Research Article **Biotechnology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

DETECTION OF *SALMONELLA TYPHIMURIUM* IN LIVESTOCK PRODUCTS BY PCR TECHNIQUE

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ABSTRACT

Livestock products were screened for *Salmonella* spp. and *Salmonella typimurium* by PCR assay. Two sets of primers derived from *inv* A gene and *fli* C gene were employed to standardize the PCR assay for detection of *Salmonella* spp. and *Salmonella typhimurium* respectively. Buffered peptone water as primary enrichment broth followed by tetrathionate broth as selective enrichment broth was used in this study. Out of 150 samples of livestock products like milk, meat and fish screened 45 samples were positive for *Salmonella* spp. and out of these 45 samples, 3 samples were positive for *Salmonella typhimurium*. Two step enrichment and followed by PCR assay is giving good results than one step enrichment for screening of *Salmonella typhimurium* in Livestock products. Hence accurate screening of livestock products by above method decreases the number of Salmonellosis cases.

KEYWORDS: ***inv* A gene, *fli* C gene, livestock products, PCR assay, *Salmonella typhimurium***

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INTRODUCTION

Salmonella is one of the most important pathogen involved in human food borne illness both in developed and developing countries. *Salmonella typhimurium* is the most frequently isolated serovar from global food borne outbreaks[1]. Non-typhoidal salmonellosis causes high incidence of infections worldwide due to food poisoning in humans which is associated with contaminated food products of animal origin[2]. The most common clinical manifestation of non-typhoid salmonellosis is of acute gastroenteritis with bacteraemia and may lead to complications like osteomyelitis, cardiac inflammation or neural disorders. Food Safety hazards caused by food borne pathogens such as *Salmonella typhimurium* remain a major problem for the food industry[3]. The number of *Salmonella*, to be swallowed in order to cause infection is rather small, i.e. fewer than 10 [4]. Livestock products must be tested for the presence of salmonella, due to its potentially low infective dose[5]. The PCR represents a major advance in terms of the speed, sensitivity and specificity of diagnostic methods and has been increasingly used to identify several bacterial species from food and clinical samples[6]. The rapid, cost effective and automated diagnosis of food borne pathogens throughout the food chain continues to be a major concern for the industry and public health. Because of these requirements, the PCR became a powerful tool in microbiological diagnostics during the last decade[7]. The present study was carried out for the presence of *Salmonella* spp. and *Salmonella Typhimurium* in livestock products collected from in and around Hyderabad, Andhra Pradesh, India.

MATERIAL AND METHODS

1. Sample collection

Fifty samples each of milk (20ml), meat (50g) and fish (50g) were collected aseptically from local markets, vendors and slaughterhouses.

2. Bacterial strains used in the study

The bacterial strains like *Salmonella typhimurium* and *Salmonella virchow* were obtained from Department of Veterinary Microbiology, College of Veterinary Science, Rajendranagar.

3. DNA isolation

The genomic DNA isolation was carried out by phenol: chloroform: isoamyl alcohol method from the above mentioned bacterial strains to standardize PCR assay for detection of *Salmonella* and *Salmonella typhimurium*. Template prepared from sample by this method. About 1000 µl of the 24h old broth culture was centrifuged at 6000 rpm for 5 min and resuspended in 50 µl of molecular grade water. The suspension was then kept in a boiling water bath for 10 min and immediately transferred onto ice (boiling and snap chilling method), later it was centrifuged at 13000 rpm for 5 min. for PCR technique, and 5 µl of supernatant was used as template.

4. Polymerase Chain Reaction

a) Oligonucleotide primers

The primers used from the *inv* A gene and *fli* C gene for the detection of *Salmonella* spp. and *Salmonella typhimurium* respectively were custom synthesized by integrated DNA technologies (IDT) are given in Table 1.

