

# Identification and detection of salmonella risk assessment from frozen buffalo meat exported from India

## Abstract

Salmonella found in worldwide in cattle and buffalo meat when even it is in frozen condition and even considered among the most important animal related zoonotic disease. Salmonella is a leading cause of foodborne illness. In this paper the focus given to, whether the frozen buffalo meat contain Salmonella or absent in India when it exported to other countries and the sanitary level in abattoir during practicing food safety conditions are up to the mark or not. In the present investigation 56 frozen buffalo meat samples from different cuts like offals, silverside, knuckle, ramstick, tenderline, stripline, topside, thick flam, blade, shoulder, neck etc tested from slaughter houses. The samples were transported to the laboratory where they were examined bacteriologically for determination of their sanitary status. Among the data of 56 samples it is observed that 12 samples are exhibiting absent or negative for Salmonella and 44 samples found positive for Salmonella. The presence of invasiveness and enterotoxicity of Salmonella in frozen buffalo meat showed their ability to cause systemic infections and which may appear to be threat to the public and a health concern. The public health hazards of these microorganisms as well as the recommended measures to improve sanitary quality status of frozen buffalo meat here is discussed.

**Keywords:** salmonella, frozen buffalo meat, zoonotic disease, serotyping, foodborne illness

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**Abbreviations:** RVM, rappaport-vassiliadis medium; HE, hektoen enteric; XLD, xylose lysine deoxycholate; BS, bismuth sulphite; BGA, brilliant green agar; AABP, American association of bovine practitioners; AMI, American meat institute

## Introduction

Food borne diseases caused by *Salmonella* from frozen buffalo meat represent an important public health problem worldwide. Developed and undeveloped countries are struggling hard to fight with *Salmonella* infections. In United States, an estimated 1.4million cases of salmonellosis is thought to occur annually, of which about 200,000 cases are reported to the CDC.<sup>1</sup> Salmonellosis is a zoonotic disease causing by serious invasive infection in human. Salmonellosis starts from fever and meat of affected animal unfit for human consumption. Most infected persons develop diarrhoea, fever, and abdominal cramps 6 to 72 h after infection. The illness usually lasts 4 to 7days. However, too many people continue to get sick and die from eating contaminated frozen buffalo meat.<sup>2</sup> In Egypt, *Salmonella typhimurium* was recovered with an incidence ranging from 8.4%-19.0% from calves.<sup>3</sup>

Ionizing radiation is able to inactivate food borne pathogens on a variety of food products and can serve as a final critical control point to ensure the microbiological safety of foods.<sup>4</sup>

There are only few reports of incidence of *Salmonella* from frozen buffalo meat and offals, so, the recent study was undertaken with the aim to isolate and identify *Salmonella* from frozen buffalo meat and offals from slaughter houses practicing safe and hygienic packaging of frozen buffalo meat in India. The recovered *Salmonella* isolates were subjected to biochemical characterization.

## Salmonella morphology

*Salmonella* comes under the family *Enterobacteriaceae*. They are facultatively anaerobic, fermentative, gram negative, non-acid fast, non-spore forming rods (typically 1-5 $\mu$ m by 1-3 $\mu$ m) and are generally motile with peritrichous flagella. Growth has been recorded from temperatures just above 5°C up to 47°C with an optimum at 37°C.<sup>5</sup>

## Materials and methods

56 various cuts of Frozen Buffalo meat samples investigated in this study were collected everyday from different slaughter houses of different cut like offals, silverside, knuckle, ramstick, tenderline, stripline, topside, thickflam, blade, shoulder, neck etc and transferred in sterile food bags and analyzed on each day when collected.

## Preparation of samples

- After 25 gm frozen buffalo meat samples weighed in sterile petridishes, they were homogenized in a blender containing sterile dilution 225mL of 0.1% peptone water under the aseptic condition. The above mentioned method consider as **pre-enrichment** method and incubated at 37°C for 24hours.<sup>6</sup>
- Enrichment onto Rappaport-Vassiliadis Medium or Selenite-

Cystein Broth by transferring 1 mL pre-enriched broth to 10mL above RVM or SCM broth and incubated at 42°C for 24hours or at 40°C for 48hours.<sup>7</sup>

- c) Isolation of Salmonella - Plated out the medias Xylose-Lysine-Deoxycholate Agar, Bismuth sulphite Agar, Hektoen Enteric Agar and Brilliant Green Agar. Prepare BSA plates the day before streaking and store in dark at room temperature until streaked. All the agar plates streaked after enrichment and incubated at 37°C for 24hours.<sup>8</sup>

**Identification of salmonella isolates**

- i. **Hektoen Enteric Agar (HE agar)** - Blue-green to blue colonies with or without black centers. Many cultures of *Salmonella* may produce colonies with large, glossy black centers or may appear as almost completely black colonies.
- ii. **Xylose Lysine Deoxycholate Agar (XLD Agar)** - Pink colonies with or without black centers. Many cultures of *Salmonella* may produce colonies with large, glossy black centers or may appear as almost completely black colonies.
- iii. **Bismuth Sulphite Agar (BS agar)** - Brown, gray or black colonies. Sometime they have metallic sheen. Surrounding medium is usually brown at first but may turn black in time with increased incubation, producing the so-called halo effect.
- iv. **Brilliant Green Agar (BGA Agar)** - Pinkish white-orange colonies with surrounding media pink.

