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Prevalence of *Salmonella* in Bovine Fecal Samples, Slaughter House Samples and Environmental Samples

R. Nethra[1*], P. T. Ramesh[2], B. M. Veeregowda[3], C. Ansar Kamran[1], A. Muralidhara[4] and M. L. Sathyanarayana[5]

[1]*Department of Veterinary Medicine, Veterinary College, Hebbal, Bengaluru, India*
[2]*Peripheral Veterinary Hospital, Yelahanka, Bengaluru, India*
[3]*IAH & VB, Bengaluru, India*
[4]*Department of VCC, Veterinary College, Hebbal, Bengaluru, India*
[5]*Department of Veterinary Pathology, Veterinary College, Hebbal, Bengaluru, India*

**Corresponding author*

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Salmonella is widely distributed in bovine in different countries and is considered the most important related zoonotic diseases today which have a public health and economic importance. The present study was conducted to survey the prevalence of Salmonellosis in bovine fecal samples collected in ten different herds located at relatively different geographical areas, slaughter house samples and environmental samples by using standard microbiological and biochemical tests and confirm the isolated *Salmonella* by polymerase chain reaction targeting *invA* gene, specific at genus level. Six *Salmonella* isolates were recovered by screening 508 samples (388 bovine fecal samples from 10 different bovine herds, 57 lymphnode and gall bladder samples from slaughter house, 63 environmental samples from the bovine herds and Slaughter house) in the study. On an overall, the prevalence of *Salmonella* reported was 1.2% (6/508). The herd prevalence in the study was 20% (2 out of 10 herds). Whereas, the observed prevalence of *Salmonella* in bovine fecal samples, slaughter house samples and environmental samples were 1.04%, 1.75% and 1.6%, respectively. Polymerase chain reaction targeting *invA* gene confirmed the presence of *Salmonella* in the samples positive for *Salmonella* by culture method.

Introduction

Salmonella infections are an important cause of mortality and morbidity in subclinically infected bovines. Bovines thus constitute an important reservoir for human infections. Interestingly, despite decades of research into salmonellosis, the disease and its public health consequences are not really resolved.

Salmonella is transmitted to animals and humans through the fecal-oral route. Animals

can become infected after ingestion of feed and water contaminated with *Salmonella*. Similarly, humans can become infected by food borne transmission (Hoelzer *et al.,* 2011). Also, Foodborne salmonellosis has been traced to undercooked ground beef and other beef products in the past, and lymph node (LN) presence in the fatty tissues of beef carcasses is one possible source of *Salmonella* contamination (Rodriguez-Rivera *et al.,* 2014).

Bacterial isolation in culture media, followed by serotyping is considered gold standard test for confirmation of *Salmonella.* This is time consuming and labour intensive. Therefore, technique like Polymerase Chain Reaction being used for rapid detection and confirmation of *Salmonella*. Amplification of *invA* gene of *Salmonella* has been reported as a suitable target for PCR amplification, with potential diagnostic applications (Mohler *et al.,* 2009).

The purpose of this study was to isolate and investigate the prevalence of Salmonellosis in bovine fecal samples, slaughter house samples and environmental samples in bovine herds within Karnataka.

Materials and Methods

In the present study the 10 randomly selected bovine herds and slaughter house in Karnataka were sampled.

Samples from bovine: Out of these 10 bovine herds, a total of 388 fecal samples were collected from all the animals. Approximately 10-20 grams of fecal samples were collected by rectal retrieval using sleeves and placed in labeled sterile plastic container.

Samples of slaughter house: 23 gall bladder content samples and 34 lymphnodes like subiliac, popliteal and mesenteric lymphnodes were collected in sterile plastic bags separately and water sample in sterile screw capped glass tube.

Samples from environment: From each herd 10-20 grams of sample of feed, soil and slurry were collected in separate plastic containers. Sterile swabs were used for sample collection from floor and manger in each farm, before which they were presoaked in sterile phosphate buffer saline. The sample from each floor and manger was collected by wiping it for ten meter and placed into sterile plastic bag containing PBS. About 20 ml of drinking water (tap /bore well/ pond water) from each herd and slaughter house were collected in labeled sterile screw capped glass tubes.

