

# Prevalence of *Vibrio cholerae* and its probiotic interactions in frozen buffalo meat at abattoir

## Research Article

### Abstract

*Vibrio cholerae* isolated from buffalo meat in India which exported to rest of the world. The aim of the study attempted for detection and isolation of *V. cholerae* and their probiotic study for safe production of meat. This research paper focuses on presence or absence of *Vibrio cholerae* in frozen meat identified in conventional way. Present investigation revealed that out of 46 samples of frozen buffalo meat 41 samples are positive for *Vibrio* species, 5 samples are negative, between 41 samples 39 observes as *Vibrio cholerae* and 27 as *Vibrio parahaemolyticus*. 25 samples of probiotic interactions tested against *V. cholerae*. The isolates antibiogram revealed sensitive against vancomycine and amoxyclave. The sanitary and hygiene condition of abattoir tested in air, water, swab study of equipments, workers and butchers for safe production. Overall, our study found frequency of *Vibrio cholerae* in significant number exits in frozen meat that might pose serious public health hazards.

**Keywords:** *Vibrio cholera*, Frozen Buffalo Meat, Probiotics, Abattoir

Volume 6 Issue 1 - 2018

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**Received:** April 17, 2017 | **Published:** February 26, 2018

## Introduction

Raw buffalo meat is most susceptible to any kind of disease. Pre - processing and post - processing of buffalo meat at abattoir undergo frozen at - 42°C believed to be reduce *Vibrio cholera* and *Vibrio parahaemolyticus* with other species of *Vibrios*. *Vibrio* species belongs to a family *vibrionaceae*. *Vibrio cholerae* has long been known to be responsible for the life threatening secretory diarrhea termed as asiatic cholera or epidemic cholera.<sup>1,2</sup> Robert Koch isolated the strain known as *Vibrio cholerae*. Cholera enterotoxin (CT) produced by *V. cholerae* primarily virulence factor for disease cholera and even produce a wide array of extracellular enzymes including proteases, nucleases, lipases and chitinase. However in 1993 a large outbreak by serogroup O139 occurred in India and Bangladesh. *Vibrio cholerae* merely a waterborne disease but, in this research recognize as water - borne, similarly a zoonotic food borne pathogen. While consumption of frozen meat contaminated with invaded *Vibrio cholerae* causes series of food - borne illness like cholera, gastroenteritis, open wound infection cause septicemia, diarrheagenic cholera etc. The global disease burden has been estimated to be 3-5 million cases and accounts for a total of 100,000-130,000 death per.<sup>3</sup> A number of studies reported outbreaks in Bangladesh like in 1991; a major epidemic caused 210,000 and 235,000 cholera cases and over 8000 deaths.<sup>4</sup> A recent surveillance carried out in Dhaka, Bangladesh, intriguingly revealed a trend of decreasing fatality rate with increasing infection rate over past 3 decades.<sup>5</sup> Some studies reports meat and meat products become contaminated with *Vibrio* species through improper handling, undercooking, washing with unhygienic water and by the use of untreated night soil.<sup>6-8</sup> The risk of disease from ingesting pathogens found in raw meat is significantly higher than cooked meat, although both can be contaminated.<sup>9</sup> Meat can be incorrectly or insufficiently cooked, allowing disease-carrying pathogens to be ingested. In addition, meat can be contaminated during the production process at any time, from the slicing of prepared meats to cross-contamination

of food in a refrigerator. All of these situations may lead to a greater risk of disease.<sup>10</sup> The statutory inspection system and standard should be considerable while exporting frozen buffalo meat to other countries. Therefore studies were undertaken to assess the quality of commercially frozen buffalo meat export from New Delhi, India and to identify the probable source of bacterial contamination. The present paper deals with the bacterial quality of frozen buffalo meat for export and also enlightens the possible sources of contamination during the different stages of processing and probiotic effects at abattoir.

