Journal of Asia Pacific Family Medicine, Vol. 18, No. 1, 2019
https://doi.org/10.22146/apfm.v18i1.62

Food Poisoning Outbreak Caused by *Diarrhoeal Bacillus Cereus* in Tegalkenongo Village, Bantul, Yogyakarta, Indonesia: a Retrospective Study

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ABSTRACT

Background: On May, 13 2019, a food poisoning outbreak of diarrhoeal *B. cereus* associated with contaminated chicken satay occurred in Tegalkenongo village involving villagers after attending mass *iftar* in a mosque. Health office of Bantul District revealed two attendances were hospitalized in the hospital of *PKU Muhammadiyah* after consuming food served during mass *iftar.* Based on the information, we immediately conducted an epidemiological investigation to make sure of the existence of the outbreak. **Objectives:** This study aimed to investigate the causative agents, source of food poisoning and mode of food poisoning transmission in Tegalkenongo Village. **Methods**: A retrospective cohort study was used in the epidemiological investigation during one week from 13 May 2019 – 20 May 2019. **Results:** Based on the investigation, Of the 303 villagers involved in the event, 188 villagers were ill with the median age of cases was 38 years old, the average incubation period was 8 hours, and the predominate symptoms were diarrhea (93.62%), nausea (84.57%), and abdominal cramps (64.89%). Contaminated chicken satay was determined as the source of contamination with adjusted Risk Ratio (aRR) was 4.36; 95% CI 1.1538, 16.5285. Initial epidemiological features and cultures from food items served in the event and stool sample of one patient suggested that the causative agent was *Bacillus Cereus* which was supported by *Klebsiella Pneumoniae*. **Conclusion:** Based on the field investigation result related to symptoms and incubation period and laboratory identification, we conclude that the causative agent was *diarrhoeal B. cereus*.

Keywords: *Bacillus cereus,* food poisoning , *klebsiella pneumoniae*

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Background

One of the causative pathogens of food poisoning is *B. cereus*. It is widely distributed in nature and isolated from soil and growing plants. From these habitats, it is easy to spread to foods, where it may cause an emetic or a diarrhoeal type of food-associated illness that is becoming increasingly important in the industrialized world [1]. In addition, it also closely related to spore-forming species *B. Cereus, Bacillus mycoides, Bacillus thuringiensis, Bacillus anthracis* and *Bacillus weihenstephanensis* [2,3].

Interestingly, the occurrence of foodborne diseases has significantly increased worldwide especially in developing countries. They are caused by ingesting food and water contaminated with chemical and biological agents, mainly viruses and bacteria[4]. Approximately, food poisoning causes 550 million people to fall ill and 230.000 deaths every year worldwide [5]. The frequency of food poisoning outbreaks has doubly increased since 2015 in Indonesia. Meanwhile, the same situation also occurred in Yogyakarta where the outbreak frequency of food poisoning had increased significantly from 37 cases in 2007 to 94 cases in 2018[3,4].

Even though the outbreaks of food poisoning tend to increase during two years in Yogyakarta, the burden of *B. cereus* was poorly unknown because people with infections rarely seek medical advice and the samples are mostly inadequate for laboratory investigation. Therefore, we conducted an investigation to identify the connection between *B. cereus* and the outbreak of food poisoning including mode of transmission during food preparation process in the kitchen and cross-contamination process during *iftar* in Tegalkenongo Village.

Methods

2.1 Study Design

A retrospective cohort was conducted including all villagers who ate any served food during *iftar* in Tegalkenongo Village. The observation period covered 9 days, from 13

to 21 May 2019. A case of food poisoning was defined as any person who attended the *iftar* in Tegalkenongo Village and experienced with diarrhea, abdominal cramps, nausea or two or more of the following symptoms: dizzy, vomiting and fever.

2.2 Data source and Epidemiological Investigation

Two data sources were used in this investigation. Firstly, data related to the total population in the village and the list of attendees during *iftar* which were obtained from village authority. Secondly, based on the list, we then interviewed the attendees to get specific data related to age, sex, sign and symptoms, food consumed during *iftar*, and time of onset of symptoms.

2.3 Microbiological Collection

Food samples from served meals during *iftar* and stood sample of 12 years old patient were collected by the investigation team on the first day of investigation and immediately distributed for bacteriological testing to the reference laboratory (*Balai Laboratorium Kesehatan*) in Yogyakarta.

2.4 Environmental Investigation

On 14 May 2019, we interviewed two food handlers to get information related to the number ordering food for *iftar* and to evaluate food safety procedures including the process of cooking, storing, serving as well as utensils which were used during food preparation. In addition, a checklist was also developed to assess kitchen sanitation.