Culture method: Faecal samples, feed samples, slurry, soil and rodent droppings were tested by mixing one gram sample in 10 ml buffered peptone (pre enrichment) water and incubated for 37°C in incubator for 12-16 hrs. One ml of grown aliquots was transferred to 10ml Rappaport and Vassiliadis (RV) medium and incubated at 42°C for 24 hours in incubator and subsequently plated on to agar plates. Aliquots of the incubated broth culture were plated on Mac Conkey agar, XLD agar and Xylose-lysine tergitol-4 (XLT-4 agar) agar plates and incubated at 37°C for 24-48 hours. The colonies showing typical characters were selected for biochemical characterization. The collected drinking water samples from the herds and slaughter house were cultured by adding 10 ml sample to 100 ml of double strength enrichment broth (RV medium) followed by incubation and sub cultured on selective media as described above. The floor swabs and manger swabs were placed in 250-500 ml of enrichment broth and followed by incubation and sub-cultured on selective media as described above. Biochemical characterization: Suspicious colonies were inoculated on to triple sugar iron (TSI) agar slants and

incubated for 18-24 hours at 37°C. The isolates showing reaction typical of *Salmonella* were further tested by urease test, sulfide indole motility test and phenyl alanine agar test.

Polymerase Chain Reaction: Culture isolated and biochemically identified *Salmonella* isolates were confirmed further by more specific molecular technique, PCR targeting genus specific *InvA* gene.

Results and Discussion

Bacterial isolation

In the present study, a total of 388 fecal samples from ten herds in and around Bengaluru *viz* ILFC Bengaluru, Chickaballapura, Yenigadale, Ganjigunte, Rachahalli, Byappanahalli, Bashetahalli, Shidlagata, Upparahalli and Gauribidanur and 63 environmental samples involving feed, slurry, rodent droppings, soil and drag swabs each from every farm and water from 10 farms and slaughter houses were cultured for *Salmonella.*

On culture two calves out of 52, one heifer out of 108 and one lactating animal out of 228 were positive for *Salmonella*. Whereas one water sample was found positive among 63 environmental samples collected. The prevalence of *Salmonella* was 5.0%, 0.0%, 0.0%, 5.9%, 0.5% and 0.0% in cattle calves, buffalo calves, cattle heifers, buffalo heifers, lactating cattle and lactating buffalo respectively (Table 2). Approximately 1.04% (4 of 388) of the total fecal samples (Table 1) and 1.6% of the total environmental samples (Table 4) were positive for *Salmonella.*

Out of 10 subiliac lymphnodes, 6 femoral lymphnodes, 4 popliteal lymphnodes, 14 mesentric lymphnodes and 23 gall bladder samples collected from the slaughter houses one mesentric lymphnode sample yielded positive for *Salmonella* spp. on bacterial culture and identification by biochemical characters. Whereas no organisms were recovered from gall bladder samples and the three bore well water samples at the slaughter houses. Approximately 1.7% of the slaughter house samples were positive for *Salmonella* spp. (Table 3).

Identification of *Salmonella* by biochemical characterization

On microbiological analysis of 508 samples, 86 samples revealed presumptive *Salmonella* colonies on XLT4 agar plate. Further, on biochemical characterization, 6 samples revealed biochemical profile suggestive of *Salmonella* genus.

Polymerase Chain Reaction

All six isolates were visualized by UV illumination which showed the expected bands of about 284 bp (Figure 1). The method of PCR demonstrated the specificity of *invA* primers for detection of *Salmonella* in the fecal, slaughter house and environmental samples after culture enrichment.

In the present investigation, a total of 508 samples (388 fecal, 34 LN, 23 Gall bladder and 63 environmental samples) were screened for *Salmonella* by culture method. The 86 presumptive *Salmonella* colonies were further characterized on the basis of biochemical characters. Six *Salmonella* isolates were identified based on biochemical characterization.

Herd prevalence of *Salmonella*

In the present investigation, a total of ten herds were screened for *Salmonella,* from which 388 fecal samples and 60 environmental samples were collected and 2 (20%) herds were found to be positive. The

results indicated that prevalence of *Salmonella* in herds of Karnataka was 20%. The herd prevalence reported in the present study was lower than some studies of *Salmonella* in dairy animals where it was reported as 43% by Callaway *et al.,* (2005), 40% by Bhoyar (2009) in Punjab, 28% by Wells *et al.,* (2001) in US and 31% by Huston *et al.,* (2002) in Ohio dairy farms. The difference in the prevalence of *Salmonella* in animals of various geographical areas may be due to difference in management, barn sectioning, herd size, feeding strategies and concurrent infections (Neilson *et al.,* 2004) and host related risk factors that include age, breed, the physiological state of the animals, feeding strategies, vaccination status.

