**Abstract**

Prior to 1970 *Campylobacter* known to veterinary microbiologist as causative agent of spontaneous abortions in cattle and sheep along with other animals. Positively *C. jejuni* isolated from frozen bovine meat according to Stern et al. *Campylobacter jejuni* is microaerophilic, produces heat labile enterotoxin, can cause in human campylobacteriosis with symptoms of meningitis, pneumonia, miscarriage and a severe form of Guillain - Barré syndrome, non-life threatening, diarrhoea to mimicking acute appendicitis. Microscopically *C. jejuni* is gram negative rods with comma shaped. This research finds the result, out of 61 samples of frozen buffalo meat 49 samples found positive and 12 samples negative for *C. jejuni* in frozen condition. Campylobacter Agar Base with charcoal and sheep blood exhibit thick translucent white or gray or colorless growth with spreading, film-like transparent flat or moist growth at 37°C and 42°C for 2 - 5 days. Antibiotic assay test revealed *C. jejuni* more sensitive to 10 µg amoxyclave & little sensitive or resistant to 5 µg methicillin. Approximately other sources of contamination and risk assessment tested at Abattoir like air - out of 25 sample 100% samples are positive, out of 25 samples of water 35% samples are positive, out of 25 samples of slaughter instrument 100% are positive & Food handlers hand swab out of 25 samples 100% are positive. Therefore, the tolerances are very high in frozen buffalo meat and resulted due to unhygienic practice at abattoir, processing of buffalo meat and create food borne diseases which decreases quality and increases the level of risk of food borne disease.

**Keywords**

*Campylobacter jejuni*; Frozen Buffalo Meat; Campylobacteriosis

**Introduction**

*Campylobacter jejuni* S-shaped, curved or spiral Gram negative bacillus. The bacteria are highly motile with corkscrew like motion. It grows mostly on microaerophilic condition i.e. at reduced 5% O₂ & added 10% CO₂. At present Campylobacteriosis is a major human food borne disease noticed worldwide [1,2], which is dominantly believed to be associated with consumption of improperly handled and unhygienically processed frozen buffalo meat at abattoir in India. *C. jejuni* is a zoonotic foodborne pathogen and one of the causes of colitis in zoonotic countries. The main reservoir of this bacteria in alimentary tract of different animals and at present here in buffalo alimentary tract in New Delhi, India abattoir. The optimum growth temperature is 42°C and also can grow in 37°C. Two or five days after consumption of injected or infested contaminated frozen bovine meat gastrointestinal
symptoms including abdominal cramps, fever and diarrhea will appear, vomiting usually not present but diarrhea may sometime become horrific, turns to bloody diarrhea or dysentery [7,18,19]. *Campylobacter* is highly susceptible to environmental stresses, like variations in temperature, humidity, osmolarity, presence of sunlight and atmospheric oxygen. [2] This study conducted on frozen bovine meat after processing at very low temperature but, sampling resulted and busted out with tolerance and survivability of the organisms O₂ at bovine abattoir in India. Studies elsewhere have demonstrated that *Campylobacter* survive in raw and cooked poultry meat during refrigerated or frozen storage. [3,4,5,6] The objective of this study were to determine potential sources of human disease along with prevalence and survivability of the organism connection in zoonotic foodborne illness at a point in slaughter house after thorough processing samples collected and examine the influence of organism and recovery of *C. jejuni* when gaining familiarity with *C. jejuni* isolation procedures.

**History and Disease Report**

Veterinary Microbiologist prior to 1970 known *Campylobacter* as an organism that cause spontaneous abortions in cattle and sheep and cause of other animal pathogens. It appears sensitive to freeze. *C. jejuni* reported isolated from frozen bovine muscle meat. [9] *C. jejuni* is occasionally invasive and infections are manifested as meningitis, pneumonia, miscarriage and a severe form of Guillain - Barré syndrome. [10,11] *C. jejuni* produces heat labile enterotoxins. It is reported that in 2007 in Kuala Lumpur, Malaysia different samples of chilled and frozen chicken carcasses have been tested and *C. jejuni* confirmed present in the samples. [12] Human Campylobacteriosis also reported in chilled and frozen poultry meat in Brazil. [13] It is also reported in USA and New Zealand during storage of beef trimming in freezing and frozen condition *C. jejuni* causes a wide range of debilitating, but non – life - threatening, symptoms, from diarrhoea to mimicking acute appendicitis. In addition, the rare immune - mediated neuropathic Guillain – Barré syndrome that is characterized by ascending paralysis and occasional death. [14] In a study of raw meat of beef and chicken it is reported that samples contaminated with *C. jejuni* [15].

