Presumptive Count of Clostridium perfringens in Iron Milk Medium from Meat Samples

Division of Veterinary Public Health, Indian Veterinary Research Institute, Izatnagar-243122

(Received 14.04.2011; accepted 25.02.2013)

ABSTRACT

The study was undertaken to determine the presumptive count of Clostridium perfringens in 90 meat samples (30 samples each of buffalo, goat and poultry,) using iron milk medium (IMM). The meat samples were collected from retail shops and slaughter houses in and around Bareilly city. In the study, most of the samples (57.75%) showed MPN counts of C. perfringens to be >1100 with the highest in goat meat samples (76.6%), followed by poultry (50%) and then buffalo (46.6%). Lower MPN counts were observed as 8.8%, 12.2% and 21.1% in meat samples, in which the MPN counts ranges between 501-1100, <100 and 100-500, respectively.

Keywords: C. perfringens, IMM, meat, presumptive counts.

Clostridium perfringens is a zoonotic pathogen and present in the intestine of both humans and domestic animals (Jay, 2005; Songer, 2010). The organism is ubiquitous in nature and is considered to be the most widely distributed pathogen on the earth surface (Garcia-Alvarado et al., 1992). In the developed countries like U.S.A., UK, Germany and Canada where disease reporting system is strong, the organism is commonly encountered in human food poisoning cases (Adak et al., 2002, McClane, 2006). In India, various studies indicate the presence of C. perfringens in different foods (Ampratwun, 1993; Agarwal et al., 2001, Singh et al., 2005). The involvement of this organism in food poisoning and diarrhoeic cases has also been reported from time to time (Chakrabarty et al., 1977; Kulshrestha et al., 1982; Vaishnavi and Kaur, 2008).

Keeping the above fact in consideration, the present study was carried out to observe (i) the presumptive count of C. perfringens in buffalo, goat and poultry meats using iron milk medium.

Ninety meat samples (30 each of buffalo, goat and poultry) were collected from retail shops and slaughter houses in and around Bareilly city. The samples were collected aseptically in UV irradiated polyethylene sachets and transported to laboratory under chilled condition for microbiological analysis.

The meat samples were subjected to presumptive C. perfringens count by most probable number test (MPN) as per method described by St. John et al. (1992) with modification. Three tubes MPN technique was performed using iron milk medium (IMM). Ten gm of meat samples was homogenized in 90 ml of normal saline solution in UV irradiated polyethylene sachets with the help of stomacher for 2-3 min. Further, ten fold dilutions were made upto 1:1000 dilutions. After mixing well, 1 ml of inoculum from each dilution (1:10, 1:100 and 1: 1000) were transferred into 3 tubes containing iron milk medium. The tubes were incubated at 45°C to 46°C for 14 to 18 h and tubes showing stormy clot were considered

*Corresponding author: rvsvet24oct@gmail.com/yahoo.com
as positive. The positive tubes in each dilution were counted and most probable number of *C. perfringens* was arrived at using McCaryd table.

The results of presumptive counts of *C. perfringens* in buffalo, goat and poultry meat samples using iron milk medium (IMM) are presented in Table 1. From the table it is evident that all the samples turned out to be positive for presumptive count for *C. perfringens*. Majority of the samples (57.75%) showed MPN counts of *C. perfringens* to be >1100, with highest in goat (76.6%), followed by poultry (50%) and then buffalo (46.6%). Lower MPN counts of <100, 100-500 and 501-1100 were observed in 12.2%, 21.1% and 8.8% of the samples, respectively.

IMM has been adopted as the media of choice by AOAC international for detection of *C. perfringens* in shellfish (Abeyta and Wetherington, 1994). During the growth of *C. perfringens* in iron milk medium, lactose is converted into lactic acid which coagulates the casein protein (acid clot). The vigorous gas production leads to disruption of the clotted milk and the shreds of clot stick to the sides of test tube. The whole process is known as stormy fermentation.

The estimation of counts of *C. perfringens* in the suspected food sample and clinical material is very important in establishing and confirming the etiology of food poisoning (St.John et al., 1982). Besides, presumptive counts of *C. perfringens* in raw meat give an idea about sanitary condition in which the animals is slaughtered and meat is being handled. In present investigation, all 90 samples turned out to be positive for *C. perfringens*. The observed MPN counts were above 1100 for majority of the samples tested. This indicates improper slaughter practices and handling of the carcass. Our results are in agreement with the previous report of Guzman et al. (1990), who reported high percentage (81.6%) of positive sausage sample. *C. perfringens* counts reported by these workers ranged from 10 to 10³ however, most of the sample had the range of 10 – 10³.

IMM role as an enumeration media has been found to be better and efficacious than other media (SPS, TSN, TSC, and TSC without egg yolk agar) for counting *C. perfringens* for food / environmental samples (St. John et al., 1982). During a comparative study of 515 meat samples, no significant differences were observed between counts in iron milk medium and tryptose–sulfite–cycloserine agar (Patricia et al., 2002). Abeyta et al. (2006) examined IMM, TSC and SFP agar for enumeration of *C. perfringens* in the samples of clams, oysters and turkey meat and observed no significant differences in recovery abilities of the three media. Moreover, IMM is inexpensive, rapid and simple to prepare. The recommended temperature for incubation of *C. perfringens* in IMM is 45°C to 46°C for 14-18 h. This temperature favours growth of mainly *C. perfringens* and reduced generation time interval to 8-10 min (Topley and Wilson, 1998). Thus, this temperature provides conducive environment for presumptive count of *C. perfringens* (Abeyta and Wetherington, 1994). Advantages of IMM over other media is its suitability for screening large number of food / clinical / environmental samples and enumeration in samples contaminated with even low number of organism (St. John et al., 1982).

Thus, the study highlighted the poor hygienic condition of retail outlets and slaughter houses due

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of samples</th>
<th>&lt;100</th>
<th>Number of samples with MPN counts</th>
<th>&gt;1100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>100-500</td>
<td>501-1100</td>
</tr>
<tr>
<td>Buffalo</td>
<td>30</td>
<td>3 (10%)</td>
<td>8 (26.6%)</td>
<td>5 (16.6%)</td>
</tr>
<tr>
<td>Goat</td>
<td>30</td>
<td>3 (10%)</td>
<td>4 (13.3%)</td>
<td>0%</td>
</tr>
<tr>
<td>Poultry</td>
<td>30</td>
<td>5 (16.6%)</td>
<td>7 (23.3%)</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>11 (12.2%)</td>
<td>19 (21.1%)</td>
<td>8 (8.8%)</td>
</tr>
</tbody>
</table>
higher presence of *C. perfringens* in meat samples. Further, IMM can be used as medium of choice for presumptive count of *C. perfringens* from food samples.

**References**