Prevalence of *Salmonella* in bovines

The prevalence of bovine fecal salmonellosis recorded was 1.04%. This observation was in accordance with Williams *et al.,* (1978), Dargatz *et al.,* (2000), Mc Evoy *et al.,* (2003) and Lombard *et al.,* (2012). While the prevalence of present study was lower than that reported by Huston *et al.,* (2002).

Fossler *et al.,* (2004), Younis *et al.,* (2009), Addis *et al.,* (2011), Youssef and El-Haig (2012) and El-Seedy *et al.,* (2016). On contrary, this result was higher than that reported by Opuda-Asiba *et al.,* (1990), Gay *et al.,* (1994), Van Donkersgoed *et al.,* (1999), Alemayehu *et al.,* (2003) and Davies *et al.,* (2004).

Fricker (1987) elucidated that lower prevalence might be due to less number of colony forming units of bacteria which were below the detection limit of the assay (especially in case of *Salmonella* in feces). On the other hand, it is also plausible that some animals with a positive *Salmonella* culture result and compatible clinical signs were actually symptomatic because of another primary disease process; this would lead to an overestimation of the incidence of salmonellosis. Additionally, the presence of antibiotic residues may explain falsely negative bacteriological results because the withdrawal time is not regarded in our herds.

Prevalence of *Salmonella* in Slaughter house

The prevalence of *Salmonella* in gall bladder was 0% and 2.94% from the lymphnodes in slaughter houses. The organism was isolated from mesenteric LN. *Salmonella* enters the animal and then is captured within the lymphnodes. The observation of the present study was in accordance with Kore *et al.,* (2017). Whereas Beyene *et al.,* (2016) observed higher prevalence of *Salmonella* in mesenteric lymphnodes and reported as 13%. One possible explanation is that it escapes the gastrointestinal tract and enter mesenteric lymph nodes, and then disseminates systemically.

Additionally, epidemiological patterns of *Salmonella* differ greatly between geographical areas depending on climate, food harvesting and processing technologies and consumer habits.

Hanson *et al.,* (2016) reported evidence for vertical transmission from the dam to her fetus. Further, serotypes recovered mesenteric lymph nodes more closely resemble those recovered from the feces [15].

One among 57 samples of slaughter houses was positive for *Salmonella* and the prevalence recorded was 1.75% in slaughter house. The observations were in accordance with Webb *et al.,* (2017). The reason could be associated with the hygienic status of the abattoir and cross contamination among the materials used in the slaughtering operation and processing of food might be less.

Table.1 Prevalence of *Salmonella* spp in faecal samples of bovine herds by isolation and biochemical characterization

Bovine Herd	Bovine fecal samples						Total samples	Total positive samples
	Calves (1-6) months		Heifers		Lactating animals			
	No. of samples		No. of samples		No. of samples			
	Tested	Positive	Tested	Positive	Tested	Positive		
1	6	0	24	0	53	0	83	0
2	3	0	5	0	16	0	24	0
3	4	0	10	0	16	0	30	0
4	5	0	8	0	15	0	28	0
5	7	1	9	0	21	1	37	2
6	6	1	13	0	23	0	42	1
7	5	0	11	0	20	0	36	0
8	4	0	10	0	16	0	30	0
9	6	0	11	1	27	0	44	1
10	6	0	7	0	21	0	34	0
	52	**2**	**108**	**1**	**228**	**1**	**388**	**4**

Table.2 Age wise prevalence of *Salmonella* spp in cattle and buffao herds.

		No. of fecal samples	No. of positive samples	Prevalence (%)
Calves	**Cattle**	40	2	5.0
	Buffalo	14	0	0.0
Heifers	**Cattle**	84	0	0.0
	Buffalo	17	1	5.9
Lactating	**Cattle**	197	1	0.5
	Buffalo	31	0	0.0
		388	**4**	**1.04**