Table 1

Details of primers used in this study

Primer	Target gene	Length	Primer sequence	Amplification Product(bp)	Reference
Salm-3	*inv* A	20	GCT GCG CGC GAA CGG CGA AG	389	Cocolin *et.al.*
Salm-4	*inv* A	19	TCC CGG CAG AGT TC CAT T	389	(1998)
Fli 15	*fli* C	22	CGG TGT TGC CCA GGT TGG TAA T	620	Olivera *et.al.*
Typ 04	*fli* C	16	ACT GGT AAA GAT GGC T	620	(2002)

b) Standardization protocol

PCR amplification of the *inv* A gene fragment and *fli* C gene was set up to 25 µl reactions. The PCR protocol was initially standardized by optimizing the concentration of the components of the reaction mixture in the PCR assay and by varying the annealing temperature and cycling conditions.

i) Standardization of PCR for inv A gene

Initial experiments to optimize PCR reaction conditions for Salmonella template involved the empirical variation of annealing temperature (58^oC-66^oC), concentration of primer (5-15pmol), magnesium chloride (1.5mM-3Mm), template volume (2µl-8µl) and the cycling conditions. Optimal results were obtained using 5µl of bacterial lysate or 20 ng of diluted DNA as template in a reaction mixture consisting of 2.5µl 10X assay buffer for Taq polymerase containing 1.5mM magnesium chloride, 1µl of 25µM each dNTP, 1µl (4pmol) of each primer and 0.9 U/µl of Taq DNA polymerase in a final reaction volume made upto 25µl with molecular grade water.

ii) Standardization of PCR for fli C gene

Initial experiments to optimize PCR reaction conditions for *Salmonella typhimurium* template involved the empirical variation of annealing temperature 42^oC - 62^oC, concentration of primer (5-15pmol), magnesium chloride (1.5-3mM), template volume (2µl-8µl) and the cycling conditions.

5. Reaction mixture

The components of the reaction mixture were finally optimized as given in Table 2. The master mix was made up to 25µl using molecular grade water. Routinely, master mix was set up and 20µl each was distributed to the PCR tubes, to which 5µl of the template was added. PCR assay was performed in Eppendorf gradient Thermal cycler with a heated lid. The cycling conditions used were given in Table 3. PCR products were stored at -20^oC until further use. The amplification products were analyzed by agarose gel electrophoresis using 1.5% agarose gel containing 0.5 µg/ml ethidium bromide at constant voltage 5 V/cm in 1x TAE.

Table 2
Components of reaction mixture

S. No.	Name of the Reagent	Quantity (µl)
1.	10X Taq polymerase buffer	2.5
2.	dNTP mix	1.0
3.	Primer-F	2.0
4.	Primer-R	2.0
5.	Taq DNA polymerase	0.3
6.	Purified DNA /Bacterial lysate	5.0

Table 3
Cycling conditions used for two sets of primers

S. No	Step	***inv*** **A (*Salmonella* spp.)**	***fli*** **C (*S.typhimurium*)**
1.	Initial denaturation	95^oC /5min	94^oC /5min
2.	Final denaturation	95^oC/1min	94^oC/1min
3.	Annealing	58^oC/80sec	45.1^oC/30sec
4.	Initial extension	72^oC/45 sec	72^oC/38sec
5.	Final extension	72^oC/7min	72^oC/7min
6.	Hold	4^oC	4^oC

RESULTS AND DISCUSSION

The PCR assay for the detection of *Salmonella* spp. and *Salmonella typhimurium* from livestock foods was standardized by using the primers derived from targeting genus conserved *inv* A gene (salm-3, salm-4) and *fli* C gene (Fli 15, Typ 04). Genomic DNA isolation (Phenol: chloroform: isoamyl alcohol method) and bacterial lysate prepared from *Salmonella typhimurium* culture and samples respectively was used as template. Upon standardization, the PCR yielded an amplicons of 389 bp and 620 bp from *inv* A and *fli* C genes (Fig 1) without any spurious product respectively. The primers derived from *inv* A gene and *fli* C gene have been the most frequently targeted genes for primer selection in PCR based *Salmonella* spp. detection[8, 9] and *Salmonella typhimurium*[10, 11] respectively.