2.5 Database and Statistical Analysis

Data were analyzed by using STATA 13 software. A bivariate descriptive analysis was used to describe the attack rate of age groups, sex, symptoms, and food-specific attack rate. Bivariate analysis of the individual food items served during *iftar* using risk ratio (RR) with 95% confidence interval (CI) was also used to measure the statistical strength of association. Finally, the significant P-values in the bivariate analysis were adjusted using binomial regression.

Results

3.1 Attack Rate and Clinical Symptoms

A total of 188 cases were identified among 303 villagers who had eaten the *iftar* dishes. The overall attack rate (AR) was 62% (95% CI .5641, .6738) with the median age of cases was 38 years (9-80 years). Cases were mostly found in under 20 years old (AR 65.33; 95% CI .5381, .7529) and were dominated by female (AR 64.47; 95% CI .5649, .7171). Of these 188 cases, the most clinical symptoms were diarrhea 93.62% (176/188), abdominal cramps 84.57% (159/188) and nausea 64.89% (122/188).

Table 1. Attack rate by sex and age groups, 95% confidence interval and p-values, food poisoning outbreak, Tegalkenongo Village, Bantul, Yogyakarta

Demographic Variable	Population at Risk	Number of cases	Attack Rate (%)	(95% CI)
Sex				
Male	151	90	59.60	.5153 - .6718
Female	152	98	64.47	.5649 - .7171
Total	303	188	62.05	.5641 - .6738
Age				
<20	75	49	65.33	.5381 - .7530
21-40	108	68	62.96	.5341 - .7160
>40	120	71	59.17	.5009 - .6765
Total	303	188	62.05	.5641 - .6738

source: primary data

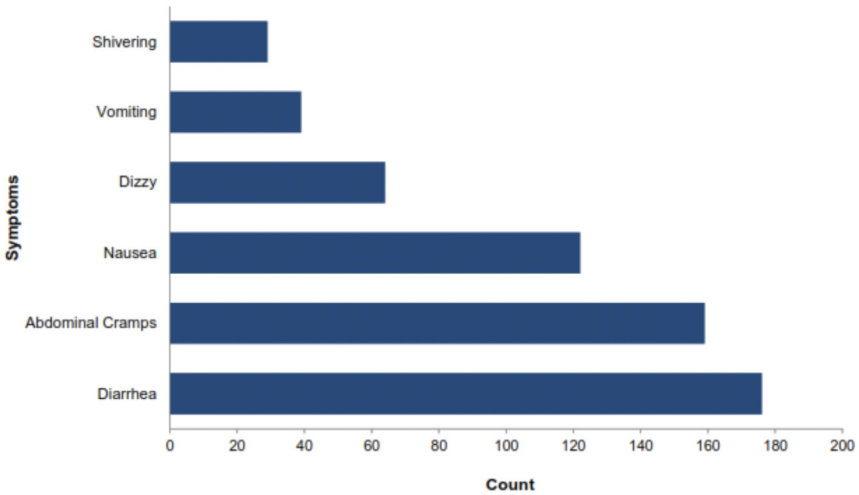

Figure 1. Clinical symptoms of the food poisoning outbreak in Tegalkenongo Village, Bantul, Yogyakarta (see figure 1)

