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Campylobacteriosis in An Outbreak of Foodborne Disease at Bantar Gadung Village Palabuhan Ratu

Luhung Budiailmiawan[1], Ida Parwati[1], Coriejati Rita[1], Dewi Lokida[2]

[1] Department of Clinical Pathology, Faculty of Medicine, Padjadjaran University/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia. E-mail: luhungbudiailmiawan@yahoo.co.id
[2] Indonesia Research Partnership on Infectious Diseases (INA-RESPOND) Laboratory, Tangerang, Indonesia

ABSTRACT

Campylobacteriosis is a rare enteritis case in Indonesia. On September 12, 2019, there was an outbreak of foodborne diseases with the symptoms of fever, abdominal cramps, and bloody diarrhea after eating food from the Tahlilan (funeral prayer) ceremonial at Bantar Gadung village Palabuhan Ratu Sukabumi. The laboratory and epidemiological investigation were performed. Fresh stool samples were collected from 18 suspected cases. Gram stain, routine stool examination, and PCR were performed. The PCR method was used based on the nested PCR method by Platts-Mills *et al*. for *Campylobacter spp*. The positive result was identified by visualization in gel electrophoresis at 816-bp. Interview, environmental investigation, and analysis of incidence rate were performed for epidemiological study. The majority of patients were males aged 33-48 years old. The main symptoms were fever (100%), abdominal cramps (100%), and bloody diarrhea (11/18;61%). Nine of them (9/11; 82%) were confirmed by Gram staining as Gram-negative bacteria, with comma, s-shaped, or seagull appearance, as presumptive *Campylobacter spp*. Two patients (2/18;11%) were confirmed infected with *Campylobacter spp*. by PCR and sequencing tests. It has been found that this outbreak was caused by food contaminated with *Campylobacter spp*. The incidence rate of *Campylobacteriosis* in this outbreak was 11%.

Keywords: Outbreak, *Campylobacter spp.*, PCR

INTRODUCTION

Campylobacter spp. is a pathogenic bacteria in humans that causes enteritis and sometimes systemic invasion, especially in infants.[1] This bacterium causes diarrhea accompanied by mucus and blood (also called bloody diarrhea). It is one of the top four bacteria that cause foodborne diseases (such as *E.coli, Shigella, Salmonella*, and *Campylobacter*) worldwide.[2] It is estimated that 2 million Campylobacteriosis cases occur each year in the United States.[1] Tjaniadi *et al*. reported that *Campylobacter jejuni* is one of the diarrhea causes in Indonesia. It was also reported that 3.6% of diarrhea cases in Indonesia were caused by *Campylobacter jejuni*.[3]

Campylobacteriosis is a disease caused by *Campylobacter spp*. infection. The incubation period usually occurs 2-5 days after infection, but it can also range from 1 to 10 days. The most common clinical symptoms of *Campylobacter spp*. infection include diarrhea (frequently bloody), abdominal pain, fever, headache, nausea, or vomiting. Mortality cases caused by Campylobacteriosis are rare and usually confined to very young children or elderly patients, those already suffering from another severe disease such as Human Immunodeficiency Virus (HIV), or immunosuppressive patients. Complications such as bacteremia, hepatitis, pancreatitis, and miscarriage have been reported with various degrees of frequency. Post-infection complications may include reactive arthritis and neurological disorders. One of them is Guillain Barre syndrome, a polio-like paralysis that can result in respiratory and severe neurological dysfunction in a small number of cases.[2]

CASE

On September 12, 2019, there was an outbreak of food poisoning after eating food from Tahlilan's ceremony at Bantar Gadung village Palabuhan Ratu Sukabumi. Eighteen outbreak patients came to Palabuhan Ratu Hospital with the chief complaint of abdominal cramps.

History

There were 137 people involved in this outbreak, and two people passed away; a 37-year-old female and a 9-year-old child. Both were suspected of having died due to sepsis. This village has been

reported to have three times food poisoning outbreaks in 2019.[4,5] Eighteen outbreak patients came to Palabuhan Ratu Hospital with abdominal cramps, fever, and bloody diarrhea. All patients had been given antibiotics with Ceftriaxone injection and Metronidazole infusion for two days. However, most of them did not show any improvement. Finally, after changing the antibiotics to a single dose of Azithromycin for three days, the patient recovered.

