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An outbreak of *Bacillus cereus* toxin-mediated emetic and diarrhoeal syndromes at a restaurant in Canberra, Australia 2018

C. E. Thirkell, T. S. Sloan-Gardner, M. C. Kaczmarek and B. G. Polkinghorne

Abstract

A cluster of gastrointestinal illness was detected following receipt of a complaint of becoming ill after a multi-course dinner at a restaurant in Canberra, Australian Capital Territory (ACT), Australia. The complaint led to an investigation by ACT Health.

Food samples retained by the restaurant for microbiological analysis returned an unsatisfactory level of *Bacillus cereus* in beef (19,000 colony forming units/gram [cfu/g]) and a satisfactory level in arancini (50 cfu/g). These positive samples underwent whole genome sequencing and genes encoding diarrhoeal toxins were detected with no laboratory evidence of the emetic toxin. No stool specimens were collected.

A cohort study was undertaken and 80% (33/41) of patrons took part in a structured interview. There was no significant difference in age or sex between those ill and not ill. Due to universal exposure most foods were unable to be statistically analysed and no significant results were found from the food history. The ill cohort diverged into two distinct groups based on incubation period and symptoms suggesting this outbreak involved *B. cereus* intoxication with both diarrhoeal and potentially emetic toxins. Some hygiene practices during food preparation were noted to be inadequate and heating and cooling procedures were unverified when questioned.

A combination of the incubation periods and symptom profile, food laboratory evidence, and genomic sequencing of the *B. cereus* diarrhoeal gene suggest a probable aetiology of *B. cereus* intoxication. Public health action included the restaurant rectifying hygiene practices and documenting heating/cooling procedures.

Keywords: *Bacillus cereus*, universal exposure, emetic syndrome, diarrhoeal syndrome, genomic sequencing, gastroenteritis, foodborne disease.

Introduction

Four people reported gastrointestinal illness to a restaurant in Canberra, Australian Capital Territory (ACT), Australia the morning after attending a multi-course dinner. The restaurant subsequently self-reported the cluster of illness to ACT Health. The restaurant had three seatings each night during the period of concern: early, middle and late. This report describes the

outbreak investigation of *Bacillus cereus* toxin mediated emetic and diarrhoeal syndromes associated with that multi-course dinner, and the first published use of whole genome sequencing (WGS) in a *B. cereus* foodborne outbreak investigation in Australia.

Foodborne illness is estimated to cause 4.1 million cases of gastroenteritis and 3,350 episodes of *B. cereus* each year in Australia; the majority of

these are not reported.¹ *B. cereus* toxin-mediated gastroenteritis is not notifiable in Australia; however outbreaks are reported by all states and territories and captured in the OzFoodNet outbreak register.¹ A foodborne outbreak is defined as a similar illness in ≥ 2 people after consuming a common food and epidemiological and/or microbiological evidence implicates food as the source of illness.² *B. cereus* was officially attributed to just ten outbreaks on the register between 2001 and 2013, likely due to the short duration of illness and relatively mild symptoms limiting reporting.³

B. cereus is ubiquitous in the environment, particularly in soil and vegetation⁴ and can cause two type of illnesses referred to as emetic-type and diarrhoeal-type illness.⁵ Emetic-type illness occurs from the production of the toxin cereulide. This toxin is pre-formed in the food with a temperature production range of 12–37 °C. It is heat stable to 100 °C for >2 hours and results in emetic illness which typically has an average incubation of 1–6 hours and symptom duration of 6–24 hours.^{5,6} Foods commonly associated with emetic illness include rice, pasta and pastries and are generally linked to improper surface cleaning, cross contamination or temperature abuse through inadequate heating and cooling.^{6–8} The infective dose has not been determined for the emetic toxin. However, although it has been reported as low as 10^3 cfu/g, in most cases it is above 10^5 cfu/g.⁹

