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Detection of *Clostridium perfringens* in full cream pasteurized cow milk in Dhaka city

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Abstract

The *Clostridium perfringens* causes food poisoning in men and animals. Pasteurized full cream cows' milk samples (n = 100) were collected from Dhaka North City Corporation (DNCC) in Bangladesh between July 2022 and June 2023. One sample showed the characteristic properties of *C. perfringens* in Dhanmondi area of DNCC in winter season. Hygienic management should be followed during processing of milk for human consumption. (*Bang. vet.* 2024. Vol. 41, No. 1 – 2, 7 – 12)

Introduction

Milk is an optimal dietary food for people (Waser *et al.*, 2007; Ahsanullah *et al.*, 2019), but can easily harbour a range of spoilage organisms. The *Clostridia* are ubiquitous, zoonotic, obligate anaerobic, spore-forming and Gram-positive bacteria. *Clostridium perfringens* are lethal food-borne microorganisms (Gopal *et al.,* 2015). This bacterium produces more than 17 toxins causing abdominal cramps and diarrhoea. Two types of gastroenteritis are recorded, one with abdominal cramps and watery diarrhoea is common, and another, enteritis necroticans (pig-bel disease) is rare (Grass *et al.*, 2013). The spores of this bacterium are generally resistance to chemicals, pH, heat, osmotic stress, and UV light. These bacteria can grow in dairy environment with wide range of temperature (15^{o}C to 90^{o}C) and spoil milk and milk products (Doyle *et al.*, 2015).

Its vegetative forms are often killed at temperatures of 60^{0}C and above. Spores in cooked food vary in their ability to withstand heat (Cremonesi *et al.*, 2012; Lee and Labbé, 2018). These bacteria can survive frequently in pasteurized milk and milk products (Ivy *et al.*, 2012). Generally, pasteurizing temperature does not exceed 90^{0}C, which destroys vegetative cells but not bacterial spores. Proper heat treatment (like HTST- high temperature short time) can make milk safe. These bacteria may occur in pasteurized milk due to post-pasteurization contamination. Findings on these microbes in the cooked or pasteurized milk or milk products are scanty in Bangladesh. Therefore, this study was conducted to identify the *C. perfringens* from

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full cream pasteurized cow milk along with assessment of risk factors associated with microbial contamination and an outbreak in Dhaka City.

Materials and Methods

Isolation and identification of *C. perfringens* was done from July 2022 to June 2023.

***Study area and samples*:** The samples were high temperature short time (HTST) treated pasteurized full cream cow milk from different companies. The samples were collected from shops in Mohammadpur, Dhanmondi, Farmgate, Agargong and Shyamoli in the Dhaka North City Corporation (DNCC) of Bangladesh. The three seasons are summer (February to April), winter (November to January) and rainy (August to October). The samples were collected once a week.

Study design and sampling strategy: A Cross-sectional longitudinal study design was followed. Multi-stage simple random sampling was practised and 25 samples were collected from each group (n = 4) of selected area (n = 5). The individual samples (200 ml) were placed in a sterile plastic bag and transported in a cool box.

Laboratory tests

i. **Cultural properties:** The samples were prepared for bacteriological culture as described by Ezatkhah *et al.* (2016) in Nutrient broth, Nutrient agar, Blood agar, MacConkey agar, and Triple Sugar Iron (TSI). At first, the nutrient broth was kept in a plastic container with a candle. Then the candle was extinguished and container was sealed to make it anaerobic. The containers were incubated at 37°C for 24 hours, following Anju *et al.* (2021) procedures. The turbidity in the nutrient broth indicated the presence of anaerobic bacteria or other microbes. The cultural properties of the colonies were recorded. The motility test was performed by hanging drop method described by Agarwal *et al.* (2009).

ii. **Staining properties:** The Gram-staining was conducted as per standard protocol (Shelke *et al.,* 2018).

iii. **Biochemical test:** Carbohydrate fermentation tests (Maltose, Mannitol, Glucose, Lactose, Dextrose), oxidase, catalase, indole, methyl red (MR), and Voges Proskauer (VP) tests were done.

