



## Original Research Article

### Detection of *Salmonella* and *Yersinia* spp. in uncooked retail chicken meat in Kerala by multiplex PCR

P.Anju<sup>1\*</sup>, C. Latha<sup>2</sup>, B. Sunil<sup>3</sup> and C. Sethulekshmi<sup>4</sup>

<sup>1</sup>MVSc Scholar, Department of Veterinary Public Health, College of Veterinary and Animal Sciences, Mannuthy, Kerala Veterinary and Animal Sciences University, Wayanad, Kerala, India

<sup>2</sup>Associate Professor and Head, Department of Veterinary Public Health, College of Veterinary and Animal Sciences, Mannuthy, Kerala Veterinary and Animal Sciences University, Wayanad, Kerala, India

<sup>3</sup>Associate Professor and Head, Department of Veterinary Public Health, College of Veterinary and Animal Sciences, Pookode, Kerala Veterinary and Animal Sciences University, Wayanad, Kerala, India

<sup>4</sup>Assistant Professor, Department of Veterinary Public Health, College of Veterinary and Animal Sciences, Mannuthy, Kerala Veterinary and Animal Sciences University, Wayanad, Kerala, India

\*Corresponding author

## ABSTRACT

### Keywords

Chicken meat,  
Multiplex  
PCR,  
*Salmonella*  
enteritidis,  
*Salmonella*  
typhimurium,

The present study was undertaken to determine the incidence and distribution of *Salmonella* spp. and *Yersinia enterocolitica* in raw chicken meats collected from different retail markets in Thrissur and Ernakulam, in Kerala. A total of 225 raw chicken meat samples were collected and analysed for the presence of *Salmonella enterica subspecies enterica* serotype Enteritidis and Typhimurium and *Yersinia enterocolitica*. Single step enrichment using Tryptic Soy Yeast Extract broth followed by multiplex PCR was carried out. Out of 225 raw chicken samples analysed, ten samples were contaminated with *Salmonella enterica subspecies enterica* serotype Enteritidis. Detection of *Salmonella* on chicken meat indicates the need for greater awareness of the risk associated with the production and handling of meat.

## Introduction

Food borne illnesses acts as a major challenge for the development of food industry and has been one of the major concerns in each country as well as in the world. *Salmonella enterica* and *Yersinia enterocolitica* are well-recognized human food borne pathogens, which are

implicated in food-borne bacterial diseases and Outbreaks (Bonardi et al., 2013). Food borne Salmonellosis is important public health problem in many parts of the world, causing gastrointestinal illness, substantial morbidity, and hospitalisation and economic burden worldwide (Fearnley et

al., 2011). *Yersinia enterocolitica* is rapidly emerging worldwide as an enteric pathogen and has become a major cause of human enterocolitis characterised by fever, diarrhoea and abdominal cramps in most of the industrialized world (Bolton et al., 2013). Consumption of contaminated poultry meat can be act as the main source of contamination of these food borne bacterial illnesses. Human cases of salmonellosis are linked with the prevalence of *Salmonella* infection in poultry and other meat sources. There are evidences that consumption of foods of animal origin especially chicken meat with *Y. enterocolitica* which are responsible for human infections (Estrada et al., 2012). The *Salmonella* serovars most frequently isolated from humans are *Salmonella enteritidis* and *Salmonella* Typhimurium and recent studies have shown that *Salmonella enteritidis* is the most prevalent global serovar of *Salmonella*. (Hassanein et al., 2011).

Increased public awareness related to health and the economic impact of food borne illness demands for rapid, sensitive, and specific techniques for detection of these food borne pathogens. The identification of the organisms using conventional bacteriological analysis is laborious and time consuming and not compatible with routine processing of large numbers of samples. Fast and accurate diagnosis of food borne diseases possible by the use of genotype based identification tests, targeting specific genes encoding for the production of virulence factors of the organisms. Multiplex PCR allows fast and accurate detection of more than one virulence factor in a single PCR reaction. This assay not only reduces the cost for testing but also provides data on the presence of different pathogens in a single experiment

In the present study, multiplex PCR was used as a tool to identify the virulence genes of *Salmonella enteritidis*, *Salmonella* Typhimurium and *Y. enterocolitica* in chicken samples collected. For *Salmonella enteritidis* and *Salmonella* Typhimurium primers targeting the STM4495 and SEN1392 genes, coding for a putative cytoplasmic protein, which are sensitive and specific for the detection of these serotypes (Liu et al., 2012) were used. The chromosomal *ail* gene has been shown to be a stable virulent marker contribute to adhesion, invasion, and resistance to complement-mediated lysis, limited only to the pathogenic strains of *Y. enterocolitica* (Vanantwerpen et al., 2013)

The global increase in chicken consumption is stimulated by its high protein content and its accessible price, has drawn the attention to the necessity of rapid detection of the food borne pathogens in the chicken meat. In light of that, the present study was carried out for the screening of raw chicken meat samples collected from retails markets for the presence of food borne pathogens under study.