Table 4
PCR results of different natural samples

Type of sample	No. of Samples	Positive results for *Salmonella* spp	Positive results for *S. typhimurium*
Milk samples	50	2	0
Meat samples	50	10	1
Fish samples	50	18	2

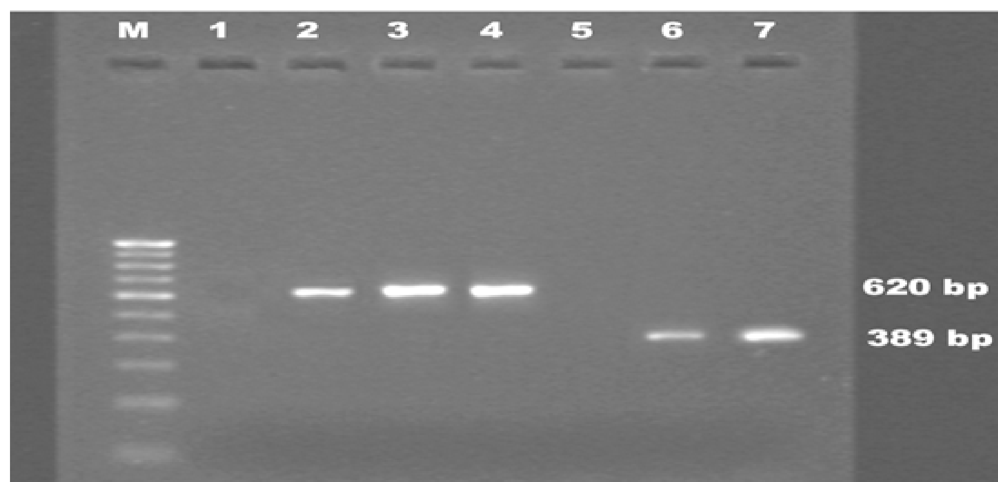

Figure 1
Comparison between amplicon products obtained from genes *fli* C and *inv* A of *Salmonella typhimurium* and *Salmonella* spp respectively

Lane M: 100 bp DNA ladder
Lane 1 and 5: Negative control for Salmonella typhimurium and Salmonella spp respectively
Lane 2 to 4: Amplicon product obtained by using primers from fli C gene (S.typhimurium)
Lane 6 and 7: Amplicon product obtained by using primers from inv A gene (Salmonella spp)

