V = vomiting, F = fever, CH = chills, FA = fainting, DZ = dizziness

According to the workers, the 1-day job involved catching and transporting a total of 13,000 6-week-old turkey poults from a brooding barn to a growing barn on another farm. No designated lunch time or breaks were scheduled. Six of the nine workers reported eating as they worked. One of the workers smoked as he

worked. The remaining two did not consume anything except for water brought in a container from home. These latter two individuals were the only members of the crew who did not report any illness. Handwashing facilities were not available; for those workers who wanted to wash their hands, a bucket of cold water was provided at the end of the day without provision of soap or disinfectant. The workers were not advised of the risks of pathogenic bacteria being shed by the birds or the need for thorough handwashing before eating or smoking. Masks were offered, but their use was not encouraged and gloves were not worn.

This was the first time casual farm workers had been employed to move birds on the farm. Four permanent workers, including the manager, reported diarrheal illness within 24 to 48 hours after handling the same birds. Biosecurity measures on the farm, such as rodent control, cleaning and disinfection of facility between flocks, restricted entry onto premises and proper handwashing procedures were not strictly adhered to. The resident flock of turkeys showed no obvious signs of disease.

Two of the 20 environmental samples (10%) yielded presumptive-positive *C. jejuni* isolates. The LCDC confirmed the species to be *C. jejuni* ss. *jejuni*, but these isolates were serologically different from the human isolates.

Discussion

Over the past 10 years the reported incidence of human *Campylobacter* infection has increased dramatically. In 1993, there were 6,738 cases reported in Ontario, more than double the number of *Salmonella* cases[2]. Infection with *Campylobacter* spp. generally causes a self-limiting gastroenteritis; however, long-term sequelae, such as Guillain-Barré syndrome, have been described[3]. The average cost per case of acute campylobacteriosis is estimated to be $916.00[4].

Consumption of undercooked poultry meat is reported to be the major cause of sporadic campylobacteriosis[5]. In a Canadian study during 1983-86, *Campylobacter* spp. was isolated from 73.7% of turkey carcasses and 38.2% of chicken carcasses sampled at slaughter[6].

Poultry farm safety recommendations have been produced for Ontario farmers[7,8], but these do not address the occupational hazards posed by zoonotic disease agents.

This investigation identified *C. jejuni* ss. *jejuni* serotype 82 in stool samples of two individuals suffering diarrheal illness. Working on the farm was the only common history of these individuals. The environmental samples failed to yield the same serotype as the patient samples, but it has been documented that many different species and serotypes of *C. jejuni* may be present on any given farm[9,10]. In addition, the environmental samples were collected from the facility 1 month after the outbreak, at which time a different flock of birds was present.

Most diagnostic laboratories do not differentiate between *C. jejuni* and *C. coli*. While this information does not alter the treatment of the patient, it is an important preliminary step in an outbreak investigation. Serotyping provides further evidence to epidemiologically link cases in an outbreak investigation.

The current surveillance system in Ontario would have failed to expose this outbreak if the agricultural office had not contacted the health unit. Even though five members of the work crew reported their illness to a physician, only one of the physicians suggested a link to the patient's work history and only two requested stool samples. The two laboratory-confirmed cases would have been misclassified as sporadic cases.

This outbreak illustrates the need for a comprehensive educational package on zoonotic disease prevention on farms. This package should be made available to farm workers and agricultural employment offices. Public health and agricultural agencies need to collaborate to address this issue. Additionally, poultry producers need to work with their producer organizations to develop and adhere to strict biosecurity measures to reduce the on-farm bacterial load.

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RESISTANCE TO QUINOLONES AMONG *CAMPYLOBACTER* SPECIES — ONTARIO

Introduction

Campylobacter jejuni and *C. coli* have been recognized as the most frequent causes of bacterial enterocolitis in Canada and other developed countries. Nalidixic acid (NA) in the form of a 30 µg disk has traditionally been used as a laboratory tool for the differentiation of *C. jejuni* and *C. coli* (both species are usually susceptible to NA) from NA-resistant campylobacters including *C. lari* and *C. fetus*. In the past, there have been few reports of problems in identification of NA-resistant strains of *C. jejuni* and *C . coli*[1,2]. However, more recently, investigations in several European countries have reported increasing frequencies of resistance to NA with high percentages of these strains demonstrating cross-resistance to fluoroquinolones[3,4,5,6]. The present study traces the emergence of quinolone-resistant strains of *C. jejuni* and *C. coli* in Ontario and compares the antimicrobial susceptibilities of selected NA-susceptible and NA-resistant strains isolated recently.

Surveillance Data

Identification records of *C. jejuni* and *C. coli* cultures submitted between 1981 and 1993 to the Enteric Reference Laboratory of the Central Public Health Laboratory were examined.