## Morphology

*Vibrios* are gram negative, pleomorphic (curved or straight) or comma shaped, short rods which are motile with sheathed, polar flagella and their size about 2 X 0.5µM. Most *Vibrio* species are halophilic, facultatively anaerobic in nature. Most of the *Vibrios* secrete enterotoxins in food.<sup>11</sup>

*Vibrio* species growth is stimulated by 6-10% NaCl and optimally 3% NaCl in growth media due to their halophilic in nature. But, *V. cholerae* is non-halophilic i.e. can grow well in ordinary media at optimum pH 8.2. Though growth occurs freely between 7.4 and 9.6. Hence, they are facultatively anaerobic and grow best under alkaline conditions.

Most *V. cholerae* strains recovered from epidemic cholera cases contain a common somatic antigen and include serogroup O1.<sup>12</sup> Over 150 known somatic antigenic types have been identified. Strains that are agglutinable in Inaba or Ogawa serotypes of O1 antiserum are well-documented human pathogens. Until recently, only the O1 serogroup was associated with cholera epidemics. Until recently, only the O1 serogroup was associated with cholera epidemics. However, in 1993, a large outbreak of cholera occurred in India/Bangladesh from a new, until then unknown serogroup, O139.<sup>13</sup> But, this research paper does not provide information regarding *V. cholerae* and *Vibrio parahaemolyticus* isolates serotyping.

## Sample preparation and sample collection

This sampling plan applies to the collection of finished products under surveillance for determination of compliance for regulatory consideration. The collection of factory samples of frozen buffalo meat in identifiable lots of processed units and finished products where regulatory action is possible. It does not apply to the collection of in-line process sample units at various stages of manufacture since those samples do not necessarily represent the entire lot of food under production. The actual techniques involved in sampling are covered in the Investigations Operation Manual.<sup>14</sup>

A sample unit consists of a minimum of 100 g and is usually a consumer-size container of product. Take sample units at random to ensure that a sample is representative of the lot. When using sample containers, submit a control consisting of one empty sample container that has been exposed to the same conditions as those under which the sample was collected. Collect more than one sample unit from large institutional or bulk containers when the number of sample units required exceeds the number of containers in the lot. A sample unit will consist of more than one container when containers are smaller than 100g (e.g., four 25g containers could constitute a sample unit). Overall present paper represent as collect 100gm of frozen buffalo meat sample, transport them in insulated shipping container enough gel - type to maintain refrigerant at 6°C or below. Upon receipt in the laboratory, store the samples at 4°C and analyze as soon as possible. If analysis cannot be started within 4 days after collection, freeze samples promptly and store at -20°C until examined. Thaw at room temperature and proceed with analysis as usual. Maintain frozen samples at -20°C until examined. Among the sample 25gm of frozen buffalo meat sample are weighed and add 225mL of alkaline peptone water, homogenized the mixture in a blender and used as pre-enrichment solution.<sup>15</sup>

## Methods of microbiological analysis

The above pre-enrichment solution incubated overnight or at 37°C for 24 hours. Pour plate 1 mL on Thiosulphate citrate bile - salt sucrose agar or TCBS agar from the pre-enrichment. Incubate the TCBS plates at 37°C for 24 hours.<sup>16</sup>

The samples of frozen buffalo meat analyzed microbiologically. The observation exhibit as the positive colonies of *Vibrio cholerae* identified on TCBS agar as yellow color colonies and the colonies of *Vibrio parahaemolyticus* on TCBS agar observed as green color colonies. Further identification confirmed *V. cholerae* and *V. parahaemolyticus* below few positive biochemical observations.