Diarrhoeal-type illness results from one or a number of associated toxins including: enterotoxin FM which is not pathogenic but contributes to the severity of diarrhoeal illness; haemolysin BL (Hbl); cytotoxin K (CytK); and non-haemolytic enterotoxin (Nhe).¹⁰ The illness is caused by the ingestion of dormant spores which then germinate and proliferate in the small intestine and then produce the toxins. Diarrhoeal-type illness has an average incubation of 8–16 hours and symptom duration of 12–24 hours.⁶ Spores should be eliminated by heating to 100 °C for 3 minutes when appropriate heating and cooling processes are adhered to. Foods commonly associated with diarrhoeal

illness include protein foods such as meat products, soups, vegetables and sauces, and practices linked to contamination including poor heating and cooling in particular.⁶ The infective dose is generally considered to be 10^5 – 10^8 cfu.⁹

Methods

Epidemiological investigation

A retrospective cohort study was performed using a standard gastrointestinal outbreak questionnaire adapted to the menu used on the implicated night. A contact from each table was obtained from the restaurant and phone interviews were conducted. Non-respondents were called back either until they responded or six attempts to make contact were unsuccessful. A case was defined as someone who ate dinner at the specified restaurant on the implicated date and experienced gastrointestinal symptoms (nausea, vomiting, diarrhoea and abdominal pain) within 24 hours. A line-list was entered into Microsoft Excel 2013 and analysed using STATA 15.0.¹¹ Univariate analysis included generating odds ratios using logistic regression, and exact logistic regression where this was not possible due to zero cell counts. Age was compared using a two-sample t-test, after confirming a normal distribution. Gender was compared using a two-tailed Fisher's exact test. Ethics approval was not sought as this is not required under the ACT *Public Health Act 1997* for the purposes of a public health investigation.¹² Australian National University has a waiver of consent for research performed as part of an outbreak investigation under protocol: 2017/909.

Environmental health investigation

An Environmental Health Officer from ACT Health attended the restaurant to perform a routine food premises inspection. Samples of all retained food served on the implicated night were taken for laboratory analysis as required by the ACT *Food Act 2001*.¹³ Two follow-up visits were conducted with ACT Health staff to discuss food preparation techniques and laboratory results.

Laboratory investigation

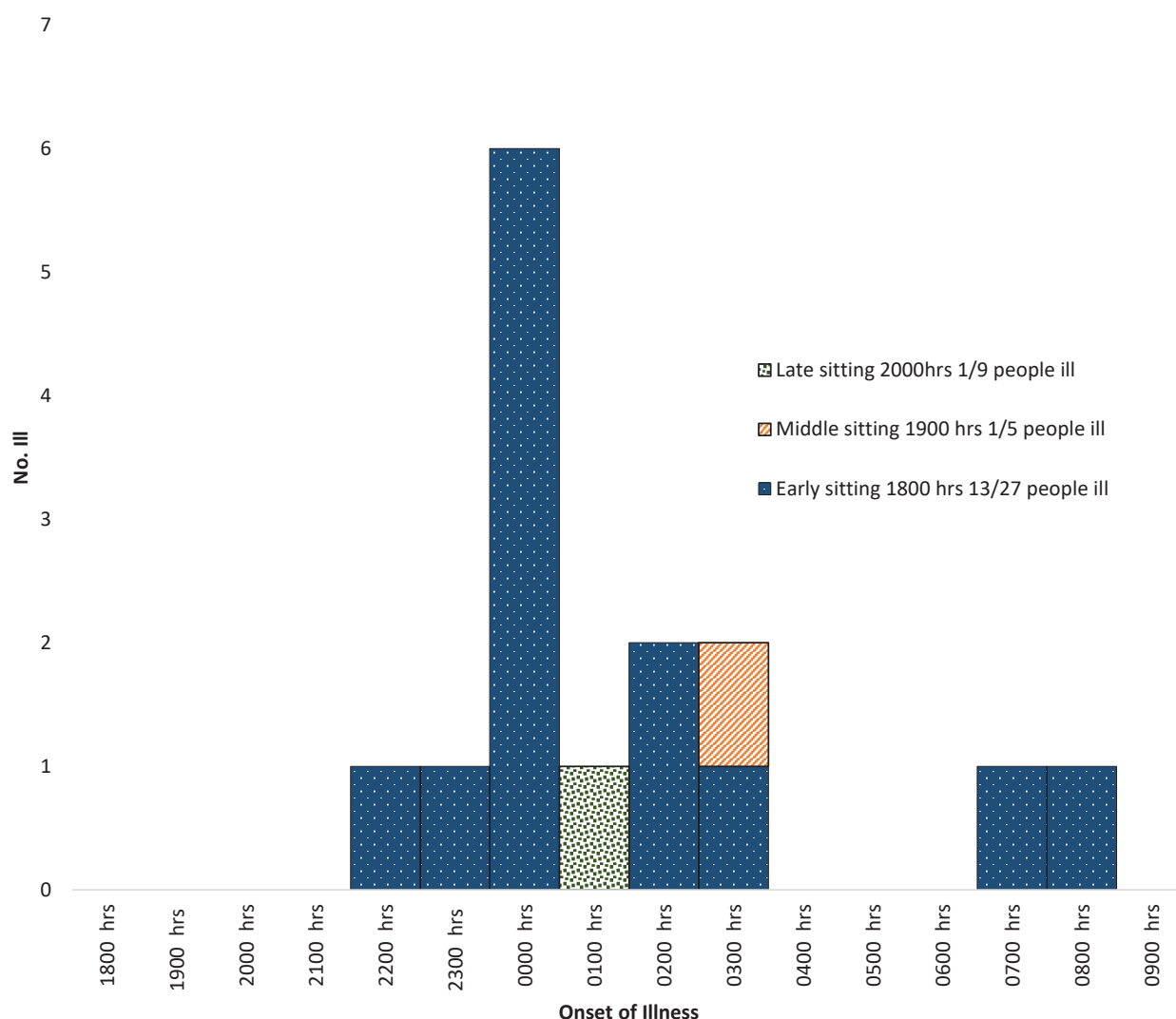
No stool specimens were received from cases. Food samples were tested at the ACT Government Analytical Laboratory (ACTGAL) for *Staphylococcus aureus*, *B. cereus* and *Clostridium perfringens* using a spread plate method and standard plate counts. Microbiological results for *B. cereus* are classified as satisfactory (<100 cfu/g), marginal (100 – <1,000 cfu/g), unsatisfactory (1,000 – <100,000 cfu/g) and potentially hazardous (>100,000 cfu/g).¹⁴ Further into the investigation WGS was performed by the Queensland Health Molecular Epidemiology Unit utilising the Illumina NextSeq genome sequencing platform to detect enterotoxin genes for all food isolates which grew *B. cereus*.

