

Foodborne disease outbreaks linked to consumption of bakery products in Bhutan

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Abstract - Foodborne disease (FBD) is a global public health issue. Foodborne diseases are caused by consumption of contaminated foods. Food poisoning outbreaks associated with bakery stores have major public health significance and the common pathogen isolated being *Salmonella* species. Data on FBD associated with bakery products is very scarce; therefore this paper presents the available data on FBD outbreaks suspected due to consumption of bakery products (cream cake) in Bhutan. Data was retrieved from National Early Warning and Alert Response Surveillance System (NEWARS) and Food and Nutrition Laboratory, Royal Centre for Disease Control (RCDC). Four districts (Thimphu, Tsirang, Pemagatshel and Tashigang) reported at-least one event of FBD suspected from consumption of cream cake (from 2017 to 2021). Suspected cake samples were collected and analyzed for microbiological culture. Three of the FBD events were associated with consumption of cream cake samples contaminated with *Salmonella* (*Salmonella* Enteritis) pathogen. The *Salmonella* species were also isolated from egg samples. Good manufacturing practices with proper cleaning of raw materials and good personal hygiene is of paramount importance to prevent FBD incidences.

Keywords: Bhutan, foodborne disease, food safety, pathogen, *Salmonella*

1. Introduction

The World Health Organization (WHO) estimates that globally, foodborne diseases (FBD) cause 600 million illnesses with 420,000 deaths and 33 million disability-adjusted life-years (DALYs) annually (Devleesschauwer *et al.*, 2018). The highest burden was recorded in the Africa region (1300 DALYs per 100000 population) followed by South East Asia (1200 DALYs per 100000 population) with the second highest burden of food borne diseases (FBD) incidences per population among WHO regions (World Health Organization, 2015). The World Health Assembly (WHA) in 2000 urged all member states to identify food safety as an essential public health concern (Bager & Halgaard, 2002). In the South East Asian region approximately 50% of FBD's are caused by diarrheal agents (*E. coli*, *Salmonella*, *Campylobacter*, Norovirus). The FBDs can be mild (self limiting) to fatal, depending upon the type of hazard (microbial, chemical or toxins) and its dose (World Health Organization, 2016). The Foodborne Disease Burden Epidemiology Reference Group (FERG) stated FBD burden is caused by 31 foodborne hazards (11 diarrheal disease agents, 7 invasive disease agents, 10 helminths, and 3 chemicals and toxins) (Devleesschauwer *et al.*, 2018).

In Bhutan diarrhea causes significant morbidity and FBD is mostly associated with diarrheal symptoms. More than 60 events of food borne disease have been recorded with the Royal Centre for Disease Control (RCDC) from 2012 to 2020 (www.rcdc.gov.bt). The FBD can be caused by consumption of pathogen contamination in ready to eat foods, pre-packaged foods or any bakery products. Food poisoning

outbreaks associated with bakery stores can have major public health significance and the common pathogen isolated being *Salmonella* species (Evans *et al.*, 1996, Humphrey, 2004a). *Salmonella* is identified as an important foodborne pathogen across the world due to its ability to survive environmental changes and survive the food chain. Relatively small doses (15 to 20 colony forming units) of *Salmonella* have been shown to cause illness but the infective dose also depends on species and immunity of person (Humphrey, 2004b). The high fat containing foods (cakes, cheese and chocolate) are also contaminated by *Salmonella* due to its high stress tolerance (Humphrey, 2004a, Humphrey *et al.*, 1994). Several studies have isolated *Salmonella* species associated from bakery products causing FBD outbreaks (Majtanova *et al.*, 2011, Lu *et al.*, 2004, Nesbitt *et al.*, 2012).

There is limited data on FBD associated with bakery products in Bhutan; thus, we retrieved the available information and data to bridge the gap. A few well documented FBD outbreaks in Bhutan are *Campylobacter* outbreak in Bumthang (2012) (Darnal *et al.*, 2017) and Shigellosis outbreak that occurred in Mongar and Pemagatshel (Darnal *et al.*, 2016) but there are no published article on FBD outbreak from bakery products. This paper aims to analyze the available laboratory data on FBD outbreaks suspected due to consumption of bakery products in Bhutan.

