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Epidemiology of *Campylobacter jejuni* Outbreak in a Middle School in Incheon, Korea

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On July 6, 2009, an outbreak of gastroenteritis occurred among middle school students in Incheon. An investigation to identify the source and describe the extent of the outbreak was conducted. A retrospective cohort study among students, teachers, and food handlers exposed to canteen food in the middle school was performed. Using self-administered questionnaires, information was collected concerning on symptoms, days that canteen food was consumed, and food items consumed. Stool samples were collected from 66 patients and 11 food handlers. The catering kitchen was inspected and food samples were taken. Of the 791 people who ate canteen food, 92 cases became ill, representing an attack rate of 11.6%. Thirty-one (40.3%) of the 77 stool specimens were positive for *Campylobacter jejuni*. Interviews with kitchen staff indicated the likelihood that undercooked chicken was provided. This is the first recognized major *C. jejuni* outbreak associated with contaminated chicken documented in Korea.

Key Words: *Campylobacter*; Epidemiology; Disease Outbreaks

INTRODUCTION

Campylobacter infection is the most commonly identified cause of bacterial gastroenteritis in developed countries (1). Infection can lead to serious sequelae such as Guillain-Barré syndrome (GBS) and reactive arthritis (2). Over 90% of human gastroenteritis is caused by *Campylobacter jejuni* with most of the remaining cases being caused by *C. coli* (3). The epidemiology of human *Campylobacter* infection is complex, with food (4), water (5), and environmental sources (6) all having a role. Especially, the handling of raw poultry and eating undercooked chicken carry high relative risks of *Campylobacter* infection (4). However, until the present report, few studies have linked *Campylobacter* outbreaks with undercooked chicken in Korea.

On July 6, 2009, a local clinic doctor informed a regional public health office of 7 cases of gastroenteritis in students from a middle school near the clinic. An outbreak control team of the regional public health office was arranged at short notice and investigations were instituted. About 2 hr later, the public health department of Incheon City Hall was notified by the public health office of an outbreak of gastroenteritis affecting 232 students from a middle school located in Incheon.

The goals of this study were 1) to identify the causative organism of the outbreak, source of the infections and mode of transmission; 2) to describe the outbreak; 3) to find the preventive measures against outbreaks similar to this case. Herein, we describe a food-borne outbreak of gastroenteritis associated with *Campylobacter jejuni* among middle school students.

MATERIALS AND METHODS

Outbreak epidemiology

A retrospective cohort study was conducted among students, teachers, and food handlers who were exposed to canteen food in the affected middle school. Self-administered questionnaires were distributed to these individuals from July 6–8 and information was collected on demographic details, symptoms, time of onset and duration of illness, and individual canteen food items consumed from July 1–3. A case was defined as a school member who consumed canteen food from July 1–3 and who exhibited abdominal pain accompanied by diarrhea and/or tenesmus and/or headache from July 3–6, 2009. The average incubation period and the common point of exposure were estimated using an established incubation period model (7).

As part of the investigation, the catering kitchen was examined including specimens taken from utensils, and interviews were

conducted with kitchen staff about food handling practices and illness. Preserved foods, drinking water and kitchen utensil specimens were collected and examined at the public health office. Symptomatic people and food handlers were asked to submit stool samples for standard bacteriological, virological, and protozoan examinations (Table 1). Food and water specific attack rates (ARs), relative risk (RR), 95% confidence interval (95% CI) were calculated, and ARs were compared among groups or grades using chi-squared test. The association between consumption of drinking water or food and the risk of disease was determined using chi-squared or Fisher's exact tests. Upon menu analyses on each day (July 1–3), cases that had occurred before lunch were excluded. Statistical analyses were made using SPSS for Windows (version 10.0; SPSS Institute, Chicago, IL, USA).

Microbial identification and pulsed-field gel electrophoresis (PFGE)

Each sample (stool or rectal swab) was planted to Preston broth (Oxoid, Basingstoke, UK) and incubated at 42°C for 48 hr under microaerobic conditions (5% O_2, 5% CO_2, 85% N_2). The sample was then streaked onto *Campylobacter* blood free selective agar (Oxoid). One presumptive *Campylobacter* isolate from each sample was identified to the species level on the basis of Gram staining, catalase and oxidase production, hippurate hydrolysis and API Campy system (Biomerieux, Marcyl'etoile, France). Further identification was performed with PCR using primer sets specific to *hipO* gene in *C. jejuni*. Template DNA for PCR was prepared by boiling method. PCR was performed using a *C. jejuni* kit (Rapigen, Gunpo, Korea) in accordance with the manufacturer's instruction.