Antimicrobial Susceptibility Testing

Twenty clinical isolates of *C. jejuni* and *C. coli*, forwarded to the Enteric Reference Laboratory in 1992 and 1993 from various hospitals, private clinical laboratories and public health laboratories in Ontario, were analyzed. Eleven of the isolates were susceptible to NA as determined by a preliminary disk diffusion test (30 µg disk) and nine were resistant. Cultures were identified as *C. jejuni* and *C. coli* by using standard laboratory criteria[7]. Susceptibility testing was performed by using an agar dilution method with Mueller-Hinton agar containing 5% sheep blood. The inocula were prepared in Brain Heart Infusion Broth to approximate the turbidity of a No. 5 McFarland turbidity standard and then diluted 1:10. The inocula were transferred to the surface of agar plates by means of an inocula-replicating device and the plates were incubated microaerophilically at 35° C for 48 hours. The minimum inhibitory concentration (MIC) was recorded as the lowest concentration of antimicrobial agent that inhibited visible growth. The MIC interpretive standards were those recommended by the National Committee for Clinical Laboratory Standards[8].

Table 1
Summary of isolation of nalidixic acid-susceptible and nalidixic acid-resistant isolates of *Campylobacter jejuni* and *Campylobacter coli* in Ontario from 1981 to 1993

Year of Isolation	*C. jejuni*			*C. coli*		
	Total No. of Isolates	No. of Nalidixic Acid-Susceptible Isolates (%)	No. of Nalidixic Acid-Resistant Isolates (%)	Total No. of Isolates	No. of Nalidixic Acid-Susceptible Isolates (%)	No. of Nalidixic Acid-Resistant Isolates (%)
1981	255[a]	255 (100)	0 (0)	0	0 (0)	0 (0)
1982	453	453 (100)	0 (0)	0	0 (0)	0 (0)
1983	64[b]	64 (100)	0 (0)	2[c]	2 (100)	0 (0)
1984	88	88 (100)	0 (0)	6	6 (100)	0 (0)
1985	112	103[d] (92.0)	9[e] (8.0)	15	15 (100)	0 (0)
1986	123	123[f] (100)	0 (0)	16	16 (100)	0 (0)
1987	86	86[g] (100)	0 (0)	38[h]	38 (100)	0 (0)
1988	113	106[i] (93.8)	7 (6.2)	18	17[j] (94.4)	1 (5.6)
1989	215	205 (95.3)	10 (4.7)	17	16 (94.1)	1 (5.9)
1990	218	203[k] (93.1)	15 (6.9)	28	26[l] (92.9)	2 (7.1)
1991	234	218[m] (93.2)	16 (6.8)	44	40[n] (90.9)	4 (9.1)
1992	82	61[o] (74.4)	21[p] (25.6)	26	16 (61.5)	10 (38.5)
1993	65	57[q] (87.7)	8 (12.3)	20	17 (85.0)	3 (15.0)

a Includes 4 canine strains.
b Includes 26 strains from a nursing home outbreak.
c Porcine strains.
d Includes 46 strains from two waterborne outbreaks and 1 strain from a sample of liverwurst.
e Related to 1 of 2 waterborne outbreaks (see footnote [d]).
f Includes 1 blood isolate.
g Includes 2 blood isolates.
h Includes 1 canine strain and 1 porcine strain.
i Includes 11 isolates from two different outbreaks (day-care centre and banquet).
j Includes 1 canine strain.
k Includes 6 blood isolates.
l Includes 1 blood isolate.
m Includes 4 blood isolates and 1 canine strain.
n Includes 1 blood isolate.
o Includes 7 blood isolates.
p Includes 1 blood isolate.
q Includes 4 blood isolates

Results

No resistance to NA was observed in *C. jejuni* and *C. coli* strains until 1985, when *C. jejuni* isolates resistant to this compound were identified during the investigation of a waterborne outbreak in a southern Ontario community[9] (Table 1). Resistance to NA was first identified among *C. jejuni* and *C. coli* isolates associated with sporadic cases of campylobacteriosis in 1988. During that year, seven (6.2%) *C. jejuni* strains and one (5.6%) *C. coli* strain were found to be resistant to this compound. Between 1989 and 1992, the percentage of NA-resistant strains ranged between 4.7% and 25.6% for *C. jejuni* and 5.9% and 38.5% for *C. coli*, respectively (Table 1).

The results of susceptibility testing of a total of 20 clinical strains of *C. jejuni* and *C. coli*

against NA, ciprofloxacin, norfloxacin and erythromycin, as determined by an agar dilution procedure, are shown in Table 2. Eleven of these strains were susceptible to NA (as determined by disk diffusion testing) and susceptibility to this compound was confirmed by agar dilution testing (MIC range, 4 to 16 mg/L). All of these strains were susceptible to ciprofloxacin (MIC range, ≤ 0.06 to 1.0 mg/L), norfloxacin (MIC range, 0.25 to 2.0 mg/L) and erythromycin (MIC range, 0.25 to 4.0 mg/L). Nine of the strains were resistant to NA as determined by disk diffusion testing and these results were confirmed by agar dilution testing (MIC range, 64 to 256 mg/L). All of the NA-resistant strains were cross-resistant to ciprofloxacin (MIC, 16 mg/L) and norfloxacin (MIC range, 32 to 128 mg/L). In addition, two of the NA-resistant strains (1 *C. jejuni* and 1 *C. coli*) were resistant to erythromycin (MIC, 128 mg/L).