2. Materials and methods

This is a descriptive retrospective study on the FBD events suspected to be caused by consumption of bakery items (data from 1st January 2017 to 31st December 2021). The

data were extracted from National Early Warning and Response Alert Surveillance System (NEWRAS) maintained by RCDC and laboratory analysis report retrieved from Food and Nutrition Laboratory (FNL).

Outbreak Notification or case definition for outbreak notification: The medical team from the field (Hospitals and Primary Health Centers) upon suspicion of FBD; when two or more patients visit with common signs/symptoms (acute gastroenteritis) acquired after consumption common food source is alerted through NEWARS event report. The surveillance unit from RCDC confirmed the outbreak and initiated the outbreak investigation.

Sample collection and transportation: The suspected food samples were collected by food inspectors (Bhutan Agriculture and Food Regulatory Authority-BAFRA) aseptically both from the bakery shop and left-over from the patient. The common ingredients (sugar, butter and egg) samples were also collected when the suspected FBD is from consumption of bakery products. The samples were sent to the laboratory for microbiological analysis. Biological samples such as vomits and stool samples from patients were also collected and shipped to FNL, RCDC maintaining appropriate cold chains and inoculated for microbial culture.

Sample processing: The samples are processed following the standard operating protocol (SoP) in line with the AOAC method (Feldsine *et al.*, 2002). Briefly, 25 gram of samples are homogenized with 225mL of buffered peptone water, homogenized for 30 seconds at 230 rotations per minute (RPM) by stomacher and serial dilution prepared.

Microbiological analysis: The food samples were cultured for indicator test organism; Total plate count (total plate count agar), total yeast and mould count (yeast extract agar), Total coliform count (ECC plate) and *Enterobacteriaceae* (MacConkey agar). The pathogenic organism were screened using selective/enrichment media plates; *Staphylococcus aureus* (Baird Parker media), *Bacillus cereus* (Mannitol-egg-yolk-polymyxin agar media), *Salmonella/Shigella* (*Salmonella Shigella* agar), *Campylobacter* (Modified Charcoal Cefoperazone Deoxycholate Agar) (Refai, 1979). The plates were incubated aerobically for 24–48 hours at 37 °C, except for mCCDA which was incubated at 35°C ±2°C for 48 hours with 5% CO₂ and 25°C for yeast/mould agar. To improve recovery and detection, pre-enrichment media, Preston broth was used for *Campylobacter* and Rappaport Vasiliadis Soy broth for *Salmonella*, *Shigella*. The tubes were incubated aerobically at 37°C for 12–24 hr and at 35°C ±2°C for 48 hours with 5% CO₂ for *Campylobacter*. The isolated pathogenic organism was identified by colony growth on specific media and then by performing Gram staining and Biochemical reaction (indole, methyl red, citrate, Voges–Proskauer, nitrate reduction, and carbohydrate fermentation tests) (Aktar *et al.*, 2016). Furthermore, API 20E (BioMerieux) test was conducted for species identification and confirmation.

A correlation between the three samples (cream cake samples from the bakery, leftover cream cake samples collected from patients and biological samples) was made based on the isolation of pathogenic organisms.

3. Results

Four districts (Figure 1) notified suspected FBD events due to consumption of bakery

items as presented in Table 1 from January 2017 to December 2021. At least a total of 58 person got infected and few requiring hospitalization.

Table 1. List of FBD events suspected from consumption of bakery items

Sl. No	Event reported from	Suspected sample	Event month/year	Number of people affected	Common signs and symptoms reported	Total food samples collected
1	Thimphu	Cream Cake**	July 2017	15	Acute gastroenteritis	Two from bakery and two from left over*
2	Pemagatshel	Cream Cake	18/04/2020	22	Acute gastroenteriti, Fever, water diarrhea, nausea/vomiting, headache	One From bakery and one from left-over
3	Tashigang	Cream Cake	02/05/2021	13	Diarrhea, fever, abdomen pain, vomiting	One from left over
4	Tsirang	Cream Cake	03/05/2021	8	diarrhea, vomiting, fever, headache, abdominal pain	Two from bakery and one from left over

* left over (the left over cakes samples collected from patient's home)

** Cream cake: butter, sugar syrup and whipped egg white are used to make butter cream. The eggs are not pasteurized or heated to a high temperature.