A total of 31 *Campylobacter* spp. were analysed by PFGE using the restriction enzyme *Sma*I. The isolates were suspended in 2 mL of cell suspension TE buffer (100 mM Tris, 100 mM EDTA, pH 7.5). A 200 μL aliquot of adjusted cell suspensions and an

equal volume (200 μL) of melted 1.2% SeaKem Gold agarose were mixed by pipetting. The agarose cell suspension mixture was dispensed into the wells of plug molds (Bio-Rad Laboratories, Hercules, CA, USA) and solidified at 4°C for 5 min. The plugs were transferred to 2 mL tubes containing 1.5 mL of ES buffer (0.5 M EDTA, 1% sodium-lauroyl-sarcosine) and 40 μL of proteinase K (20 mg/mL). Lysis was allowed to proceed for 90 min at 55°C in an orbital shaker water bath. After lysis, the plugs were washed five times with plug wash TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5).

A 2 mm wide slice from each plug was cut with a single-edge razor blade and transferred to a tube containing 100 μL of the restriction enzyme mixture (40 unit *Sma*I 1 μL, 100 BSA 1 μL, buffer 10 μL, DW 88 μL). The plug slices were incubated at 25°C for 4 hr. The electrophoresis conditions consisted of an initial switch time of 6.76 sec and a final switch time of 35.38 sec (gradient of 6 V/cm and an included angle of 120). The gels were electrophoresed for 18 hr, depending on the equipment used (CHEF Mapper, Bio-Rad), in 0.5× TBE. After the electrophoresis run was finished, the gels were stained with 500 mL of ethidium bromide solution (50 μg/mL), and the band pattern was observed under UV illumination. Matching and dendrogram UP-GMA (unweighted pair group method with averages) analysis of the PFGE patterns was performed using the Dice coefficient with a 1.0% tolerance window.

RESULTS

Epidemiologic findings

Of the 921 school members (854 students, 48 teachers, 11 food handlers, eight other employees) who consumed canteen food, 791 (85.8%) completed the questionnaire. Of these 791 people (760 males, 31 females), 738 (93.2%) were students, 38 (4.8%) were teachers, and 11 (1.3%) were food handlers. Ninety-two school members met the case definition, representing an overall AR of 11.6% (92/791). AR did not differ significantly among students, teachers, and food handlers, but was significantly higher in second-grade students than students in other grades ($P \leq 0.01$) (Table 2).

Day of illness onset for the 92 cases is shown in Fig. 1. The epidemic curve showed characteristics of a point source out-

Table 1. Examination of environmental specimens and rectal swabs

Samples	Number of specimens (Number of persons)	Microorganisms
Rectal swabs	77 (77)	Bacteria (10): *Salmonella, Shigella, Staphylococcus aureus, Vibrio* species, *Listeria monocytogenes, Yersinia enterocolitica, Bacillus cereus, Escherichia coli, Clostridium perfringens, Campylobacter jejuni* Viruses (5): Rotavirus, Norovirus, Adenovirus, Astrovirus, Sapovirus Protozoa (3): *Cryptosporidium, Giardia, Entamoeba*
Drinking water*	13	Bacteria (10): *Salmonella, Shigella, Staphylococcus aureus, Vibrio* species, *Listeria monocytogenes, Yersinia enterocolitica, Bacillus cereus, Escherichia coli, Clostridium perfringens, Campylobacter jejuni*
Preserved foods	7	
Kitchen utensils	4	

*Water purifiers were located in the feeding facility, classroom corridors and teachers' office.