Table 2
Antimicrobial susceptibilities of selected nalidixic acid-susceptible and nalidixic acid-resistant isolates of *Campylobacter jejuni* and *Campylobacter coli*

	MIC (mg/L)			
STRAIN	NALIDIXIC ACID	CIPROFLO-XACIN	NORFLO-XACIN	ERYTHRO-MYCIN
CJ*5	8	≤ 0.06	0.25	1.0
CJ 6	4	0.12	0.50	0.50
CJ 7	4	0.12	0.50	1.0
CJ 9	4	0.12	0.50	1.0
CJ 11	16	0.50	2.0	4.0
CJ 12	4	≤ 0.06	0.25	2.0
CJ 13	8	1.0	2.0	2.0
CJ 15	4	0.12	0.50	1.0
CJ 16	4	≤ 0.06	0.50	1.0
CC*1	8	≤ 0.06	0.50	0.25
CC 14	8	0.25	0.50	4.0
CJ 2	256	16	64	0.50
CJ 17	64	16	64	> 128
CJ 19	128	16	64	1.0
CJ 20	64	16	32	2.0
CJ 22	64	16	32	0.50
CJ 27	64	16	64	1.0
CC 4	128	16	64	0.50
CC 18	64	16	64	> 128
CC 26	128	16	128	1.0

* CJ - *Campylobacter jejuni*; CC - *Campylobacter coli*

Discussion

The results of our investigation indicate that increased resistance to quinolones has emerged in clinical strains of *C. jejuni* and *C. coli* isolated in Ontario in recent years. The actual rates of resistance in these closely related species are currently unknown. The percentages of resistant isolates reported in the present investigation may not necessarily reflect the actual rates of resistance because our data are based on clinical isolates sent to a reference laboratory for identification. Nevertheless, the emergence of quinolone resistance among *Campylobacter* spp. demonstrated in this study has several important implications.

From the standpoint of the clinical laboratory, susceptibility to NA can no longer be regarded as a reliable means of differentiating *C. jejuni* and *C. coli* from other members of the genus *Campylobacter*. Laboratories that encounter NA-resistant campylobacters may misidentify these strains as *C. lari* or other species that are intrinsically resistant to this compound. To avoid such errors, we recommend the use of the indoxyl acetate hydrolysis test as a rapid, reliable and inexpensive method for differentiation between *Campylobacter* species[2]. This test is commercially available in Canada.

The emergence of NA resistance in *C. jejuni* and *C. coli* may also have implications for the treatment of infections caused by these organisms because each of the NA-resistant isolates analyzed in the present study were shown to be cross-resistant to ciprofloxacin and norfloxacin. *Campylobacter* enteritis is usually a mild, self-limiting illness and normally does not require antimicrobial therapy. However, the use of antimicrobial agents is indicated in cases of severe, prolonged or relapsing illness[10]. Traditionally, erythromycin has been the antimicrobial of choice for *Campylobacter* infections, but more recently fluoroquinolones have been used increasingly for therapy of gastrointestinal infections caused by campylobacters and other bacterial pathogens[11].

Since 1990, several European investigations have demonstrated increasing resistance to NA and cross-resistance to fluoroquinolones in *C. jejuni* and *C. coli* strains isolated from human sources[3,4,5]. In Spain, levels of resistance to these compounds have been reported to be as high as 50%[6]. Compared with other bacterial enteropathogens, relatively little is known about the current antimicrobial susceptibilities of *Campylobacter* spp. isolated in Canada. In 1981, no resistance to NA and only 1% resistance to erythromycin was identified among a collection of *C. jejuni* clinical strains isolated in Ontario and Alberta[12]. Similarly, in 1986 it was reported that only 0.6% of *C. jejuni* strains isolated in Quebec were resistant to NA or erythromycin and none of these strains was resistant to norfloxacin[13]. More recently, no resistance to ciprofloxacin was found in a study of *C. coli* strains isolated from humans in Quebec, but only 21% of these strains were susceptible to erythromycin[14].

The present study indicates that, although the usefulness of routinely testing the susceptibility of campylobacter strains to NA for species identification purposes has been diminished, the results of such testing can provide useful preliminary information regarding the susceptibility of the isolates to fluoroquinolones. In addition, our findings emphasize the need for ongoing regional surveillance of resistance patterns among clinical strains of *C. jejuni* and *C. coli* in Canada.

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