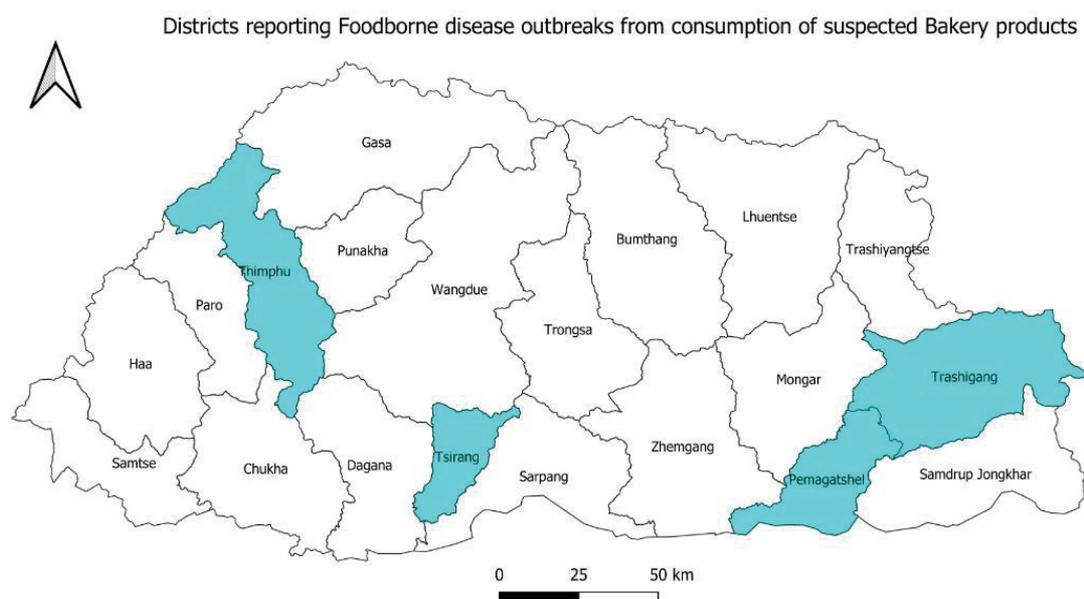


Figure 1. Districts reported with FBD event suspected from consumption of bakery products in Bhutan (2017 – 2021)

Pathogenic organism (*Salmonella* spp.) was isolated from four cream cake samples collected from bakery and four left over samples (as shown in table 2), except the samples collected from Tsirang outbreak. The reference range or the acceptable limits for each organism is adapted from Food Safety and Standards Authority of India (FSSAI) (FSSAI, 2015) and WHO guidelines. The results of the microbiology culture report are presented as in Table 2.

The correlation was done between cream cake sample microbiological findings and biological specimens (fecal

and vomit samples collected from few patients); *Salmonella* spp. was isolated from cream cake samples collected from bakery and left over collected from patients. The biological sample microbiological culture findings were in concordance with the cake samples; *Salmonella* isolates were positive from fecal/vomits samples (complete report not presented). The common signs and symptoms were acute gastroenteritis with diarrhea, fever and vomiting developed within 6-12 hours after consumption of suspected food item (Table 1).

Table 2. Microbiology culture results of suspected FBD cream cake sample

Outbreaks sites	Indicator test organism (CFU/g*)					Pathogenic test organism (CFU/g)				
	Aerobic Plate Count (APC)	Yeast & mould count (Y/M)	<i>E. coli</i>	<i>Enterobacteriaceae</i>	<i>Environmental Listeria</i>	<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>	<i>Salmonella</i> spp.	<i>Shigella</i> spp.	<i>Campylobacter</i> spp.
Thimphu	1.27 x 10 ⁵	<100	TNTC**	ND***	ND	Absent	Absent	Present	Absent	Absent
Pemagatshel	TNTC	>100	TNTC	TNTC	<1 x 10 ¹	Present (>10 ² CFU/g)	Present (>10 ² CFU/g)	Present	Absent	Absent
Tashigang	1.20 x 10 ⁶	>100	<1 x 10 ¹	<1 x 10 ¹	<1 x 10 ¹	Absent	Absent	Present	Absent	Absent
Tsirang	<1 x 10 ¹	52	<1 x 10 ¹	<1 x 10 ¹	<1 x 10 ¹	Absent	Absent	Absent	Absent	Absent

* CFU/g= Colony Forming Unit per gram of sample, **TNTC= Too Numerous to Count, ***ND=Not Done

4. Discussion

This is the first study report from Bhutan on FBD outbreaks linked to consumption of bakery products. The outbreaks were notified by the District Hospitals when the suspected patients with similar signs and symptoms (AGE, vomiting, fever) were admitted to the hospital after the consumption of the same source of foods (cream cake) purchased from the same shop.