Table 2. Attack rate by group in a middle school in Incheon, Korea, 2009

Group	No. of subjects	No. of patients	Attack rate (%)
Students			
First-grade	257	18	7.0
Second-grade	253	51	20.1
Third-grade	228	21	9.2
Teachers	38	2	5.2
Cooking workers	11	0	0.0
Other employees	4	0	0.0
Total	791	92	11.6

break. The canteen food and drinking water were provided during lunch time (12:00–13:00). The first patient developed symptoms beginning at approximately 1:30 a.m. on July 3. After a slight increase beginning on July 3, the number of cases rose sharply to a distinct peak on July 4 and decreased exponentially over the subsequent 2 days. The average incubation period for this outbreak was 2.13 days (95% CI: 0.66–6.81 days). The date of exposure was considered to be July 1. The main symptoms were abdominal pain (100%) and diarrhea (80.4%). Tenesmus (62%) and headache (48.9%) were less frequent (Table 3).

Laboratory findings

A total of 77 stool samples were provided from 66 symptomatic people and 11 food handlers. Of the 66 patients who provided stool samples (Table 4), 31 samples (46.9%) were positive for *C. jejuni*. All these samples came from students. Among these 66 patients, only 36 completed the questionnaire; of the 36 respondents, 10 were positive for *C. jejuni*. Five (7.5%) samples were positive for enteropathogenic *Escherichia coli* (EPEC). EPEC was simultaneously detected in two students who were positive for *C. jejuni*. The small number of EPEC cases effectively excluded the bacterium as the causative pathogen of the outbreak.

Pulsed-field gel electrophoresis (PFGE) of *Sma*I-digested DNA isolated from patients revealed three patterns: DBRS16.001 (n=29 strains), DBRS16.002 (n=1), and DBRS16.003 (n=1). The
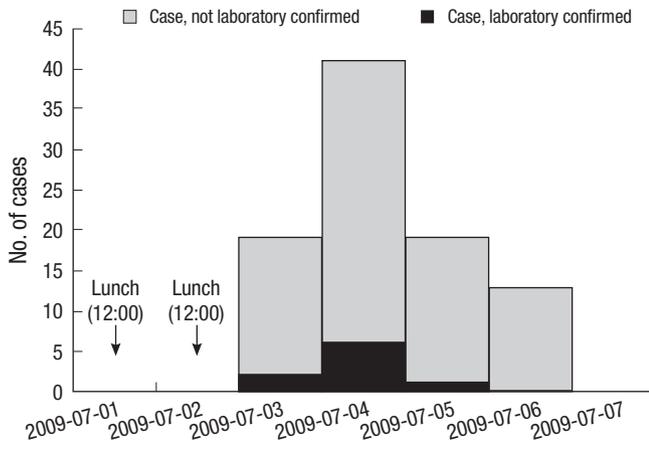
DBRS16.001 pattern shared 92.3% and 85.5% similarity with the DBRS16.002 pattern and DBRS16.003 pattern, respectively (Fig. 2), consistent with a single source of infection in the outbreak.

The culture of drinking water and the other environmental specimens showed negative results. Only seven preserved foods were obtained (Korean cabbage kimchi, boiled barley rice, potato and hot pepper paste soup, grilled scabbard fish with curry, steamed sesame leaf, diced radish kimchi, yogurt). The remainder had already been discarded by school officials prior to the investigation. Excluding Korean cabbage kimchi, the foods on July 2 were not kept despite the legal preservation period.

Statistical analyses of food and water

Upon menu analysis, consumption of grilled scabbard fish with curry on July 3 was significantly associated with illness (RR=2.39). The other food items were not significantly associated with illness (Table 5). Based on cross-classification analysis of chicken soup with ginseng and grilled scabbard fish with curry, consumption of both food items was associated with a higher AR than consumption of either food item alone (Table 6).
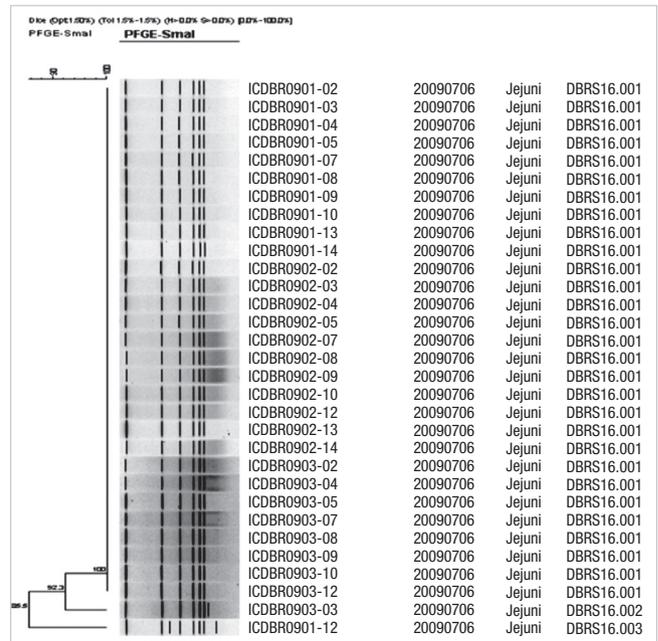

