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Prevalence of *Salmonella* in Meat of Food Animals

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Abstract:
An attempt was made to isolate and identify Salmonella bacteria from meat samples of different animals around Pantnagar area. A total of 716 meat samples were screened by conventional cultural method and further confirmed by biochemical, serological and PCR based molecular technique. The samples were plated on various media like HEA, BGA, XLD, BSA and MLA from where the putative isolates were tested biochemically followed by serological identification by latex agglutination kit and molecular confirmation using specific genes. A total of 9 salmonella could be isolated.

1. Introduction

Salmonella is the second major cause of food borne diseases in the world. Over one million and twenty-seven thousand cases of food borne illness are caused by nontyphoidal *Salmonella*, which resulted in 19,336 hospitalizations and 378 deaths in USA alone. Currently, there are more than 2579 serotypes of *Salmonella enterica*. In India, the most frequently associated serotype among non typhoidal *Salmonella* in chicken meat is *S*. Typhimurium (87.8%). Information on prevalence of *Salmonella* in India is fragmentary and no systematic literature is available on prevalence of *Salmonella* from production to consumption level which is essential in understanding the dynamics of *Salmonella* contamination. Therefore, the present investigation was carried out to study the prevalence of *Salmonella* in meat of different animal species and human stool.

2. Material Method

During the period of September 2014- February 2015, a total of 716 meat samples comprising of poultry (289), pork (127), chevon (162), fish (97) and carabeef (41) were collected from the district Nainital and Udham Singh Nagar of Uttarakhand state if India and processed on medium as Brilliant Green Agar (BGA), Xylose Lysine Deoxycholate (XLD) Hekoen Enteric Agar (HEA) plates and Bismuth Sulphite Agar (BSA). Morphological (Old, 1996), biochemical (Chandrashekhar, 2012) and serological (Kumar, 2013) characterization of *Salmonella* isolates was carried out.

Salmonella isolates identified on the basis of cultural, morphological, biochemical reactions and agglutination tests were sent for serotyping to *Salmonella* Typing Centre, Division of Bacteriology and Mycology, IVRI, Izatnagar, Bareilly (U.P.) Further, isolation of DNA from pure culture was undertaken using Hi-pura DNA extraction kit (Hi-media), following the manufacturer's instructions supplied along with kit. These isolates were then subjected to PCR based molecular characterization. All the suspected isolates got confirmed with PCR and yielded products at 244 bp, 617 bp and 220 bp for the primer pairs targeting *inv*A, *stn* and *sop*B gene respectively. The amplified PCR products were analyzed using horizontal submarine gel electrophoresis in 1.8% agarose gel.

3. Results and Discussion

In this study, a total of 9 isolates of *Salmonella* were obtained from 716 meat samples of different animal species and 50 human stool samples. In meat, the prevalence rate of *Salmonella* in Pantnagar and nearby areas based on the above findings was calculated as 1.17%, but lower prevalence of 0.94% was reported by Chandrashekhar, (2012). The prevalence rate of *Salmonella* in chicken meat

was recorded as 2.42% concomitant with Kumar and Lakhera, (2013) who reported 2% of chicken meat samples positive for *Salmonella.* While in pork it was calculated to be 1.57%, although in another Danish study, which estimated prevalence on pig carcasses between 0.9–1.2percent (Nielsen *et al.,* 2001).

All the chevon (162) samples failed to reveal the presence of *Salmonella* during the course of the study agreeing with a study on 600 samples examined from goats, only 4 (0.7%) were *Salmonella* positive (Molla, 2006). None of the fish samples were found to be positive for *Salmonella* in the present study, however, in India Hatha and Lakshmanaperumalsamy (1997) reported 14.25% of the fish samples and 17.39% of crustacean samples contaminated with *Salmonella*. The absence of *Salmonella* in fish might be due to the different epidemiological conditions prevailing in this area. The prevalence of *Salmonella* in carabeef was found to be 0% which is in agreement of the study conducted in Canada, where none of the carabeef samples (n= 134) tested were positive for *Salmonella* (Aslam *et al*., 2012). There was a significant finding that *Salmonella* organism could not be recovered from stool samples of human in Pantnagar. In a study of 232 samples examined, only one sample (0.43%) yielded *Salmonella* serovar (Bisht, 2010).

The overall prevalence of *Salmonella* in different meat samples was 1.17%. Higher prevalence was reported in chicken meat (2.42%) followed by pork (1.57%), as these are the main reservoirs of *Salmonella* bacteria. Both poultry and porcine meats are generally considered higher risks than other meats for *Salmonella* (Duffy *et al.,* 1999). The reason of such low prevalence could be the use of antibiotics and other feed additives given regularly to the animals. Further, samples collected from healthy individuals, less number of processed samples, low endemicity of salmonellosis in this area and good manage mental and hygienic practices might be the other reasons for these observations.

S.No.	Source of sample	No. of processed samples	Total no. of isolates recovered	Serovars identified
1.	Chicken meat	289	7 (2.42%)	*S.* Typhimurium (6) *S*. Enteritidis (1)
2.	Pork	127	2 (1.57%)	*S*. Weltevreden (2)
3.	Chevon	162	-	-
4.	Fish	97	-	-
5.	Carabeef	41	-	-

Table 1: Prevalence of Salmonella in different samples

In this study, a total of 9 isolates of *Salmonella* were recovered with maximum prevalence were *S*. Typhimurium (66.66%) followed *S.* Weltevreden (22.22%) and *S.* Enteritidis (11.11%) as depicted in Table: 2 in agreement with data in the European Union, in which *S.* Typhimurium and *S*. Enteritidis was reported as the most prevalent serotype in broiler carcasses in 2009 (EFSA, 2011). These two serotypes are commonly reported from poultry in the Indian subcontinent (Murugkar *et al*. (2005). Since *S.* Typhimurium can infect man and animals equally, interspecies sharing of this serovar have also been observed (Singh *et al.,* 2010). The dominant serotypes identified from beef, poultry and pork meat were *S*. Typhimurium (12.7%), followed by *S*. Enteritidis (12.5%) (Roseliza, *et al.*2011).

S. No	Source	Serovars Identified	Antigenic Structure
1.	Poultry	*S.* Typhimurium	4,5,12:i:1,2
2.	Poultry	*S*. Typhimurium	4,5,12:i:1,2
3.	Poultry	*S*. Typhimurium	4,5,12:i:1,2
4.	Pig	*S*. Weltevreden	9,12:-:-
5.	Poultry	*S*. Enteritidis	9,12:gm:-
6.	Poultry	*S*. Typhimurium	4,5,12:i:1,2
7.	Poultry	*S*. Typhimurium	9,5,12:g,m:-
8.	Pig	*S*. Weltevreden	9,12:g,m:-
9.	Poultry	*S*. Typhimurium	4,5,12:i:1,2

Table 2: Salmonella serovars recovered from the samples.

4. Summery

Out of 716 samples comprising of chicken meat (289), pork (157), chevon (162), fish (97) and carabeef (41) collected from the areas of Nainital and Udham Singh Nagar, only 9 cold divulge *Salmonella* spp. by conventional cultural method and confirmed by biochemical, serological and PCR based molecular techniques. Six *S*. Typhimurium, two *S*. Weltevreden and only one *S*. Enteritidis could be isolated.

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