### Biochemical observations

Positive isolates of *V. cholerae* and *V. parahaemolyticus* identification differentiation tabulated below --

### Antimicrobial susceptibility testing

Positive isolates of *V. cholerae* from TCBS agar inoculated in a test tube containing nutrient broth. After overnight incubation when cell concentration reach  $10^7$  to  $10^8$  cells/mL and 1mL of *V. cholerae* culture placed in TCBS agar and after solidification placed 30µg of Vancomycin, 10µg of amoxyclave and 5µg of methicillin discs on the

agar. After 24 hours of incubation at 37°C observed that methicillin does not produce any zone of inhibition. Hence, it is resistant against *Vibrio cholerae*. Amoxyclave produce clear 1.5 mm zone of inhibition. Hence it is sensitive against *Vibrio cholerae*. Vancomycin form clear 1 cm zone of inhibition. Hence, it is sensitive against *Vibrio cholerae*.

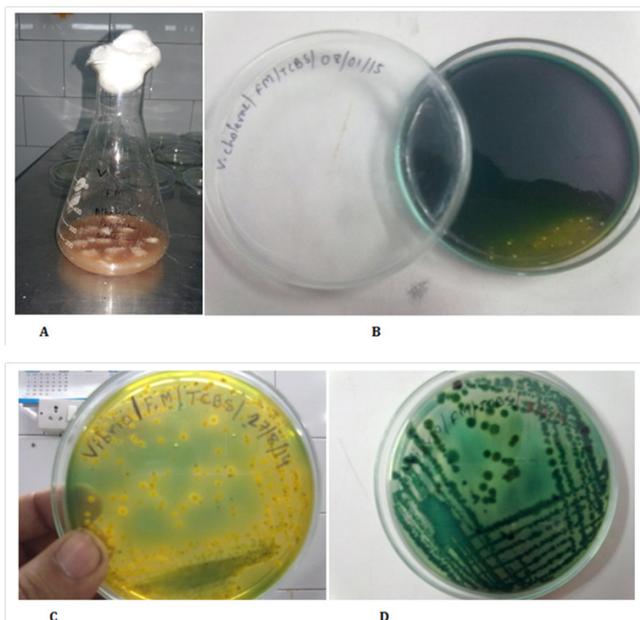
### Probiotic interaction *V. cholerae*

The concept of use probiotics at abattoir during processing of raw buffalo meat to slicing and frozen, explore the ideas to generate inhibiting and fighting capabilities within the meat itself against all food contaminating enteropathogens. The concept of probiotics is the colonization of beneficial bacteria in the intestinal tract, promoting efficient functioning of digestion, helping prevent digestive upsets and stimulating and maintaining the natural immunity of the body. Probiotic bacteria are normal inhabitants of the intestines and normally found in the healthy gut of all humans. Probiotic bacteria comprises of genera: *Lactobacillus*, *Streptococcus*, *Bifidobacterium* according to Shah. 2007, Chow 2002. The organism Lactic acid bacteria (LAB) contain bacteriocin (bactericidal peptides or proteins), nicin (bacteriocin), lantibiotics (amphiphilic polypeptide), also produce flavoprotein oxidases, degradative enzyme catalase, hydrogen peroxide which has antimicrobial activity, LAB also produces ethanol which is potential antimicrobial which can inhibit the growth of other organisms.<sup>18</sup> Hence, according to this concept such as lactic acid or acetic acid if use can preserve the meat for longer time and increase the quality with increasing frozen meat shelf-life. On the other hand, if concentration of above probiotic organisms sprayed in processing area during over meat can inhibit other pathogenic organisms, increase shelf-life and durability, increase quality, safe for consumption, safe for public health condition. In this paper, experiments carried out probiotic organisms Vs. *Vibrio cholerae*. A paper represent that the antagonistic effect against *Vibrio cholerae* observed by use of *Lactobacillus species*.<sup>19</sup> On the other hand, these experiments exclusively include certain method of tests like spot test, well diffusion test methods. Eventually, results obtained directly through well diffusion test *Lactobacillus species* Vs. *Vibrio cholerae*. The test organisms were prepared in a broth culture overnight when cells density reached to  $10^7$  per mL and inoculated in agar plates. Probiotic organisms such as - *Lactobacillus*, Yeast and *Enterococcus* used to culture overnight in broth culture and when it reached to  $10^7$  cell density per mL in the culture. After solidification of the agar plates with test organisms, at the centre of the agar plate bore or punched with a sterile 7 mm cork borer or with a sterilized scalpel. 50µL of each probiotic culture organisms (not a cell free supernatant) added to the punched well and allow it diffuse for 4 hours at 4°C. A well filled with distilled water will serve as control. Zone of inhibition was checked after incubating plates at 37°C for 24 hours under aerobic condition following the protocol indicated in mentioned research paper.<sup>19-21</sup> At the end it is observed that *Lactobacillus*, *Enterococcus* and Yeast cultures within the agar well diffuse and zone of inhibition denotes inhibition of *Vibrio cholerae* within the agar plates as shows in below figures.