Fig. 2. Results of PFGE analyses.

Fig. 1. The epidemic curve by symptom onset date in a middle school.

Table 3. Prevalence of symptoms in cases* in a middle school in Incheon, Korea, 2009

Symptoms	Number of patients	Positive rate (%)
Diarrhea	74	80.4
Tenesmus	57	62.0
Headache	45	48.9
Nausea	36	39.1
Chills	32	34.8
Fever	26	28.3
Vomiting	9	9.8

*A school member who had abdominal pain, accompanied with one or more symptoms among diarrhea, tenesmus, and headache during July 3–6, 2009.

Table 4. Result of microbiological examinations

Group	No. of subjects	*Campylobacter jejuni*	*Campylobacter jejuni* +EPEC*	EPEC	No growth
Students	65	29	2	3	31
Teachers	1	0	0	0	1
Cooking workers	11	0	0	1	10
Otheremployees	0	0	0	0	0
Total	77	29	2	4	42

*Enteropathogenic *Escherichia coli*.

Table 5. Relative risk of suspected foods

Date	Food*	Intake			Non-intake			Relative risk (95% C.I.)	P value
		No.	Cases	AR (%)	No.	Cases	AR (%)		
7/1	Chicken soup with ginseng	690	83	12.0	91	6	6.6	1.82 (0.82-4.05)	0.12
	Leek and pumpkin panfried food	570	62	10.9	218	28	12.8	0.84 (0.55-1.28)	0.43
	Cuttlefish salad	621	75	12.1	166	15	9.0	1.33 (0.78-2.26)	0.27
	Radish kimchi	576	67	11.6	209	23	11.0	1.05 (0.67-1.65)	0.80
	Purified water in feeding facility	632	76	12.0	148	11	7.4	1.61 (0.88-2.96)	0.11
7/2	Black boiled rice	738	85	11.5	41	5	12.2	0.94 (0.40-2.19)	0.89
	Spicy beef soup	688	82	11.9	97	8	8.2	1.44 (0.72-2.89)	0.28
	Pork and quail's egg boiled down in soy	729	84	11.5	56	6	10.7	1.07 (0.49-2.35)	0.85
	Cham cooked potherb	549	59	10.7	236	31	13.1	0.81 (0.54-1.22)	0.33
	Korean cabbage kimchi	650	74	11.4	135	16	11.9	0.96 (0.57-1.59)	0.87
	Purified water in feeding facility	614	73	11.9	162	14	8.6	1.37 (0.79-2.37)	0.24
7/3	Boiled barley rice	712	81	11.4	64	4	6.3	1.82 (0.68-4.80)	0.29
	Potato and hot pepper paste soup	617	67	10.9	164	18	11.0	0.98 (0.60-1.61)	0.96
	Grilled scabbard fish with curry	660	79	12.0	120	6	5.0	2.39 (1.06-5.36)	0.02
	Steamed sesame leaf	518	58	11.2	262	27	10.3	1.08 (0.70-1.67)	0.70
	Diced radish kimchi	624	67	10.7	156	18	11.5	0.93 (0.57-1.51)	0.77
	Yogurt	715	82	11.5	66	3	4.5	2.52 (0.82-7.76)	0.09
	Purified water in feeding facility	583	68	11.7	188	13	6.9	1.68 (0.95-2.98)	0.06

*Foods were provided during lunch time (12:00-13:00).
No., Number of subjects; AR, Attack rate.