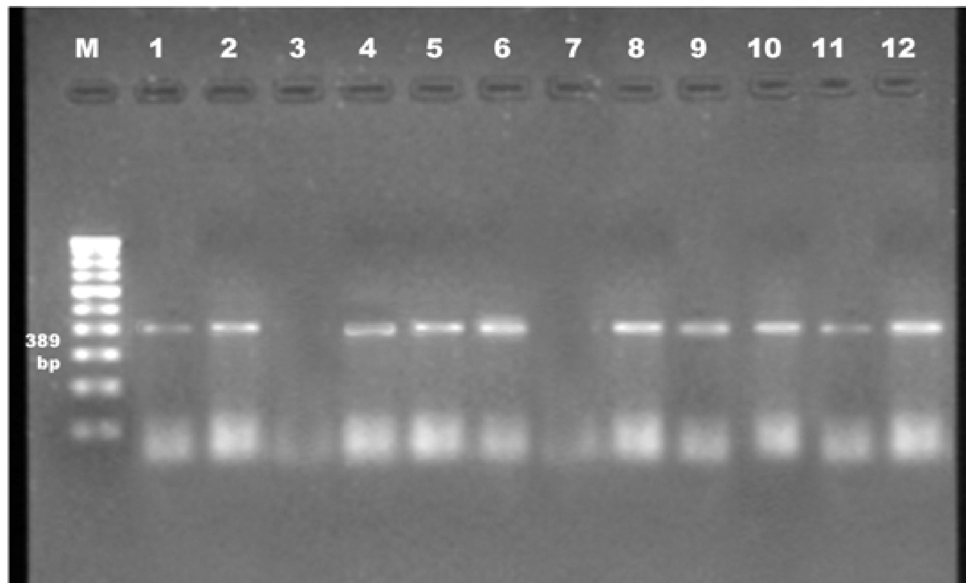

Figure 2
Results of some samples for Salmonella

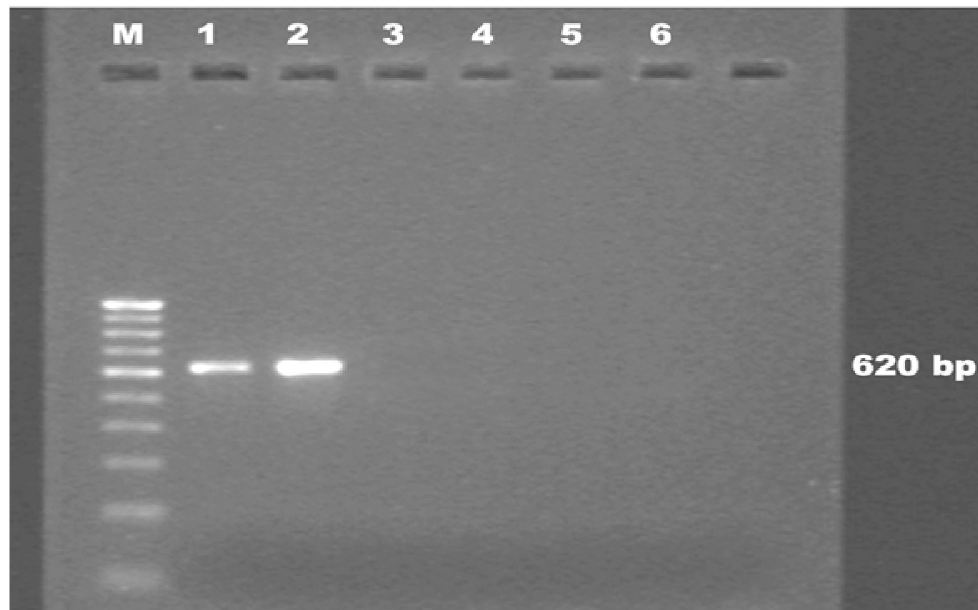

Figure 3
Results of Salmonella typhimurium from positive Salmonella samples

Screening of 150 samples revealed (Table 4) a wide variation in detection of *Salmonella* spp. and *Salmonella typhimurium* in livestock foods. Out of 150 samples screened, 45 samples were positive for (Fig 2) *Salmonella* spp. Out of these 45 positive samples, 3 samples were positive for (Fig 3) *Salmonella typhimurium*. Out of the 50 milk samples screened for the presence of *Salmonella* in this study, two samples were positive for Salmonella and all samples were negative for *Salmonella typhimurium*. Out of 50 meat samples, PCR assay detected 10 samples were positive for *Salmonella* and only one sample shown positive result for *Salmonella typhimurium*. In case of fish samples, out of 50, 18 samples were positive for *Salmonella* whereas 2 samples were positive for *Salmonella typhimurium* out of 18 salmonella positive samples. Non-typhoidal salmonellosis causes high incidence of infections worldwide

due to food poisoning in humans which is associated with contaminated food products of animal origin[1]. Serovar *typhimurium* is also the most frequently isolated serovar from global food borne outbreaks and thus a rapid detection and identification method of this serovar is necessary in the food industry[2]. These observations were more or less similar with the findings of [12, 13, 14.]

CONCLUSION

The present study indicates the presence of *Salmonella* spp and particularly *Salmonella typhimurium* in livestock product samples in and around Hyderabad, Andhra Pradesh, India. Keeping in view the very low infectious dose of *Salmonella* (< 10 viable bacteria), much greater efforts are required for detection of these organisms. Therefore, good livestock management practices and proper hygienic measures for the handling of various food products need to be adopted in order to bring down the presence of these organisms in livestock products, thereby safeguarding the health of human beings from foodborne outbreaks.

ACKNOWLEDGEMENT

The authors are thankful to the Sri Venkateswara Veterinary University for providing necessary facilities and financial support to carry out this work
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