Results

Epidemiological

A total of 45 patrons dined in three separate sittings at the restaurant on the night, across 15 tables. We were able to contact 41 patrons (91%) from 14 tables. Of the 41 who were contacted, an ill status was gathered for all (100%) and a food history was obtained for 33 patrons (80%). The attack rate for those who attended on the night was 37% (15/41) (Figure 1).

Figure 1: Epidemiological curve of cases by onset of illness time after attending dinner at restaurant (n = 15)



There was no statistically significant difference in gender ($p=0.51$) or age ($p=0.93$) between those ill and not ill. No cases required treatment in-hospital.

More than eight courses were served and ten foods were captured during interview (Table 1). Universal exposure was present for four foods (pastry, crocodile, arancini and salmon) and >90% ate another four foods (beef, apple lolly, apple sour and scallops). The two other foods were alternate foods provided for taste or intolerance to menu foods (oysters, raspberry). Statistical analysis of ill status and exposure to foods provided no evidence of a contaminated food source. All confidence intervals crossed one and no odds ratios with significant p -values were produced.

Illness was concentrated in the early sitting (1800 hrs) with an attack rate of 48%, 20% for the middle sitting (1900 hrs), and 11% for the late sitting (2000 hrs). No significant differences ($p>0.05$) were noted in age, sex or general health between those who were ill or not ill in the early sitting.

No clusters among tables were observed with a broad distribution of illness across the 15 tables. Food history was available for 13 of 15 ill patrons and 20 of 26 not ill patrons. No staff were interviewed but all staff were reported to eat the same food on the night prior to opening except for the beef and none were reported to be ill.

Two distinct ill cohorts emerged based on incubation period and duration of symptoms (Figure 2). All emetic syndrome cases (nine) had vomiting and all diarrhoeal syndrome cases (six) had diarrhoea.