Table 6. Attack rate by chicken soup with ginseng and grilled scabbard fish with curry

	+Chicken soup with ginseng			−Chicken soup with ginseng		
	No.	Cases	AR (%)	No.	Cases	AR (%)
+Grilled scabbard fish with curry	603	72	11.9	49	4	8.2
−Grilled scabbard fish with curry	78	4	5.1	41	2	4.9

+Chicken soup with ginseng, subjects who ate chicken soup with ginseng.
−Chicken soup with ginseng, subjects who did not eat chicken soup with ginseng.
+Grilled scabbard fish with curry, subjects who ate grilled scabbard fish with curry.
−Grilled scabbard fish with curry, subjects who did not eat grilled scabbard fish with curry.
No., Number of subjects; AR, Attack rate.

Environmental investigations

The school food service facility was located between the main building and an annex building. The facility housed its own water supply and was equipped with five water purifiers. The main building had a water purifier on the first and third floor, and one in the teachers' office. The annex building had a water purifier on the third floor. The annex building also housed second-grade classrooms, with the first- and third-grade classrooms located in the main building. The water supplies were used in the food processing but purified water was not used. The original water for the water purifiers was supplied from water supplies. Prior to the July 6 illness outbreak, the purifier filters had all been replaced on May 29 with newly-purchased filters (as verified by the purchase receipts). Water samples had been collected from the water purifiers and tested on June 12 by a contracted inspection business; total coliform counts for all samples were zero.

The residual chlorine concentration rates of the water pipes in the bathrooms in the kitchen, main building, annex building and outside the building were measured; the concentration exceeded 0.4 ppm. There was no evidence of underground water supplies in the water supplies and drainage tax receipts during the last three months. Also, a 30 ton sewage septic tank was located outside the kitchen at distance of about 5 meters. It had been sterilized on June 19 by a service company. The decontamination cleaning certificate and photographs of the cleaning operation were examined.

Additional investigations

Considering laboratory results, chicken soup with ginseng prepared and consumed on July 1 was considered to be the infection source because handling and eating undercooked poultry have consistently been shown to be important risk factors in foodborne illness. The investigation team members revisited the food service facility on July 23, 2009 and performed a thorough investigation of the cooking process and the food ingredients of chicken soup with ginseng. The raw chickens used in the chicken soup with ginseng were supplied frozen (-6°C) in the morning of July 1. The chickens had been slaughtered and processed on June 29. They were deemed acceptable for use in food until July 9 if kept in refrigerated.

The preparation pathway for the chickens used on July 1 was as follows. The raw chickens were stored in a meat refrigerator immediately after being inspected. About 1 hr later, they were cleaned at the meat sterilization sink and transported on meat carts to a couple of large iron pots. All the chickens were boiled in the pots along with jujubes, garlic, and ginseng. An estimated 460 carcasses were simultaneously boiled in each pot. While

being boiled, each chicken carcass was removed from the pot and glutinous rice was added into the pot. The central temperature of the chicken or the soup was not recorded. All the prepared chicken carcasses were put into eight food distribution containers and stored in a heating cabinet. Only one food handler was involved in the process of cooking and distributing chicken soup with ginseng. The food handler was not cooking other menus. Other vegetables were cleaned at a vegetable sink and cuttlefishes were cleaned at a fish sink. All the raw chickens had been supplied by company A. Among other schools supplied by the company A, there were no cases of food poisoning. There were no recorded food hygiene regulation violations by company A.

Cuttlefish salads were provided together with the chicken soup with ginseng. The cuttlefishes used in the cuttlefish salads were supplied in a refrigerated state after being handled by company B. Their acceptable period of use was until December 30, 2009. Company B also supplied eight other schools with the same products and no food poisoning occurred in these schools. The ingredients of the grilled scabbard fish with curry prepared on July 3 were curry, soybean oil, fry powder, and frozen scabbard fish. The frozen scabbard fishes were imported from China. Their acceptable period of use was until January 2, 2010. Their core temperature was recorded as 92–98°C. No cases of food poisoning were recorded at the other schools supplied with frozen scabbard fish by the same company. The company was free of food hygiene violations.