Investigation

Laboratory Investigation

The data of clinical symptoms, stool examination, and Gram staining from fresh stool were collected. The Gram staining was performed with quantification based on Clinical Microbiology Proficiency Testing (CMPT).[6] Table 1 shows the CMPT program recommended Gram stain reporting criteria.

Table 1. A clinical microbiology proficiency testing program recommended Gram stain reporting criteria

Grade	Description	Total Per Oil Immersion Field (x1000)	
		cells	Bacteria
1+	Rare	<1	<1
2+	Few	1-5	2-10
3+	Moderate	6-10	11-50
4+	Many	>10	>50

Adaptation from Church[6]

For microbiological culture, food specimens were taken and sent to the provincial health laboratory. Stool samples were sent to the INA-RESPOND laboratory to conduct a Polymerase Chain Reaction (PCR) and sequencing tests. The PCR method used based on Platts-Mills *et al.* nested PCR for *Campylobacter spp.* detection.[7] Principle used in the nested PCR was as follows: a portion of the16S rRNA gene of *Campylobacter spp.* DNA genome was amplified using the 16S rRNA_F and 16S rRNA_R primer pairs. The visualization of *Campylobacter spp.* identified the positive result. Specific product in gel electrophoresis at 816-bp.

The PCR procedures for detecting *Campylobacter spp.* were: Bacterial DNA was extracted from stool with QIAmp DNA stool mini kit[8]; All reagents were thawed slowly and kept cold by placing them in a 1.5 mL tube cooler box; Reagent tubes were vortexed lightly to mix and spin down, drops were removed from the inside of the lid and were pipetted; The first PCR reaction mix was prepared using the following recipe from Table 2. The reaction mix was prepared in a 1.5 mL tube for a total reaction. Once finished, it was distributed into each 0.2 mL tube in an equal 15 uL volume (minus DNA). The 0.2 mL tubes were kept cold using a PCR cooler; The reaction mix tubes were brought into the extraction room using a PCR cooler. The DNA and positive control were added to the reaction mix. Next, the tubes were vortexed lightly to mix and spin down and remove drops from the inside of the lid; The RT-PCR was run using *Campylobacter* 16S rRNA. The PCR product was then visualized by gel electrophoresis. The positive result showed that the amplicon matched the target size. The target size of the PCR product *Campylobacter sp.* was 816 bp; The positive results were conducted by sequencing. The primers sequencing of 16S_rRNA_F (5'-GGA TGA CAC TTT TCG GAG C-3') and 16S_rRNA_R (5'-CAT TGT AGC ACG TGT GTC-3').

Epidemiological Investigation

As part of the epidemiology investigation, the kitchen environment was investigated. In addition, interviews were conducted with kitchen staff about food handling practices. The incidence rate was used to describe *Campylobacter spp.* infection epidemiology. The incidence reflects the number of new cases and can be reported as a risk or incidence rate. The incidence rate can be calculated by dividing the number of subjects developing a disease by the total time at risk of getting the disease. The denominator of this formula includes a measure of time instead of just the number of subjects.[9] Below is the formula for incidence rate:

$$\text{Incidence rate} = \frac{\text{Number of subjects developing the total disease}}{\text{time at risk for the disease for all subjects followed}}$$

Table 2. The first reaction mix

Initial Concentration	Component	Concentration (uL)	Final Concentration
2x	Go Taq Green Master Mix	12.5 uL	1.25X
10 uM	16S rRNA_F	1.25 uL	0.625 uM
10 uM	16S rRNA_R	1.25 uL	0.625 uM
	DNA	5 uL	
	Total	20 uL	

DISCUSSIONS

A total of 18 people who showed symptoms of Campylobacteriosis were identified in this study. The characteristics of patients from Bantar Gadung Village are shown in Table 3.