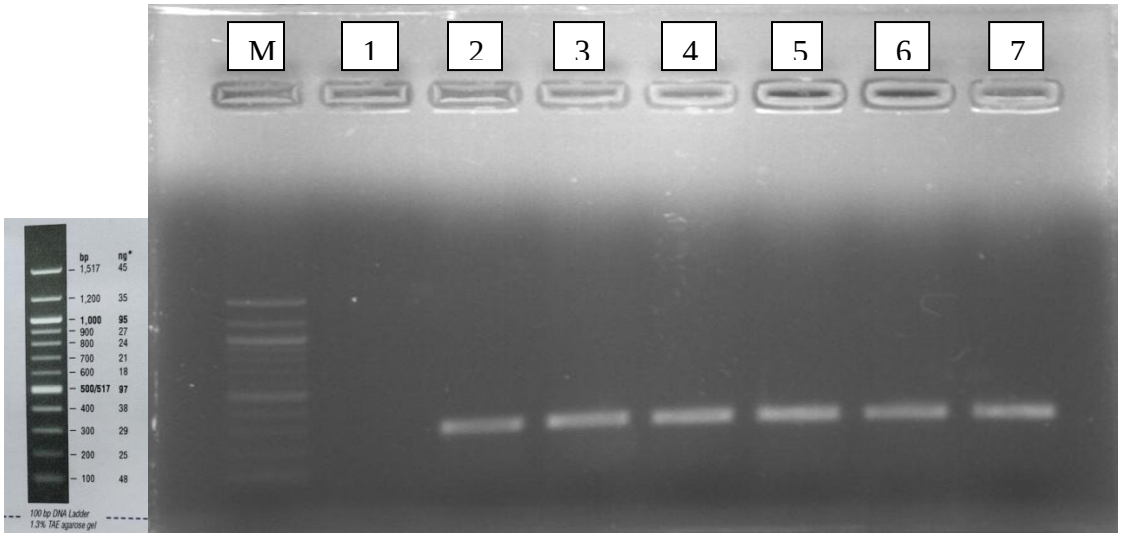
Table.3 Isolation and characterization of *Salmonella* spp from Slaughter house samples.

Slaughter House	Lymphnodes		Prevalence (%)	Gall bladder		Prevalence (%)	Total no. of samples		Prevalence (%)
	No. of samples			No. of samples					
	Tested	Positive		Tested	Positive		Tested	Positive	
Interval 1	7	0	0.0	5	0	0.0	12	0	0.0
Interval 2	11	1	5.2	8	0	0.0	19	1	5.2
Interval 3	16	0	0.0	10	0	0.0	26	0	0.0
	34	**1**	**2.94**	**23**	**0**	**0.0**	**57**	**1**	**1.75**

Table.4 Prevalence of *Salmonella* in environmental samples

Water		Other environmental samples						Total number of samples	
No. of samples		Feed	Rodent droppings	Slurry	Soil	Drag swab	Positive		
Tested	Positive	Tested	Tested	Tested	Tested	Tested		Tested	Positive
13	**1 (7.7%)**	**10**	**10**	**10**	**10**	**10**	**0**	**63**	**1 (1.6%)**

Fig.1 The PCR amplification of invA gene of *Salmonella* serovars (isolated from bovine fecal samples and Slaughter house samples) showing positive amplicons at 284 bp. DNA size marker (M). Lane 1: negative control Lane 2 to 7: Salmonella positive



Environmental prevalence of *Salmonella*

Salmonella, although being intestinal bacteria, are widespread in the environment and are commonly found in farm effluents and in any material subjected to fecal contamination [24]. Identification of *Salmonella* reservoirs is critical for understanding its dissemination in the environment.

In the present investigation the environmental samples yielded 1.6% prevalence of Salmonellosis. The environmental prevalence in the present study was higher than prevalence of Salmonellosis from bovine fecal shedding.

The findings suggest that sources other than farm animals may be a contributor to *Salmonella* in the farming environment. The results were in accordance with Warnick *et al.,* (2001), Fossler *et al.,* (2004) and Gorski *et al.,* (2011).

Whereas all the other environmental samples collected in the present were negative for *Salmonella* organisms which might be due to fecal shedding of the organism is intermittently variable and the collected sample might not have contained the pathogen.

Confirmation of the isolates by Polymerase Chain Reaction

The results demonstrated a correct genus identification of examined *Salmonella* isolates. These results were similar to those obtained by Nair *et al.,* (2009), Kaushik *et al.,* (2014) and El-Seedy *et al.,* (2016) confirmed the *Salmonella* isolates by proving the PCR test to be more specific and also a rapid test. *Salmonella invA* gene has become one of the most popular PCR target sequences and its amplification now has been recognized as an international standard for detection of *Salmonella.* Despite the challenges and limits of this study, it is clear that Salmonella is present in bovines in the study area at prevalence rate of 1.04%. Moreover, the prevalence of Salmonellosis is to some extent, associated with farm management practices. Salmonellosis is important zoonotic infection and reducing the prevalence within and between herds would benefit the human population directly as well as allow for healthier livestock.

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