### Other sources of frozen meat contamination during processing at abattoir

There are numerous other sources to contaminate frozen meat during pre and post processing, slicing, deboning, chilling and frozen.

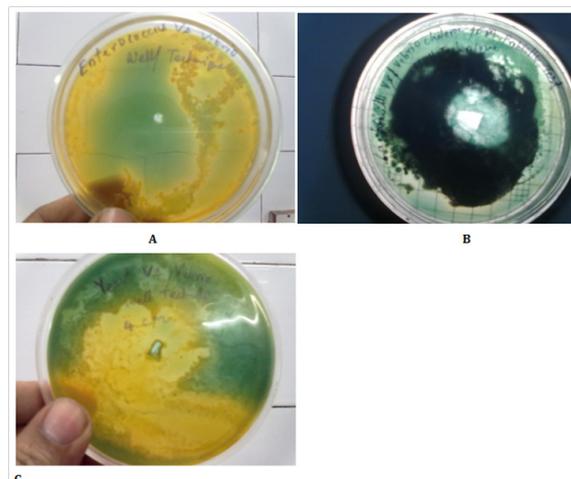
*Vibrio cholerae* and *Vibrio parahaemolyticus* are mostly water borne disease. During slicing and cutting on tables if table cannot washed with hotwater above at 95°C and chlorine based water or carcasses does washed with chlorine water on the surface of carcass chances of contaminations are more. Workers coming from outside if not used hands are chlorine washed which is potential source of contamination of *Vibrio species* on buffalo carcasses. There are different sources of water at abattoir checked and experimented where observation of *V. cholerae* and *V. parahaemolyticus* also reported. In spite of that, air quality of the plant also potential hazardous source of meat contamination.



**Figure 1** (A) indicate alkaline peptone water along with frozen buffalo meat, (B & C) indicate yellow colonies of *V. cholerae* on TCBS and (D) denotes green colonies of *V. parahaemolyticus* on TCBS.

**Table 1** Positive isolates of *V. cholerae* and *V. parahaemolyticus* identification

S. No	<i>Vibrio Cholerae</i>	<i>Vibrio parahaemolyticus</i>
1	Microscopically gram negative comma shaped (curved or straight) bacteria	Microscopically gram negative curved and rod shaped bacteria
2	Motile	Highly Motile
3	Catalase Positive	Catalase Positive
4	Oxidase Positive	Oxidase Positive
5	Indole Positive. (It produces indole that gives cholera red reaction and belongs to group 1 <sup>17</sup> )	Indole Positive.
6	VP Positive or Negative	VP Negative
7	Urease Negative	Urease Positive or Negative
8	Citrate Positive	Citrate Negative
9	Methyl Red Negative	Methyl Red Negative
10	Gelatinase Positive	Gelatinase Positive
11	ONPG Positive	ONPG Negative



**Figure 2** Probiotic Interactions: (A) denote *Enterococcus* Vs. *V. cholerae*, (B) denote *Lactobacillus* Vs. *V. cholerae*, (C) denotes *Yeast* Vs. *V. cholerae*.