Of the four outbreaks investigation conducted on FBD events suspected due to consumption of bakery products, three events were due to *Salmonella* contamination

of bakery products (cream cake). The seasonality of FBD outbreaks from bakery products could not be established due to low sample size and limited outbreaks reported, though most diarrheal cases in Bhutan are during hot and wet seasons (Wangdi & Clements, 2017).

The isolation of pathogenic organism (*Salmonella* spp.) from the cream cake samples (samples collected from bakery and left over samples) relays that the FBD outbreaks were linked with consumption of *Salmonella* contamination; otherwise should not have been detected in 25g of food samples. The common serovar

identified was *Salmonella* Enteritidis isolated from the samples. The gastroenteritis incidences caused by *S. Enteritidis* have been widely recognized, reported and concerned (Nesbitt *et al.*, 2012). *Salmonella* Typhimurium and *S. Enteritidis* are the two most common serotypes identified in India and other South East Asian countries (Arora *et al.*, 2015, Lan *et al.*, 2016, Jacob *et al.*, 2020). An outbreak investigation conducted in Australia concluded *Salmonella* as a causative agent in bakery product (Gregory *et al.*, 2003). A similar case study in Singapore also concluded that cream cakes are a vehicle for transmission of *Salmonella* (Solhan *et al.*, 2011) causing FBD outbreaks. Older evidence from Minnesota (1981–1998) suggests *Salmonella* species to be the major pathogen causing FBD (Deneen *et al.*, 2000). Makinde *et al.* reported that *Salmonella* is a major concern to food safety due to its ability to survive for long time on surfaces, transfer and/or acquire virulent genes thereby leading to the proliferation of highly virulent strains (Makinde *et al.*, 2020)

Furthermore, common ingredients (sugar, butter and eggs) samples were also analyzed to identify the source of outbreaks for three FBD events with pathogenic organism. No pathogenic organisms were isolated from sugar and butter samples. However, *Salmonella* species were isolated from the egg (egg shell) samples suggesting eggs to be a source of contamination and causing outbreak. This finding is similar to the study conducted in USA (Braden, 2006, Centre for Disease Control and Prevention, 2003) and Indian bakery industry and food safety statement that the most common cross-contamination was between products with eggs. Raw egg shell and its contents are known to be a source of *Salmonella* and

poultry is widely known to be a reservoir for *Salmonella*. More precisely eggs are considered as the main vehicle for *Salmonella* infection. It is also possible that the organisms present on egg shells may contaminate egg contents during egg breaking. Egg contents may be contaminated with *Salmonellae* by 2 routes: transovarian (vertical transmission) or trans-shell (horizontal transmission) (FAO & World Health Organization, 2002, Food, 2001). Therefore, adhering to good manufacturing practice and personnel hygiene, and quality inspection or effective cleaning of eggs is of paramount importance. The other types of pathogens occasionally isolated from cream cake samples were *Staphylococcus aureus* and *Bacillus cereus*. This indicates a very low level of sanitation and high probability for FBD incidents.

The main limitation of the study is the lack of available laboratory methods to identify and subtype (sequencing analysis) of pathogenic strains to identify or investigate outbreaks associated with widely distributed commodities or food products and link fecal isolates with food pathogen isolates. Moreover, detailed investigation of bakery premises and food handler's assessment was not carried out during the outbreaks.

5. Conclusion

Foodborne diseases are preventable. The current report suggests that the FBD suspected due to consumption of bakery products (cream cakes) to be probably contaminated by *Salmonella* from egg shells. To minimize the potential risk of Salmonellosis due to the consumption of products containing egg and egg products, good manufacturing practice, proper cleaning of eggs and good

personal hygiene should be followed at all times. Training and knowledge sharing on food safety issues should be regularly conducted by the relevant authorities.

6. Acknowledgement

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7. Conflict of Interest

Declared none

8. References

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