Table 3. The characteristics of patients from Bantar Gadung village

Characteristic	Cases n (%)
Age, years	
1-16	5(27)
17-32	5(27)
33-48	6(33)
49-64	2(11)
>64	1(2)
Gender	
Female	8 (44)
Male	10(55)
Symptoms	
Abdominal cramps	18 (100)
Bloody diarrhea	11 (61)
Fever	18(100)
Gram Stain (*Campylobacter spp*. appearance)	
Grade 2+	5(28)
Grade 3+	4(22)
Negative	9(50)

Table 3 showed that most patients were males aged 33-48 years. The main symptoms were fever (100%), abdominal cramps (100%), and bloody diarrhea (11/18;61%).

Among those patients with bloody diarrhea, Gram-negative bacteria with a morphological comma, s-shaped, or seagull appearance were found in 9 (82%) stool samples. Most of the samples were at Grade 2 (few) quantifications of Gram stain based on CMPT. Gram staining results from the patient's stool are shown in Figure 1.

From the interview of patients, it was discovered that all patients felt dizziness, nausea, and abdominal cramps several hours after consuming eggs and rendang meat. From the environmental investigation of the catering kitchen and kitchen staff interviewing, it was found that the kitchen environment was filthy with the ground floor without tiles. The kitchen staff cooked the food with firewood without a stove and cleaned the raw food with dirty water from a river. The *Campylobacter spp*. mode of transmission to humans is based on direct contact with infected animals or indirectly by consuming water and foods without proper cooking.[10] Therefore, egg and Rendang meat were suspected to be contaminated with *Campylobacter spp*. from improper food preparation with poor kitchen facilities. The main issue for cross-contamination of *Campylobacter spp*. was the use of raw chicken eggs or cow meat with undercooked food. For surveillance, the food specimens were taken for microbial culture tests at the provincial health laboratory.

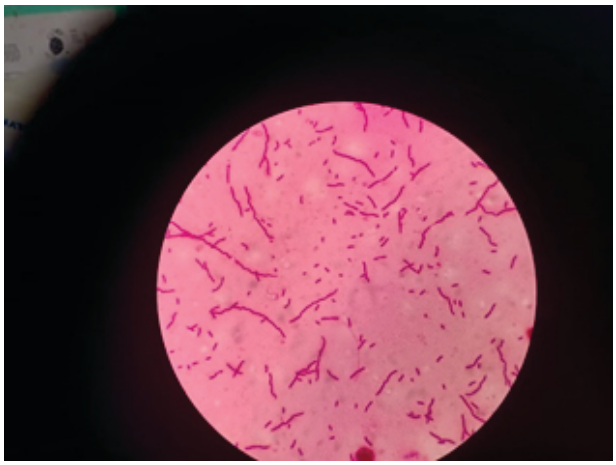

Figure 1. Gram stain of patient stool (1000x magnification) Showing: Gram-negative bacteria with comma, s-shaped, or seagull appearance

The culture results from the provincial health laboratory showed that the Rendang meat seasoning was contaminated by *Staphylococcus aureus*, and the egg seasoning was contaminated by *Candida spp*. However, both microorganisms don't cause bloody diarrhea; therefore, infection by *Campylobacter spp*. couldn't be excluded. The provincial health laboratory doesn't have specific culture media for *Campylobacter spp*. and microaerophilic incubator, leading to the inability to detect *Campylobacter spp. Campylobacter spp*. is a microaerophilic bacteria that require selective agar for its culture and lower O2 (5% O2) and more CO2 (10% CO2) for its incubation.[11] The most commonly used selective agar is the Skirrow Agar, which uses a combination of Vancomycin, Polymixin B, and Trimethoprim, and Campy BAP Agar also includes Cephalothin. Campy Blood Agar Plate (BAP) is made from Brucella Agar base, 10% sheep blood, and antibiotic combinations such as Vancomycin, Trimethoprim, Polymyxin B, Amphotericin B, and Cephalothin.[12] Research from Andriani *et al*. concluded that PCR has better sensitivity

and specificity than the culture method to detect *Campylobacter spp*.[13,14] Therefore, PCR and sequencing tests were used to detect *Campylobacter spp.*

Eighteen fresh stool samples were taken from patients and sent to the INA- RESPOND laboratory for a conventional PCR test. In addition, the two samples that showed positive infection results with *Campylobacter spp*. were tested by pulsed gel electrophoresis PCR using the method used by Platts-Mills *et al*.[7] Figure 2 shows the stool sample results tested by pulsed gel electrophoresis PCR.