## Materials and Methods

### Sample collection

A total of 225 samples of raw broiler chicken were collected from various retail outlets in Thrissur and Erankulam viz., Market A (n=94), Market B (n= 86) and Market C (n=45). The samples were taken from birds in good condition and slaughtered by halal method. Fifty gram of sample was taken from each deskinning bird and thigh, rib, and neck area were selected for sampling. The samples were collected aseptically in sterilized polythene bags and transported to the

laboratory under chilled condition in thermocool containers. The samples were processed upon arrival in the laboratory and subjected to microbiological analysis on the same day of collection.

### Processing of samples

A 25 gram portion of each sample was aseptically transferred to 225 ml of Tryptic Soy Yeast Extract Agar supplemented with 1.5 mg/100 ml cefsulodin, 0.4 mg/100ml Irgasan and 0.25 mg/100 ml Novobiocin, in a stomacher bag. Then it was homogenized in a stomacher (Smasher, AES, France) for 120 sec. and incubated at 37<sup>0</sup> C for 16 h. After incubation, two milliliter aliquot from the homogenised sample was used for DNA extraction by boiling and snap cooling method and multiplex PCR protocol was carried out.

### DNA extraction

The boiling and snap chilling technique was used for the preparation of DNA template (Lee et al., 2009). Two milliliter of aliquot from the enriched samples were taken into an eppendorf tube and centrifuged at 1000 X g for 10 min at 4<sup>0</sup> C. The supernatant was discarded and the pellet obtained at the bottom was washed twice in one millilitre of sterile milliQ water by re-centrifugation at 1000 X g for 10 min at 4<sup>0</sup> C. The pelleted cells obtained finally were resuspended in 100µl of Molecular grade water, kept in a boiling water bath for 10 min, and then immediately chilled on crushed ice for 30 min. Then the samples were centrifuged at 1000 X g for five minutes and supernatants were stored at -20 °C for further use as template for PCR. From this 4µl of template DNA were used directly for the PCR.

### Multiplex PCR

The multiplex PCR was standardised for detecting the pathogens under study simultaneously in a single reaction tube containing all the three primer sets for these organisms *i.e.*, *sen*, *stm* and *ail* primers for *S. enteritidis*, *S. typhimurium* and *Y. enterocolitica* respectively (Table-1). PCR was performed in a reaction volume of 25µl containing 4 µl of genomic DNA, 2.50 µl PCR reaction buffer (1X concentration), 2 µl of MgCl<sub>2</sub> (2mM), 0.25 µl of *taq* DNA polymerase (5U/ µl), 1 µl of dNTP mix (400 µM), 1 µl of forward and reverse set of each primers and 9.25 µl of molecular grade water. PCR amplification was performed as follows: Initial denaturation of 7 min followed by 35 cycles of final denaturation at 95<sup>0</sup> C for 1 min, annealing at 60<sup>0</sup> C for 40 sec and extension for 2 min at 72<sup>0</sup> C and one cycle of final denaturation at 72<sup>0</sup> C for 10 min. The PCR products were separated in a 1.5 per cent agarose gel and stained with Ethidium bromide(10mg/ml). The gel was visualised under UV transilluminator (Hofer, USA) and the images were documented on gel documentation system (Bio- Rad Laboratories, USA).

### Statistical analysis

Statistical analysis was carried out using 'Z' test in order to study the significant difference in the occurrence of Salmonella in chicken between different sources.

### Results and Discussion

Out of 225 raw chicken meat samples analysed by multiplex PCR, 10 samples *i.e.*, six from Market A and four from Market B, with an overall prevalence of 4.44 per cent were found to be

contaminated with *S. enteritidis* (Table-2). The result of multiplex PCR is shown in figure 1. None of the samples was contaminated with *S. typhimurium* and *Y. enterocolitica*. On statistical analysis, it was found that there was no significant difference in occurrence of Salmonella in chicken samples collected from Market A and Market B.