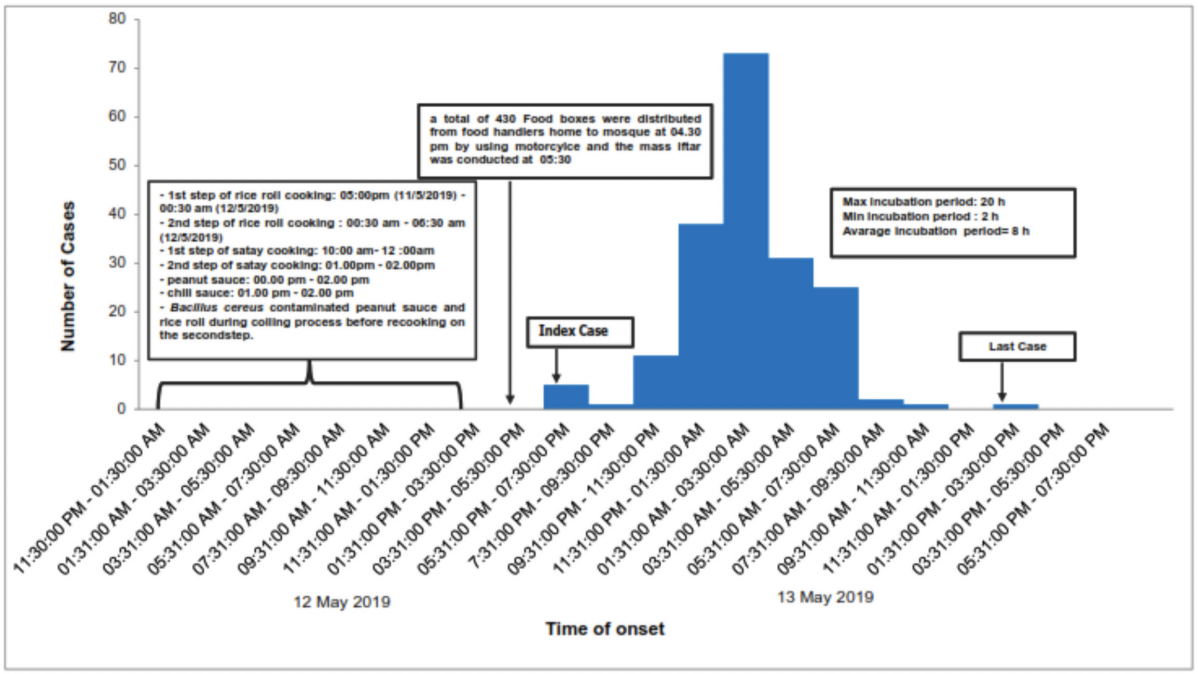

Figure 2. Epidemic curve of cases, by date of onset of symptoms, outbreak of food poisoning in Tegalkenongo Village, 12-13 May 2019 (n=188) (see figure 2)

3.2 Incubation Period

The median incubation period was 8 hours (range: 2-20 hours). The first five cases considered as index case appeared shortly at 07.30 pm on 12 May 2019 or two hours after mass *iftar* and the latest one case was reported at 03.00 pm on 13 May 2019. In addition, we suggested that the contamination of *B. cereus* on peanut sauce and rice roll occurred in the range of 00.30 am – 03.00 pm. While the cross-contamination of *B. cereus* from peanut sauce and rice roll to chicken satay occurred at 05.30 pm.

3.3 Bivariate Analysis

The attendees were served six meals during *iftar*. However, there were only three meals that significantly associated with the cases. In the bivariate analysis of food items, chicken satay had the highest risk ratio (RR 5.18; 95% CI 1.0528, 3.0384) followed by peanut sauce (RR 1.81; 95% CI 1.1653, 2.8270) and rice roll (RR 1.78; 95% CI 1.0528, 3.0384).

Table 2. Relative risk for food items served at iftar, 95% confidence interval, and p-values, food poisoning outbreak, Tegalkenongo Village, Bantul, Yogyakarta

Food Items	Outcome		AR (100%)	RR	P value (CI: 95%)
Rice Roll	**Ill**	**Not Ill**			
Exposed	179	9	64.38	1.78	.000 (1.0528-3,0384)*
Unexposed	99	16	36		
Chicken Satay					
Exposed	186	2	64.81	5.18	.000 (1.4141 – 19.0087)*
Unexposed	101	14	12.51		
Peanut Sauce					
Exposed	175	13	65.54	1.81	.000 (1.1653 – 2.8270)*
Unexposed	92	23	36.11		
Tahu Bakso					
Exposed	116	72	59.81	0.91	.281 (.7581 – 1.0807)
Unexposed	78	37	66.05		
Chili Sauce					
Exposed	44	144	72.13	1.21	.069 (1.0050 – 1.4621)
Unexposed	17	98	59.5		
Tea					
Exposed	139	49	62.33	1.01	.864 (0.8315 – 1.2454)
Unexposed	84	31	61.25		

**: Pvalue<0.05*

source: primary data

3.4 Multivariate Analysis

In the binomial regression model, we measured an adjusted RR (aRR) of 4.36; 95% CI 1.1538, 16.5285 for chicken satay which means that they who eat chicken satay had four times higher risk of becoming ill than those who did not eat chicken satay. While peanut sauce (aRR 1.41; 95% CI .0912, 2.1933) and rice roll (aRR: .95; 95% CI .6076, 1.4970) respectively had no association with the food poisoning in Tegalkenongo Village.

Table 3. Multivariate analysis of food items related to food poisoning in Tegalkenongo Village, Bantul, Yogyakarta

Food items	Adjustment Risk Ratio	Std. Err.	Z	P>z	[95% CI]
Rice Roll	.95	.219397	-.21	.837	.6076 – 1.4970
Chicken Satay	4.36	2.965627	2.17	.03*	1.1538 – 16.5285
Peanut Sauce	1.41	.316453	1.55	.121	.0912 – 2.1933
Chili Sauce	1.13	.106507	1.28	.201	-9377 – 1.3576
_cons	.11	.07507	-3.28	.001	.0304 – .4159

**Pvalue: <0.05*

source: primary data

3.5 Laboratory Investigation

The result of laboratory analysis confirmed the isolation of *B. cereus* and *Klebsiella pneumonia* where both pathogens were isolated in stool and food samples.