Sequencing was then carried out on both samples showing positive PCR results. The sequencing test result confirmed that the two patient samples were positive for *Campylobacter spp.* infections. The incidence rate of *Campylobacter spp.* infection on Bantar Gadung food poisoning outbreak was 11% (2/18;11%).

The incidence rate of *Campylobacter spp*. infection in the Bantar Gadung food poisoning outbreak was 11%. It was higher than the research by Tjaniadi *et al*., which reported that only 3.6% of diarrhea cases in Indonesia were caused by *Campylobacter jejuni* infection.[3] The rare cases of *Campylobacter spp*. difficulties in bacteria isolation can cause infection in Indonesia. Not all microbiology laboratory centers in Indonesia have a micro-aerophilic incubator and *Campylobacter spp*. culture media. From the results, most patients of the food poisoning outbreak were males aged 33-48 years, showing correspondence to the epidemiology of *Campylobacter spp*. Infection populations often show symptoms of Campylobacteriosis in children less than one year and adults aged 20-40 years.[11]

The results concluded that eleven patients with bloody diarrhea and nine were confirmed as Campylobacteriosis by Gram stain, and only two patients were confirmed positive by PCR test. Several factors that caused this condition are: Most patients consumed a small concentration of *Campylobacter spp*. It was proven from quantification by Gram stain based on CMPT that most of the samples were at Grade 2 (a few bacteria); Most of the stool specimens were collected three or four days after taking Azithromycin, and some bacteria might have been killed. *Campylobacter spp*. infection typically peaks 1 to 2 days following onset. The acute enteritis phase of the infection is self-limiting and resolves after seven days.[2]; The stool specimens were tested for PCR after 24 hours and sent to INA-RESPOND laboratory with an uncontrolled temperature; Therefore, most of *Campylobacter spp*. bacteria might have already died. Stool specimens for *Campylobacter spp*. test should be done before 24 hours. If the test is carried out after 24 hours, the samples must be cooled but not frozen and then stored in a closed container that is not exposed to oxygen and contaminants. The genus *Campylobacter spp*. is thermophilic and categorized as micro-aerophilic bacteria. The bacteria will die if they stay too long in the presence of oxygen and without a presentative temperature[15]; There might be some inhibitors for PCR that decrease PCR sensitivity. Most of the inhibitors for PCR stool specimens are polysaccharides, pectin, bile salt, lipids, and urate.[16]

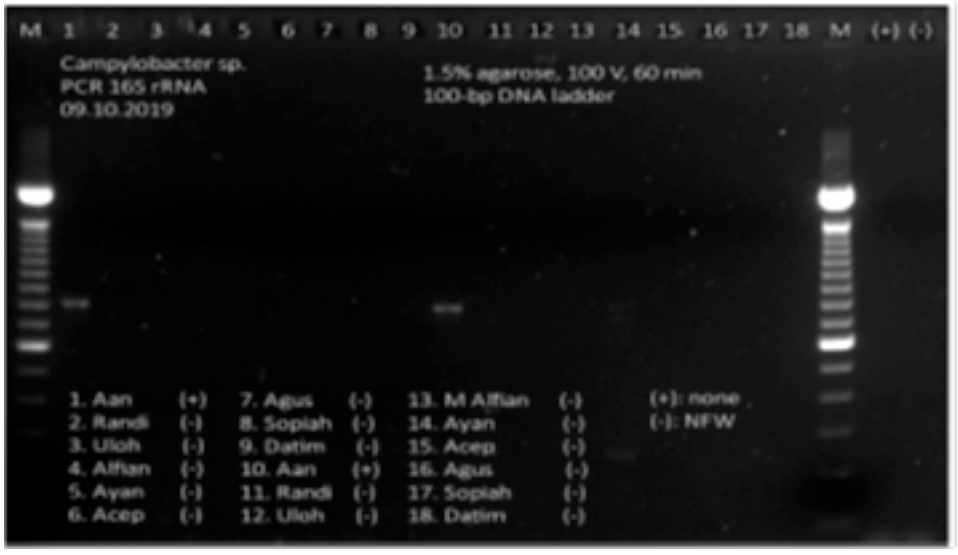

Figure 2. Stool samples tested by pulsed gel electrophoresis PCR
Showing: the two positive PCR was identified by band gel electrophoresis at 816-bp