## Results

Present research study in this paper revealed that out of 46 samples of frozen buffalo meat 39 samples appeared as positive for *V. cholerae*, 27 samples confirm as *Vibrio parahaemolyticus* 5 samples appeared as negative. All positive colonies of 39 samples from yellow color colonies of *V. cholerae* on TCBS agar and 27 positive samples from green color colonies of *V. parahaemolyticus*. Both the *Vibrio* species identified for confirmation through biochemical experiments tabulated above. Apart from that, the antibiotic study confirm *V. cholerae* 5µg methicillin resistant and 10µg amoxyclave sensitive. Besides above studies, research on positive probiotic organisms recovered from frozen buffalo meat samples tested their interaction against each other (Probiotic organisms and Pathogenic Organisms) such as, *Enterococcus* Vs. *V. cholerae*, *Lactobacillus* Vs. *V. cholerae*, *Yeast* Vs. *V. cholerae* where all tests shows positive and form zone of inhibition.

Table Continued....

S. No	<i>Vibrio Cholerae</i>	<i>Vibrio parahaemolyticus</i>
	0% NaCl - Positive	Negative
	3% NaCl - Positive	Positive
12	Growth in 6% NaCl - Negative	Positive
	8% NaCl - Negative	Positive
	10% NaCl - Negative	Negative
13	Growth at 42°C Positive	Positive
14	Lactose Negative	Lactose Negative
15	Sucrose Positive	Sucrose Negative
16	Nitrate to Nitrite Positive	Nitrate to Nitrite Positive
17	TSI - Alkali/Acid or Acid/Acid without gas and H <sub>2</sub> S	TSI - H <sub>2</sub> S Negative

## Discussion

46 frozen different cuts of buffalo meat investigated through above methodologies of test. Frozen buffalo meat in slaughter house area contaminated during processing from slaughtering, carcass washing, chilling, deboning, fresh packing and till frozen condition, whereas chilling done at 0–4°C and frozen achieved through -38 to -42°C. The result obtained which shows high risk and affinity to contaminate and invade as potential pathogen and destruct food quality of frozen buffalo meat. Generally, foods might be suspected together with water in conveying the cholera disease within communities. Foods can be faecally contaminated more frequently during preparation and handling, particularly by infected food handlers in an environment with poor hygienic condition. The risk factors associated with the high prevalence of *Vibrio cholerae* in frozen buffalo meat samples may pose impact on the cholera disease outbreaks. The irresponsible and unhygienic act of washing chicken, beef meats with sewage contaminated water/seawater could explain the reported *Vibrio* incidences (especially *V. cholerae* and *V. parahaemolyticus*) among these types of fresh meats.<sup>22,23</sup> *Vibrio cholerae* cultured in alkaline peptone water and TCBS agar medium which is highly selective for isolation of *Vibrio spp.* and *Vibrio parahaemolyticus*, contain high pH which (8.5–9.5) which suppresses the growth of other intestinal flora.<sup>24,25</sup> Cholera is a disease of great public health importance. Hence, abattoir water has been recognized as the primary vehicle of cholera transmission to meat. Thus, to interrupt the transmission cycle, effective water treatment system at abattoir placed for public health measures should be undertaken to prevent meat contamination and also releases the water to environment with suitable effluent treatment system to keep way hazardous in outside environment.

## Conclusion

Alkaline Peptone Water (APW) broth is the best media for growth of *V. cholerae*. The detection level of *V. cholerae* and *V. parahaemolyticus* should be absent in frozen buffalo meat. Further, TCBS agar culturing media helps in selectively isolate and differentiate the *V. cholerae* from *V. parahaemolyticus*. Overall, probiotic studies also finds different way to keep away contaminating *Vibrio* species from frozen buffalo meat. The results of studies suggest that in frozen condition also *V. cholerae* and *V. parahaemolyticus* survive and potential

public risk should be assessed which spread of cholera disease in humans.

## Acknowledgement

None.

## Conflict of interest

None.

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