Environmental

The environmental health officer collected three frozen samples each of crocodile, beef and arancini during their first inspection. Following discussions with the restaurant chef, it was discovered that the beef dish was cooked for 12 hours at 85 °C before being de-boned. The de-boning process was interrupted by the arrival of fresh

fruit and vegetables which the staff unloaded and then returned to meat preparation with no hand hygiene performed. The beef was then spread across large metal trays which were stacked and placed in the cool-room for 25 minutes. The meat was then pressed and placed in the cool-room overnight. The following day, 12 individual servings were placed into 2 kilogram (kg) vacuum sealed bags and then frozen. Prior to serving, the 2 kg bag was reportedly thawed in a water bath held at 55 °C for 35 minutes, then just prior to serving a single-serving size of beef was added to a *jus* sauce heated to 78 °C for <5 min. The heating, cooling and re-heating process was not documented or verified with thermometers. Three to four bags were reported to be used on the night, however the restaurant was unable to confirm whether they were from the same batch of cooking, nor whether the bags were used concurrently or consecutively.

Laboratory

Initial testing of retained food specimens demonstrated growth of *B. cereus* at unsatisfactory levels in the beef dish and at detectable but satisfactory levels in the arancini balls. No pathogens were detected in any other samples. The restaurant requested testing of a second sample of the beef dish retained by the laboratory, and another 2 kg bag of frozen beef the restaurant had retained. The second beef sample demonstrated growth of *B. cereus* at lower levels than the first sample but still unsatisfactory. No pathogens were detected in the 2 kg frozen sample (Table 2). Whole genome sequencing was performed on isolates from the two positive beef samples and one positive arancini sample. The diarrhoeal toxin genes Hbl, Nhe and CytK were detected in both the beef and arancini balls. All three positive isolates had the same multi locus sequence type (MLST) 177. The emetic toxin, cereulide, was not detected in any of the three isolates tested. No stool samples were received from cases.

Table 1: Univariate analysis of food exposures (n = 33)

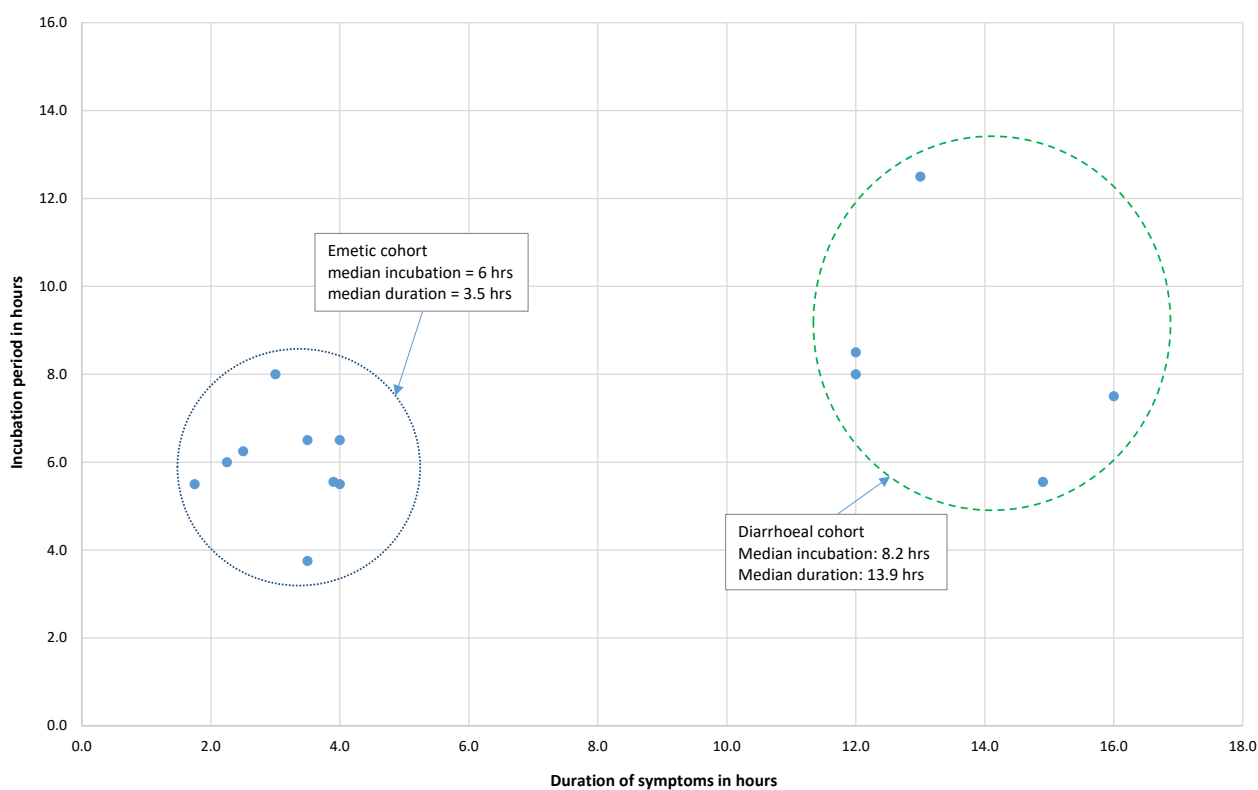
Food	Exposed			Not Exposed			Odds ratio (95% CI)	p-value
	Ill	Not ill	AR (%)	Ill	Not ill	AR (%)		
Oysters	3	4	43	10	15	40	0.76 (0.33 – 1.76)	0.52
Pastry	13	20	39	0	0	0	^a	
Crocodile	13	20	39	0	0	0	^a	
Scallops	13	19	40	0	1	0	0.65 (0.02 – ∞) ^b	1.0
Arancini	13	17	43	0	0	0	0.88 (0 – 1.18) ^b	0.42
Salmon	13	20	39	0	0	0	^a	
Beef	12	20	38	1	0	100	0.65 (0 – 25.35) ^b	0.79
Apple lolly	12	18	40	1	2	33	1.3 (0.11–16.39)	0.82
Apple sour	12	18	40	1	2	33	1.3 (0.11–16.39)	0.82
Raspberry	2	7	22	4	9	31	1.19 (0.99–1.42)	0.06