The result of the present study clearly establishes the presence of *Salmonella* spp. among chicken. Detection of Salmonella on meat indicate the need for greater awareness of the risk associated with the production and handling of meat. The primary reservoir of Salmonella is the intestinal tract of animals and birds. During slaughter, the intestinal contents can spill and contaminate the muscles and organs of the chicken, which is the important source of presence of Salmonella in meat (Paiao et al., 2013). Poultry products have consistently been identified as important sources of Salmonella infection in humans, because of vertical transfer of infection from

breeding hens to progeny is an important aspect of the epidemiology of *Salmonella* spp. infection within the poultry industry (Bae et al., 2013)

High level of contamination of chicken meat with Salmonella was reported by Thai et al. (2012). Out of 356 retail chicken meat samples analysed, Salmonella could be isolated from 38.8 per cent of the samples. Freitas et al. (2010) employed multiplex PCR for the specific

When 288 different abattoir samples screened in that study, 10 per cent were positive for *Salmonella* spp.

In the present study, in the retail markets A and B from where positive samples obtained, slaughter of birds were carried out in the market place itself, existing condition of where was quite unsatisfactory. Carcasses may expose to heavy contamination from dung and soil during evisceration. Situation can be further aggravated by inadequate ante-and post-mortem inspection practice.

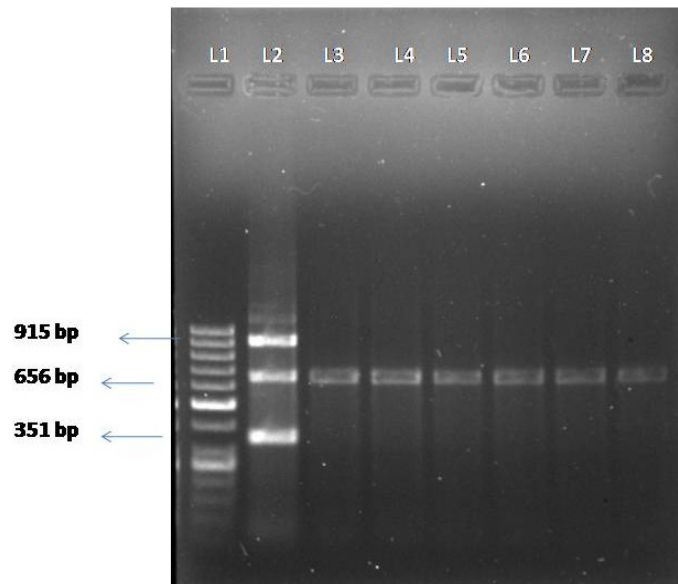
**Table.1** Primers used for the identification of *S. enteritidis*, *S. typhimurium* and *Y. enterocolitica*

Organisms	Primer	Primer sequence	Amplification product size
<i>Y. enterocolitica</i>	<i>ail</i> F	5' TAATGTGTACGCTGCGAG 3'	351 bp
	<i>ail</i> R	3' GACGTCTTACTTGCACTG 5'	351 bp
<i>S. typhimurium</i>	<i>stm</i> F	5' GGTGGCAAGGGAATGAA 3'	915 bp
	<i>stm</i> R	3' CGCAGCGTAAAGCAACT 5'	915 bp
<i>S. enteritidis</i>	<i>sen</i> F	5'GCCACTGTCTGATGCTCTTG3'	656 bp
	<i>sen</i> R	3' GAAAGGCTCCGTGGTTAGT5'	656 bp

**Table.2** Incidence of Salmonella and Yersinia in raw poultry meat by multiplex PCR

Sl. No	Source	Number of samples collected	<i>Y. enterocolitica</i> positive samples	<i>S. enteritidis</i> positive samples		<i>S. typhimurium</i> positive samples
1	Market A	94	0	6	6.38	0
2	Market B	86	0	4	4.76	0
3	Market C	45	0	0	0	0
Total		225	0	10	4.44	0

**Figure.1** Agarose gel analysis of multiplex PCR assay results for *Salmonella enteritidis*



Lane 1. Multiplex PCR control

Lane 2- 8. Chicken samples positive for *Salmonella enteritidis*

Reports of Pavlovic et al. (2007) and Mauro et al. (2008) are also in agreement with the present study where none of the samples was positive for *Y. enterocolitica* when screened 60 and 40 samples of fresh poultry meat. There are reports of isolating the organism from the rectal swabs of poultry as observed by Kechagia et al.

(2007). When 302 samples of rectal swab screened in that investigation, 4.3 per cent of samples were positive for *Y. enterocolitica*. With raw meat, contamination may be less important as cooking to an internal temperature of 60<sup>0</sup> or more prior to consumption eliminate the pathogens. However, inadequately cooked

meat and cross contamination cause greatest risk of food poisoning. Personnel involved in the production, distribution, retail handling and sale should be aware of potential risk from cross contamination and steps to be taken to limit the transfer of the pathogen between raw and cooked products.

### Acknowledgements

Kerala Veterinary and Animal Sciences University, India is duly acknowledged for providing all the necessary infra structure and facilities for doing this research.