**Morphology and Cultural Characteristics**

*Campylobacter* are microaerophilic (reduced 5% O₂ & added 10% CO₂, Oxygen quenching agents such as Hemin and Charcoal used in media), gram negative rods with comma, S or “gull - wing” shapes, 0.5–8 µm in length and 0.2 – 0.5 µm in width, cells are pleomorphic, non – spore forming, very small, slender, curved or spiral shape and one or more polar or amphi-/ribious flagella shows corkscrew movement of bacilli, as old cultures spiral or curved bacilli are replaced by round forms. *Campylobacter jejuni* are cultured in microaerophilic condition at reduced 5% O₂ & added 10% CO₂ in media, charcoal used as effective substitute in media. On blood agar it is non - haemolytic. Here *Campylobacter* Blood Charcoal Agar Base supplemented with 5-7% sterile lysed horse blood or 10% sterile defibrinated sheep blood and Campylobacter supplement I (Blaser - Wang) or Campylobacter Supplement III (Skirrow) and Charcoal is used [16].

**Materials and Methods**

**Sample Collection:** Collect 100 gm of frozen buffalo meat sample, transport them in an 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 immediately. 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.

**Method:** Within 100gm sample 25gm frozen buffalo meat samples are weighed aseptically inside laminar air flow and add 225mL of 0.1% peptone water homogenized the mixture in a blender and used as pre-enrichment solution according [16] sampling method as mentioned above. Incubate the pre-enrichment at 37°C for 24hrs. After incubation pour plate on the Campylobacter Blood Charcoal Agar Base supplemented with 5-7% sterile lysed horse blood or 10% sterile defibrinated sheep blood and Campylobacter
supplement I (Blaser-Wang) or Campylobacter Supplement III (Skirrow) and Charcoal. Incubate at 37°C and 42°C for 2 - 5 days undergo moist, microaerophilic condition in an anaerobic jar or wrapped it with aluminium foil. Observe the plates for presence or absence of colonies.

**Observation**

*Campylobacter jejuni* exhibit thick translucent white or gray or colourless growth to spreading, film-like transparent growth or watery, convex, pinpointed, round, irregular and smooth edges colonies and also show water droplets on charcoal agar.

**Biochemical Confirmation**

*Campylobacter jejuni* is microscopically gram negative rods and biochemically confirmed as motile, oxidase positive, catalase positive, ferment carbohydrates, H₂S on TSI negative.

**Antibiotic Sensitivity of C. jejuni**

Antibiogram test on recovered positive *C. jejuni* isolates conducted to look for acquired whether antibiotic resistant *C. jejuni* organisms takes part in creating food borne diseases during human consumption. These assessment measure actual potential
source may come from cattle feed and may come from insertion of antibiotic to control diseases in buffalo. These conveyed as natural phenomena and transferred through genetically acquired antibiotic resistant plasmid DNA to human after the meat consumption. As a result, when disease acquired higher dose or higher generation of antibiotic used to cure the disease. Lower dose of antibiotic become ineffective. In this research few samples of isolated positive \textit{C. jejuni} colonies analyzed. \textit{Campylobacter jejuni} positive colonies picked from few samples of frozen buffalo meat and tested with three antibiotic discs like 5µg methicillin disc, 10µg amoxyclave discs which incubated at 37°C for 24 hours. 1.7 cm zone of inhibition appears on around methicillin disc and 7mm zone of inhibition appears around methicillin disc. Hence Campylobacter more sensitive to 10µg amoxyclave disc but less sensitive to 5µg methicillin disc.

**Risk Assessment from The Potential Sources of Abattoir Environment**

The assessment of potential risk is important to signify that potential risk depend on different contaminant present in abattoir environment which contaminate frozen buffalo meat while processing and resultant in spoilage. To assess and analyze these potential risk sources are summarize and significant in all respect. Most importantly, air present inside abattoir where aerosols are major potent source which need to analyze frequently with conventional way i.e. by sedimentation technique of exposing the plates in plant environment. Food handler’s hand, equipment, machineries, cracks and crevices with wall and floor swab analysis can provide potential source to identify contaminating areas. On the other hand, potential and significant source can identify through most probable number (MPN) test of water which used in washing floors and other used utensils. During assessing the risk of \textit{Campylobacter jejuni} from air, it is observed that out of 25 samples almost 100% of the samples are positive for \textit{C. jejuni}. While screening the water samples used in plant by pour plate technique, it is evident out of 25 samples, 35% of the samples found positive. During investigation of other pathogenic organisms through swabbing slaughter house instrument after wash out of 25 swab samples analysis yield 100% of the samples are positive. Even when check and analyze 25 swabbing samples of food handlers and butchers where 100% of the samples observed as positive. It means...
during processing and post-processing an unhygienic practice prevail which can contaminate and help in spoilage of the food at very low temperature. This is significantly dangerous for human consumption and requires standard international and national level regulatory authority to set microbiological and other significant parameter before export from New Delhi, India to maintain it strictly. On the other hand, it’s a not only economic loss of the manufacturer and buyer, but, at the same time economic loss and honor for the country. At the end, safe food production and safe consumption will open the way to new era of food production.