a Logistic and exact regression unable to be calculated due to zero cell counts and collinearity

b Calculated using exact logistic regression

Denominator differs by food due to unknown data

Figure 2: Incubation period by duration of symptoms for 14 of 15 cases^a



a One diarrhoeal syndrome case excluded from graph due to duration of 48hrs (incubation of 14 hrs). This case had rice as a starter with no other differences noted (no other cases reported eating rice other than the arancini)

Table 2: Food related laboratory testing and results

Food Tested	Organism or toxin testing for	Test	Result	Interpretation
Beef first sample	<i>S. aureus</i>	Spread plate	<50 cfu/g	Satisfactory
	<i>B. cereus</i>	Spread plate	19,000 cfu/g	Unsatisfactory
	<i>C. perfringens</i>	Pour plate	<50 cfu/g	Satisfactory
Crocodile	<i>S. aureus</i>	Spread plate	<50 cfu/g	Satisfactory
	<i>B. cereus</i>	Spread plate	<50 cfu/g	Satisfactory
	<i>C. perfringens</i>	Pour plate	<50 cfu/g	Satisfactory
Arancini balls	<i>S. aureus</i>	Spread plate	<50 cfu/g	Satisfactory
	<i>B. cereus</i>	Spread plate	50 cfu/g	Satisfactory
	<i>C. perfringens</i>	Pour plate	<50 cfu/g	Satisfactory
Beef second sample	<i>B. cereus</i>	Spread plate	3,500 cfu/g	Unsatisfactory
2 kg frozen bag of beef	<i>B. cereus</i>	Spread plate	<50 cfu/g	Satisfactory

Discussion

The food laboratory evidence along with duration, incubation and symptom history provides evidence of a probable outbreak of *B. cereus* intoxication caused by diarrhoeal and potentially emetic toxins. According to OzFoodNet and the US Centers for Disease Control and Prevention (CDC) guidelines, definitive evidence would require case specimens or isolation of at least 100,000 cfu/g from food samples.^{2,15} The evidence to support this cfu/g threshold is not clear with some variance in other investigation reports¹⁰ and this outbreak suggesting lower levels may be appropriate. Re-heating of the beef for service indicates both emetic-toxin-mediated and diarrhoeal-toxin-mediated gastroenteritis are biologically plausible because being placed in 55 °C water for thirty-five minutes followed by briefly being placed in the *jus* may not destroy either the pre-formed emetic toxin or the diarrhoeal spores. Evidence suggests the risk of meat contamination increases with multiple stages of preparation¹⁶ which is consistent with the complex meat preparation implicated in this outbreak.