Results and Discussion

Cultural properties: Six samples (2 from group A and 4 from group C) showed turbidity. These were cultured in Blood agar and MacConkey agar followed by sub-culture. One sample of group A and one of group C gave the characteristics grey-white colonies with haemolysis on the Blood agar. This suspected sample of group A further produced green colonies on MacConkey agar (Fig. 1) and showed yellow in

TSI slant due to production of acid (Fig. 2). These findings were comparable to the observation of Chalmers *et al.* (2008) and Praveen Kumar *et al.* (2019).

Fig. 1: Suspected bacterial colonies on MacConkey agar

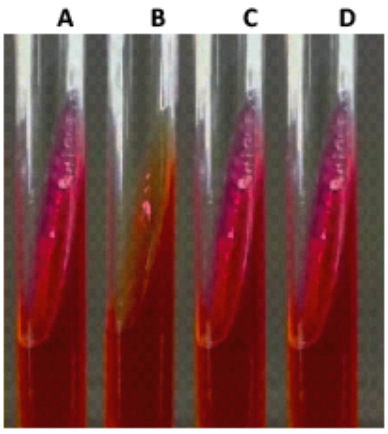

Fig. 2: Produced yellow colour (B) in TSI agar

Staining properties: The suspected colonies were stained. One sample from group A was gram-positive, rod-shaped and non-motile under microscope (100 X).

Biochemical properties: The *C. perfringens* fermented Maltose, Mannitol, Glucose, Lactose, and Dextrose, and produced acid and gas (Table 1).

The findings were all consistent with *C. perfringens* identified by Shelke *et al.* (2018).

Table 1: Biochemical tests of *C. perfringens*

Serial No.	Name of the tests	Results
1	Carbohydrate fermentation	Acid and gas
2	Indole	Negative
3	Methyl Red	
4	Voges Proskauer	
5	Catalase	
6	Oxidase	
7	Motility	Non-motile

Occurrence of C. perfringens

The prevalence of the *C. perfringens* in full cream pasteurized cow milk in Dhaka City was 1% (one positive sample out of 100). This one positive sample came from Dhanmondi area of DNCC in winter season. From the Table 2, the probability of *C. perfringens* was 6, 2% and 1% based on turbidity observation in nutrient broth,

characteristics colonies in Blood agar and MacConkey agar, respectively. One sample from the group A gave the characteristic results for *C. perfringens* in all tests.

Table 2: Probability of the *C. perfringens* isolated in different media

Serial	Sample groups	Total samples	Nutrient broth	Blood agar	MacConkey agar	TSI	Percentage (%)
1	A	25	02	01	01	01	01
2	B	25	00	00	00	00	00
3	C	25	04	01	00	00	00
4	D	25	00	00	00	00	00
Total		100	06	02	01	01	01

Typical zone of haemolysis was observed in each plate of *C. perfringens* and that was similar with Nyrah *et al.* (2017) observation. The probability of presence of *C. perfringens* was recorded in the Dhanmondi of the DNCC in winter season (Table 3 and 4).

Table 3: Probability of the *C. perfringens* in different location of the DNCC

Serial	Location	Total samples	Positive sample	Percentage (%)	*P-value*
1	Mohammadpur	20	00	00	
2	Dhanmondi	20	01	01	≤0.471
3	Farmgate	20	00	00	
4	Agargong	20	00	00	
5	Shyamoli	20	00	00	
Total		**100**	**01**	**01**	

Table 4: Probability of the *C. perfringens* in different seasons

Serial	Season	Total samples	Positive sample	Percentage (%)	*P- value*
1	Summer	35	00	00	
2	Winter	35	01	1	≤0.658
3	Rainy	30	00	00	
Total		**100**	**01**	**01**	

This finding was lower than reported by Islam *et al.* (2003) and Talha *et al.* (2001). The *C. perfringens* was found in 9% of honey samples in Kazakhstan (Maikanov *et al.*, 2019). Mannan *et al.* (2023) in cooked food in Bangladesh found 1.5% occurrences of *C. perfringens* during the winter season. Al Azad *et al.* (2020) reported *Bacillus cereus*

from buffalo milk in Bangladesh. Aksoy *et al,* (2021) in Turkey found *B. cereus* in 61% of samples of raw milk.

Conclusions

The *C. perfringens* bacterium was isolated from one out of 100 samples of full cream pasteurized cow milk in DNCC. Epidemiological investigation needs to be done for drawing conclusive results.

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