All the above plates are incubated at 37°C for 24hours<sup>8</sup> (Figure 1).



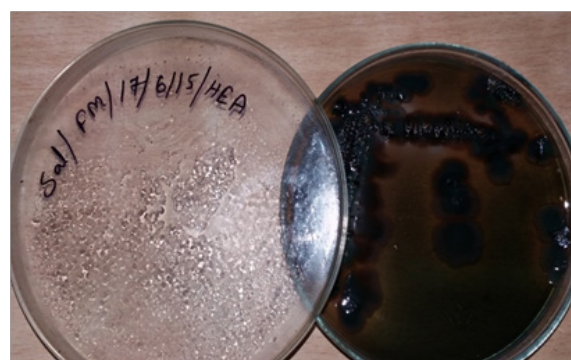
A. Greyish black color colony on BSA.



A. Black centered colony on XLD.



B. Phenol Red colonies on BGA.



C. Black centered colonies on HEA.

**Figure 1** *Salmonella* colonies.

**Biochemical and serological tests**

There are few biochemical tests which confirmed the presence of above colonies of *Salmonellae* species. *Salmonella* on triple sugar iron agar produces hydrogen peroxide and blackening at the butt and slant. Hence H<sub>2</sub>S positive and also form yellow butt on TSI which means produces acid. Other biochemical reactions like urease negative (no color changed), Indole test negative (Yellow color at surface), Voges-Proskauer test negative (No color change), Methyl Red Test Positive (Diffuse Red color), Simmon Citrate Negative (No growth no color change or green in appearance).<sup>8</sup>

Serotypically classification of these organisms by antigenic analysis is based on the original work of Kauffmann and White and is often referred to as Kauffmann-White Scheme. Here Somatic and Flagellar antigens are used. Somatic antigens are designed as O antigens, Flagellar antigen designed as H antigens and Capsular antigen designed as K antigens. K antigen lie at the periphery of the cell and prevent access of anti-O agglutinins (antibodies) to their homologous somatic antigens. The K antigens differ from ordinary O antigens is being destroyed by heating for 1h at 60°C and by dilute acids and phenol. Using H, O, K antigens determined *Salmonella* species possess it's own genetical determined specificity. Most of the *Salmonellae* observed with these antigenic characters.<sup>9</sup>

**Clinical study of higher doses use of antibiotics in slaughter animals (Buffalo)**

There are different antibiotics are used for different purposes

on food animals like buffalo before slaughter. These include antimicrobials, anti-inflammatories, growth promotants, parasiticides and insecticides. According to a survey of members of the American Association of Bovine Practitioners (AABP), the drugs most commonly used by practitioners for dairy cows were antibiotics, followed by anti-inflammatories, tranquilizers and analgesics. Some of the antibiotics used here are Penicillin, Cephalosporine, Aminoglycosides, Streptomycin, Gentamycin, Apramycin, Tetracycline, Erythromycin etc. Steroids and hormones are also used as growth promoters. These drug residues as per the age and use on animals, failure to absorb and regular extra level of use concern public health hazards including toxic and anaphylactic reactions and develop drug-resistant strains of bacteria. There are different factors used to affect after slaughter. One simple factor which affects the stability of residues in tissues is the passage of time. For example, Penicillin G degrades after time. Another factor which will affect the viability and presence of residues in tissues are the effects of chilling and cooking. Freezing would be expected to retard degradation of chemicals. Sulfonamides were found to be stable in long-term freezing conditions. There is immense risk of these drug residues and growth promoters during consumption of food as meat and meat products causes zoonotic illness. The development of drug resistance in foodborne bacteria is another area of great concern. During a survey of antibiotics in beef and chicken in Mexico, a concurrent microbiological analysis was conducted. The predominant micro-organisms found in beef were: *Staphylococcus*, *Enterobacter*, *Citrobacter*, *E.coli*, *Salmonella* etc. These organisms found resistant against Penicillin, Tetracycline, Streptomycin.<sup>10</sup>

As per the Journal of "American Meat Institute" (AMI) 80% of antibiotics used in animals, Drug residues monitored by USDA, MRSA are common in meat and Poultry products.<sup>11</sup>