3.6 Environmental Investigation

The environmental investigation performed in the food handler's kitchen revealed that none of the food handlers experienced gastrointestinal symptoms before *iftar*. However, there were three essential results which showed the mode of transmission of *B. cereus*. Firstly, the space area in the kitchen was too small, unclean and dusty. Secondly, the meals were stored for cooling in the uncontrolled room temperature and thirdly, compared to the number of foods ordering, there was a lack of kitchen utensils. Consequently, the utensils must be used to cook more than one food item which could increase the possibility of cross-contamination between foods.

Discussion

The epidemiological analysis allows us to reconstruct the possible source and subsequent transmission of food poisoning [8]. The epidemiological analysis revealed that the case of food poisoning in Tegalkenongo Village was an outbreak where at least two or more cases of a similar foodborne disease resulting from the ingestion of a common food [2,9]. In the early investigation, based on clinical symptoms, food items and incubation period, investigators hypothesized *staphylococcus areus* and *B. cereus* as the causative bacterial agents of food poisoning in Tegalkenongo Village [10]. However, in the final laboratory test, only *B. cereus* was identified as the main source of food poisoning. *B. cereus* has two distinct toxins of foodborne illness. The first form is emetic which is characterized by nausea, vomiting, and abdominal cramps. The emetic form has a short onset time of about 1-6 hours after consuming contaminated foods. In contrast, diarrhoeal form has a longer onset time of about 6-15 hours and can last approximately 24 hours which is characterized by diarrhea, nausea and abdominal cramps[3,4,11]. Compared to the onset time and symptoms we emphasized that the food poisoning in Tegalkenongo Village was strongly associated with diarrhoeal *B. cereus.*

The contamination of *B. Cereus* on sauce peanut and rice roll occurred during the cooling process before food reheating where the cooling process was approximately one hour for each food. Due to uncontrolled room temperature, open food containers and uncleaned kitchen area, sauce peanut and rice roll were easily contaminated by the spore of *B. cereus* through direct contact with dust. *B. cereus* is ubiquity found in the soil and many types of fresh food where illness usually occurs when food is improperly cooked and stored in danger zone (41^0F to 135^0F) for an extended period of time. Furthermore, when food was cooked and stored in improper temperature, the spore of *B. cereus* can resistant to the heat temperature and multiply from 10^5 to 10^8 cell/gram[3,12,13].

Based on multivariate analysis, chicken satay was considered as the most contaminated food associated with food poisoning in Tegalkenongo Village. Interestingly, there was no *B. cereus* found in chicken satay. The cross-contamination between foods might happen during the serving process which could be explained by the fact that all food items were served in the same box. Moreover, the cross-contamination might also occur during *iftar* where attendees consumed chicken satay mixed with sauce peanut or rice roll. Cross-contamination can cause food poisoning when harmful bacteria are transferred to ready-to-eat products (RTE) where the sources of cross-contamination are including raw foods, utensils (cutting board and knife), food contact surfaces and people (food handlers)[14,15,16].

Not only *Bacillus* cereus, but K. *pneumoniae* was also identified either in chicken satay or stool samples which could lead to incoherently causative bacteria of food poisoning in Tegalkenongo village. *K. pneumoniae* is a gram-negative which belongs to the tribe of *Klebsiella*,

a member of the family *enterobacteriaceae* where is normally found in the environment. However, it can cause severe infection if it spread to another part of the body and also some cases were asymptomatic[16]. *K. Pneumoniae* infection is usually corelated with the nosocomial infection where a reported case confirmed that *K. pneumoniae* was the causative agent of nosocomial infection outbreak in a hospital where 44 food handlers in the kitchen were considered as the carrier[17,18]. Although isolated in both stool and food samples, *K. pneumoniae* was not considered as the main cause of the food poisoning in Tegalkenongo village because there were no typical symptoms of *K. pneumoniae* such as shortness of breath and tachycardia. This explanation was supported by the reported case of gastroenteritis outbreak in Canada where *clostridium perfingens* and *K. pneumoniae* were isolated on turkey. However, due to dissimilarity with symptoms and incubation period, *klebsiella pneumoniae* was excluded as the main causative agent[19]. Our epidemiological investigations had several limitations. The data related to food serving during *iftar* in the mosque were not clear. Consequently, we could not clearly explain how *B. cereus* could transmit from contaminated rice roll and sauce peanut to chicken satay. Moreover, because we did not involve in the laboratory, we could not explain the method used for samples examination.

Conclusion

We can conclude that food poisoning occurred in Tegalkenongo village was an outbreak where the highest attack rate was in the female group. The etiology of the outbreak was caused by diarrhoeal *B. cereus*. In addition, it is likely that the cross-contamination of *B. cereus* from food to food also has contributed to food poisoning in Tegalkenongo Village.

Acknowledgment

We grateful to the health office of Bantul district and public health center of Kasihan II for the support provided during the outbreak investigation. Finally, we also would like to thank the Indonesian Endowment Fund for Education (LPDP) for the scholarship support.

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