Some of the patients had been given 2 grams of Ceftriaxone injection or Ciprofloxacin 1000 mg daily on the first and second day, but no improvement was shown. Tjaniadi *et al*. reported that *Campylobacter jejuni* increased the frequency of resistance to Ceftriaxone, Norfloxacin, and Ciprofloxacin but was susceptible to Erythromycin.[3] Therefore, after the antibiotics were changed to Erythromycin or Azithromycin, some improvement was seen. *Campylobacter spp*. infections are self-limiting; therefore, antibiotics treatment is not recommended unless these patients experience fever and bloody or persistent diarrhea.[2] Quinolone resistance in *Campylobacter* has been associated with a single nucleotide mutation at the level of their target (gyr A), especially the Thr86Ile Gyr A, which is the most common mutation among clinical and veterinary isolates, but also with the presence of efflux pump.[17-19] Unfortunately, an antimicrobial sensitivity test was not performed in this study.

The news reports stated that two patients, a 9-year-old child, and a 33-year-old female, had died due to septicemia. Even though *Campylobacter spp*. generally produces self-limiting gastroenteritis, but some complications, such as septicemia, meningitis, hemolytic-uremic syndrome, pancreatitis, and abortion have been reported. Moreover, post-infection sequels such as reactive arthritis and Guillain Bare syndrome have been attributed.[18,20]

From the interviews with the patients, it was reported that all patients had consumed chicken eggs and Rendang meat. The environmental investigation showed that the kitchen is filthy on the ground floor without tiles. The kitchen staff cooked the food with firewood without a stove and cleaned the raw food with dirty water from the river. *Campylobacter spp*. are widely distributed in most warm-blooded animals. The bacteria are prevalent in foods from animals such as poultry, cattle, pigs, sheep, and ostriches; and in pets, including cats and dogs. The bacteria have also been found in shellfish. The main route of transmission is believed to be foodborne, via undercooked meat and meat products, as well as raw or contaminated milk. Contaminated water or ice can also be a source of infection.[2,10] Chicken eggs and Rendang meat were suspected of being contaminated with *Campylobacter spp*. from improper food preparation and poor kitchen facilities. It seemed that the main issues for cross-contamination of *Campylobacter spp*. resulted from raw chicken eggs or meat from undercooked food.

Some strategies that can be used to prevent diseases from *Campylobacter spp*. infection. Prevention is based on control measures at all stages of the food chain, from agricultural production on farms to processing, manufacturing, and preparation of food both commercially and domestically; Prevention methods against infection in domestic kitchens are similar to those used against other foodborne bacterial diseases; Training in hygienic food handling for catering staff to keep the food from contamination; Bactericidal treatment, such as heating (for example, cooking or pasteurization) or irradiation, is the only effective method of eliminating *Campylobacter spp*. from contaminated food.

CONCLUSIONS

Campylobacter spp. was one of the causes of the outbreak. The incidence rate of *Campylobacter spp*. infection in this outbreak was 11%. Therefore, this outbreak could have been caused by food contamination with *Campylobacter spp*. The limitations of this investigation were: No *Campylobacter spp*. culture test on the poisoned food due to the lack of media and incubator for *Campylobacter spp*.; The use of small stool samples for PCR; No antibiotic-sensitive tests were performed for *Campylobacter spp*.

Despite the limitations, this outbreak highlights the danger of *Campylobacter spp*. infection from eating undercooked chicken or meat products. These data also suggest that training in hygienic food handling for catering staff is essential.

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