It is found that resistance of antibiotics are most serious global medical problem in 21<sup>st</sup> century. There is evidence that resistance in some human enteric pathogens has arisen because of transfer of resistant bacteria or resistance genes from animals to people via the food chain.<sup>11</sup> Antibiotic resistance can form in human during zoonotic transmission through following order-The first is allergic reaction and the second is development of antibiotic resistance in the gut bacteria of human. Reduction in antibiotic resistance problems in people could be assisted by improved on-farm management and farm disease control. Improvements in food production and food hygiene would reduce contamination of carcasses and meat with zoonotic food borne *Salmonella*, *Campylobacter*, *E. coli* and *Enterococci*.<sup>12</sup>

Transmission of animal to human takes place from variety of routes, food-borne route probably the most important such as *Salmonella enterica*, *Campylobacter jejuni* and *Yersinia enterocolitica* are the major pathogens takes part. In 1960, *Salmonella* in food animals and humans pass their resistance trait onto other bacteria. In 1990, detection of vancomycin-resistant *Enterococcus faecium* as well as quinolone-resistant *Salmonella* and *Campylobacter* in food animals and evidence of their spread to humans elevated the scientific and public concerns to new levels. A international community like WHO and OIE (World Animal Health Organisation) implemented termination on use of antimicrobial and growth promoters in Europe.<sup>13-15</sup>

The overuse of antibiotics in farm animals like buffalo, its impact in public health by food borne pathogens and form resistance against antibiotics used previously by administration of antibiotics on farm animals consume as food by human. European Union, the United

States and the World Health Organization, are agreed that drug-resistant bacteria are created in farm animals by antibiotic over use. Antibiotics are a precious resource in both human and veterinary medicine. They have saved countless lives since the mid-20<sup>th</sup> century. On World Health Day, 7th April 2011, the WHO Director-General, Dr Margaret Chan, warned that 'In the absence of urgent corrective and protective actions, the world is heading towards a post-antibiotic era, in which many common infections will no longer have a cure and, once again, kill unabated' and that 'The responsibility for turning this situation around is entirely in our hands'. In 1999-2001 in Europe multi-resistance food borne bacteria like *Salmonella* strains isolated from humans and farm animals showed high rates of antibiotic resistance in tests except for Cefotaxime (3<sup>rd</sup> generation cephalosporin) and ciprofloxacin (fluoroquinolone). Multi drug resistance is higher in samples from food and farm animals and are reservoir of multi drug resistance of *Salmonella*.<sup>16,17</sup>

### Different sources of contamination of salmonella in slaughter house area

Salmonellosis is described as a zoonotic infection since the major source of human illness is infected animals. Transmission is by the fecal-oral route whereby intestinal contents from an infected animal are ingested with food or water. A period of temperature abuse which allows the *salmonellae* to grow in the food. *Salmonellae* survive through cross-contamination through direct contact or indirect via contaminated equipments and contaminated handlers. Human transmission occur, if the faecally contaminated hands of an infected food handler touch during slicing or packaging. Food animals like buffalo can acquire infection in farms from wild birds and rodents or contaminated feeding stuff. Improper maintenance of hygienic condition like not using uniform, spraying of chemicals on carcass washing before enter in chillers etc. *S. enteritidis* increased 14-fold between 1981 and 1988, while those of *S. Typhimurium* less than doubled, and *S. Enteritidis* is now the commonest serotype recorded. At freezing temperature like -42°C all samples of frozen buffalo meat tested and most of the samples found positive and found contaminated with *Salmonella* survival.

### Results

In the present study 56 frozen buffalo meat samples tested, 44 samples among them found positive for *Salmonella* spp. All the 44 samples revealed characteristic features of *Salmonella* producing pink colonies with or without black centers from Xylose Lysine Deoxycholate (XLD) Agar colorless pink or pink or opaque-white colonies often surrounded by pink or red zone from Brilliant Green (BG) agar or brown, gray or black colonies from Bismuth Sulphite Agar (BS) agar or blue-green to blue colonies with or without black centers from Hektoen Enteric Agar (HE) agar. On preliminary characteristic IMViC pattern *Salmonella* produces positive tests as per the species.

### Discussion

56 frozen different cuts of buffalo meat investigated through above methodology 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 0-4°C and frozen achieved through -38 to -42°C. It is believed that in frozen condition *Salmonella* cannot



survive. But sample tested showed they survive the frozen condition too. *Salmonellae* in fact, originated through different infected touch of food handlers and butchers, improper clothing, fecal hand contamination, may be through rodent and other insects or unhygienic practice in slaughter house area. They are isolated and identified by above methodological means and observed as positive for *Salmonella*. Hence, from the public point of view, frozen buffalo meat exported in many countries from India and human being consume the same in worldwide. There is a risk of chances for developing Salmonellosis is major due to survival of *Salmonella* at very low temperature too. They may come in different serotypes *S. enteridis*, *S. typhi*, *S. typhimurium* etc.

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## Conflict of interest

The author declares no conflict of interest.

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