Results and Discussions

Indeed, present research experiments of frozen buffalo meat where 61 samples have been pursued for analysis to identify Campylobacter jejuni, but 49 samples found positive and 12 samples found negative. Hence, as a result this finding expressed processing of buffalo meat yield the results of tolerance of Campylobacter jejuni at very low temperature can withstand the temperature and survive in very low temperature too can cause spoilage of the food. The research and investigation reported organism’s tolerances are very high in frozen buffalo meat and resulted unhygienic processing of buffalo meat, compromising with quality and increases the risk of food borne diseases. The most common species of Campylobacter in animals and foods are Campylobacter jejuni. C. jejuni involved in human gastroenteritis. C. jejuni in raw meat of animals detected in Iran [17]. Hippurate hydrolysis is the only biochemical test which can differentiate between C. jejuni and other Campylobacter species. [18,19,20,21] Present investigation of frozen buffalo meat reported that C. jejuni can survive frozen storage or shipment. Freezing is harmful to the organism and can lower isolation rate. The deleterious effect of freezing has been reported. [22,23] Fresh and frozen poultry collected at slaughter and recovered C. jejuni have been reported in a study. [24] In a complete study on Bovine Genital Campylobacteriosis is a bacterial disease caused by Campylobacter fetus subspecies veneralis isolated from cows, buffalos and cattle in Australia. The disease is characterized by temporary infertility of female cattle as a result of a sub-acute diffuse mucopurulent cervicitis, endometritis and salpingitis. Abortions occurred in small percentage of cows and buffalos months after initial infection [25]. Prevalence and survivability of C. jejuni as initial level of aerobic bacteria and failure to decrease in number of beef trimmings during frozen storage has been reported. [26] One of the study investigated C. jejuni in Turky meat during storage the impact of the storage factors on its survivability based on effects of storage temperature, storage period and the interruption of the cooling chain on its survival have been evaluated. [27] According to result it suggests that freezing offers a short- to long-term solution to the safe storage of buffalo meat with respect to the survival of Campylobacter. While it does not entirely eliminate potentially infectious Campylobacter, the initial reduction achieved in its population using this method may have a substantial impact on reducing the incidence of campylo- bacteriosis [29] These results also demonstrate the existence of injured cells in the frozen bacterial population. To prevent campylobacteriosis, it is suggested to spray chlorinated water up to 50ppm in concentration during slaughtering, skinning and evisceration under aseptic conditions on buffalo carcasses. Before the consumption of frozen buffalo meat food, the processed raw frozen buffalo meat must cook undergo temperature reach at least 77°C and 82°C respectively. To model the survival kinetics of Campylobacter jejuni on frozen chicken meat studied elaborately by. [28] in Netherland.

Conclusion

Campylobacter jejuni can recovered from mostly all cuts of frozen buffalo meat. This research concentrated only on frozen buffalo meat recovery of C. jejuni and its survival capacity in very low freezing, but not concentrated on chilled meat recovery. The detection from frozen buffalo meat is pretty high and denotes C. jejuni survivability capacity and adaptation during freezing environment. Overall, as a reason of survival lead to food deterioration and spoilage. Diseases caused by Campylobacter jejuni are increasing in significance worldwide. Infact, cross contamination through mishandling of frozen buffalo meat during processing at abattoir and the failure to maintain the storage condition increasing. The result show that raw frozen buffalo meat must be considered to increased C. jejuni contamination rate. Therefore, there will be a considerable risk of C. jejuni infection in consumers either through direct contamination or cross-contamination during handling or processing of raw frozen meat. However, in order to prevent Campylobacter jejuni contamination and foodborne diseases, consistently practice good hygiene at buffalo meat processing and handling as well as consumer education mandatorily implement.

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References