Although the majority of illness was concentrated in the early sitting, the reason for this

is unknown. Contamination appears to have occurred in batches, however investigations were unable to identify differences in food preparation or plating. The fresh fruit and vegetables arrived during the deboning process following initial cooking and it is postulated that contamination may have occurred at this stage. The process of cooling then involved stacking the trays of beef directly on top of each other in the cool room. It is possible the middle trays were not cooled to the same extent or as quickly resulting in batched contamination.

The MLST identified was last reported in human isolates in Europe in 2003/04 on PubMLST (a global database for molecular typing). This is the first documented whole genome sequencing of *B. cereus* isolates following an outbreak in Australia. Although the arancini did not produce unsatisfactory levels of *B. cereus*, the isolated genes and MLST type found matched those of the beef. This suggests there was cross contamination between the beef and the arancini and potentially undetected cross contamination with other foods. Beef is one of the predominant food types associated with *B. cereus* diarrhoeal syndrome⁵ and therefore biologically plausible and consistent with the cases who presented with diarrhoeal symptoms in this outbreak. The

majority were unwell with emetic symptoms suggesting either another source of illness or the beef producing both toxins. Improvement of hygiene policies with increased hand hygiene was a public health action from this investigation which may help prevent the introduction of organisms into the food and also prevent cross-contamination within the kitchen.

Literature has traditionally suggested it is rare to have both forms of illness; however, a 2016 review of French *B. cereus* outbreaks has challenged this thought and this outbreak supports this notion.^{5,17} It was noted that 57% (42/74) of *B. cereus* related outbreaks resulted in both emetic and diarrhoeal symptoms with variability in incubation periods. The two distinct illness profiles in this outbreak present as a typical example of emetic and diarrhoeal syndromes, although the emetic cases with a median incubation of 6 hours support the suggestion of a longer incubation period because of a low dose of the emetic toxin. Isolating the *B. cereus* diarrhoeal gene in the food provides strong evidence of the cause of the diarrhoeal syndrome even though only 6/15 cases presented with typical diarrhoeal symptoms. Not isolating the *B. cereus* emetic toxin does not rule out its presence – it is still considered the likely cause of the emetic syndrome based on the case profiles and is consistent with recent evidence.¹⁷ It is well accepted that the emetic toxin is difficult to isolate in food and stool samples which emphasises the importance of epidemiological evidence.¹⁸

Limitations of this investigation included no stool samples being received; however, nearly all the ill patrons had recovered by the time they were contacted. Steps to minimise this bias included a standard detailed questionnaire. This questionnaire also helped to highlight differences in incubation/duration times. Eight people were unable to be contacted, of which two were ill. A variety of food was available on the implicated night with only three foods available for analysis. Near-universal exposure hampered statistical testing due to zero cell counts, and attack rates were not high. For example, the one person who did not report eating beef did report

being ill, hence a statistically insignificant, protective odds ratio was produced. This highlights the importance of gathering a portfolio of evidence including descriptive epidemiological and microbiological food evidence.

This investigation is a good example of a public health response that combined detailed epidemiological, microbiological, whole genome sequencing and environmental health expertise to provide strong evidence of a likely pathogen and cause of contamination. This led to recommendations regarding hygiene and food preparation practices and no further reports of illness. The restaurant in question was very cooperative and positively engaged in the investigation: self-reporting in a timely manner, retaining specimens for testing and responding to all public health requests.

Conclusion

Following a thorough and rapid investigation, the probable aetiology of this outbreak was *B. cereus* intoxication with diarrhoeal and potentially emetic toxins due to cross contamination of food. This was corroborated by the symptom profile and food sample laboratory evidence, including genome sequencing of the diarrhoeal gene. Although rarely reported in Australia, this outbreak provides evidence of the ongoing risk of *B. cereus* in food produced in restaurants, and highlights the need for continued vigilance in food preparation techniques.

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