### References

- Bae, D. H., Dessie, H. K., Baek, H. J., Kim, S. G., Lee, H. S. and Lee, Y. J. 2013. Prevalence and characteristics of *Salmonella* spp. Isolated from poultry Slaughterhouse in Korea. J. Vet. Med. Sci. 75 : 1193-1200.
- Bolton, D. J., Ivory, C. and McDowell, D. 2013. A small study of *Yersinia enterocolitica* in pigs from birth to carcass and characterisation of porcine and human strains. Food Control. 33- 521-524.
- Bonardi, S., Bassi, L., Brindani, F., D'Incau, M., Barco, L., Carra, E. and Pongolini, S. 2013. Prevalence, characterization and antimicrobial susceptibility of *Salmonella enterica* and *Yersinia enterocolitica* in pigs at slaughter in Italy. Int. J. Food Microbiol. 163: 248-257.
- Cortez, A. L. L., Carvalho, A. C. F. B., Ikuno, A. A., Burger, K. P. and Vidal-Martins, A. M. C. 2006. Identification of *Salmonella* spp. isolates from chicken abattoirs by multiplex-PCR. Res. Vet. Sci. 81: 340-344.
- Fearnley, E., Raupach, J., Lagala, F. and Cameron, S. 2011. Salmonella in chicken meat, eggs and humans; Adelaide, South Australia, 2008. Int. J. Food Microbiol. 146: 219-227.
- Freitas, C. G., Santana, A. P., Silva, P. H. C., Goncalves, V. S. P., Barros, M. A. F., Torres, F. A. G., Murata, L. S. and Perecmanis, S. 2010. PCR multiplex for detection of *Salmonella enteritidis*, Typhi and Typhimurium and occurrence in poultry meat. Int. J. Food Microbiol. 139: 15-22.
- Hassanein, R., Ali, S. F. H., Abd El-Malek, A. M., Mohamed, M. A. and Elsayh, K.I. 2011. Detection and identification of Salmonella species in minced beef and chicken meats by using Multiplex PCR in Assiut city. Vet. World. 4: 5-11.
- Kechagia, N., Nicolaou, C., Ioannidou, V., Kourti, E., Ioannidis, A., Legakis, N. J. and Chatzipanagiotou, S. 2007. Detection of chromosomal and plasmid-encoded virulence determinants in *Yersinia enterocolitica* and other *Yersinia* spp. isolated from food animals in Greece. Int. J. Food Microbiol. 118: 326-331
- Lee, S. H., Jung, B. Y., Rayamahji, N., Lee, H. S., Jeon, W.J., Choi, K. S., Kweon, C. H. and Yoo, H. S. 2009. A multiplex real-time PCR for differential detection and quantification of *Salmonella* spp., *Salmonella enterica* serovar Typhimurium and Enteritidis in meats. J. Vet. Sci. 10: 43-51.
- Liu, B., Zhou, X., Zhang, L., Liu, W., Dan, X., Shi, C. and Shi, X. 2012. Development of a novel multiplex PCR assay for the identification of *Salmonella enterica* Typhimurium and Enteritidis. Food Control. 27: 87-93.

- Mauro, A., Lagana, P., Bruno, G., Micali, M., Minutoli, E., Inutoli, E., Delia, S. 2008. Isolation of *Yersinia enterocolitica* biotype 1 A from raw meat products. *Prev. Med. Hyg.* 49: 75-78.
- Paiao, F. G., Arisitides, L. G. A., Murate, L. S., Vilas-Boas, G. T., Vilas-Boas, L. A. and Shimokomaki, M. 2013. Detection of *Salmonella* spp, *Salmonella enteritidis* and Typhimurium in naturally infected broiler chickens by a multiplex PCR-based assay. *Brazilian J. Microbiol.* 44 : 37-41.
- Pavlovic, L. B. T., Popovic, M. B., Novakovic, B. D., Pasterko, V. P. G., Jevtic, M. R. and Mirilov, J. M. 2007. Occurrence of *Compylobacter*, *Salmonella*, *Yersinia enterocolitica* and *Listeria monocytogenes* in some retail food products in Novi Sad. *Cent. Eur. J. Public Hlth.* 15: 167-171.
- Thai, T. H., Hirai, T., Lan, N.T. and Yamaguchi, R. 2012. Antibiotic resistance profiles of *Salmonella* serovars isolated from retail pork and chicken meat in North Vietnam. *Int. J. Food Microbiol.* 156: 147–151.
- Vanantwerpen, G., Houf, K., Damme, I. V., Berkvens, D. and Zutter, L. 2013. Estimation of the within-batch prevalence and quantification of human pathogenic *Yersinia enterocolitica* in pigs at slaughter. *Food Control.* 34: 9-12.