DISCUSSION

The results of the investigation conducted after the illness outbreak suggested that the chicken soup with ginseng provided on July 1 was the most probable vehicle of transmission of illness. This finding is not surprising, given that undercooked chicken is a recognized risk factor for campylobacteriosis (8-10). Indeed, a survey of raw poultry demonstrated that 50–70% of raw chickens tested at the retail level were contaminated with *Campylobacter* (11). In the soup preparation at the school, raw chickens were boiled intact. An estimated 460 chicken carcasses were cooked in an iron pot at a time, making it possible that not all the chickens were completely cooked. *C. jejuni* is inactivated <10 min after ground beef reaches an internal temperature of 70°C (12). It is conceivable that the preparation of such a large number of chickens using a double boiler was inadequate to consistently achieve this internal temperature. Moreover, the internal temperature of the chicken or the chicken soup with ginseng was not recorded.

An interview with a nutritionist revealed that there could be some defects in the process designed to determine whether all the chickens were sufficiently prepared. The large number of chickens contained in the two pots (approximately 920) was too great to permit inspection by the one food handler. Addi-

tionally, in the process of taking the chickens out of the pots for addition of glutinous rice, it is possible that the upper level chickens, which would have been the first to be removed, were undercooked because of relatively low heating temperature and short heating time. These chickens were mainly distributed to the second-grade students, who displayed the highest AR. Subsequently, the chicken soup was distributed to first-grade students followed by third-grade students.

In this outbreak, the date of exposure was estimated to be lunchtime on July 1, considering the peak of symptom development on July 4 and an average incubation period for *Campylobacter* infection of 2–5 days (13). Among the 4 food items provided on July 1, it is possible that other food items like cuttlefish salad were cross-contaminated with *Campylobacter* while the food handlers were storing, cleaning, and transporting the raw chickens. However, since every cooking step was isolated and every food item was cooked by dedicated food handlers, cross-contamination was very unlikely.

Grilled scabbard fish with curry prepared on July 3 was statistically significant. However, preserved food was negative for *Campylobacter* and the probable date of exposure (July 1) was not consistent. In seven cases, symptoms began before lunch on July 3. Since the central temperature of the fish served on July 3 was recorded 92–98°C, there was no chance that *Campylobacter* survived in the consumed fish. In addition, the food ingredients (frozen scabbard fish, curry, soybean oil and fry powder) are not generally reported risk factors of *Campylobacter*. Based on cross-classification, a relation of effect modification between chicken soup with ginseng and grilled scabbard fish with curry was possible, although how cross-contamination of grilled scabbard fish with curry by chicken soup with ginseng could have occurred is unknown.

The reports of a large waterborne outbreak of campylobacteriosis (14) suggested the possibility that the purified water at the feeding facility was the cause of the outbreak. However, testing of purified water at the feeding facility on July 1–3 was not statistically significant, and the water culture was negative for bacteria. In addition, all purifiers had been recently inspected as being in good condition. Therefore, the purified water was not determined to be an infection source.

In this study, hippurate hydrolysis and PCR were used to distinguish *C. jejuni* and *C. coli*. Previous studies have described a discrepancy between hippurate test and PCR-based assay (15, 16). False positive hippurate test results for non-*C. jejuni* species have also been recognized (17). Seventeen strains identified as *C. coli* by PCR were wrongly identified as *C. jejuni* by the hippurate test. The positive predictive value for the hippurate test was 83% (18). The present study showed that all the 31 positive specimens for hippurate hydrolysis were positive by PCR without false-positivity. This result confirmed a single source of infection in this outbreak.

In conclusion, *Campylobacter* was the cause of this outbreak. One limitation in this report was that it was impossible to detect *Campylobacter* in preserved foods, because food samples were unavailable at the time of this survey. The investigation did not begin until 3 days after the outbreak due to the notification delay. A second, and most important, limitation is that laboratory examination of the chicken soup with ginseng was not performed. The food provided on July 2 was not preserved in spite of the legal preservation period.

Despite these limitations, this outbreak highlights the danger of *Campylobacter* infection from eating undercooked chicken products. These data also suggest that authorities need to prolong the legal preservation period of preserved food and strengthen regulations pertaining to measurement of the core temperature of food.

ACKNOWLEDGMENTS

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