Screening of poultry samples for *Salmonella Typhimurium* by PCR assay

V K Anumolu* and V R Lakkineni

Department of Veterinary Public Health
College of Veterinary Science, Sri Venkateswara Veterinary University,
Rajendrangar, Hyderabad - 500 030 (AP) India
* Corresponding author email: anumoluvk2k@gmail.com
Received: 14-08-2011, **Accepted**: 20-09-2011, **Published Online**: 26-12-2011
doi: 10.5455/vetworld.2012.169-172

Abstract

Poultry samples viz., cloacal swabs, egg swabs, poultry faeces and feed were screened for Salmonella Typhimurium. A set of primers derived from fli C gene were employed to standardize PCR for detection of Salmonella Typhimurium from poultry samples, which gave specific amplification of a 620 bp fragment. Boiling and snap chilling method used for template preparation. Screening of 112 samples revealed that 12 samples positive for Salmonella Typhimurium by PCR assay.
Keywords: Poultry samples, fli C gene, PCR, Salmonella Typhimurium

To cite this article :
Anumolu VK and Lakkineni VR (2012) Screening of poultry samples for *Salmonella Typhimurium* by PCR assay, *Vet. World.* 5(3):169-172, doi: 10.5455/vetworld.2012.169-172.

Introduction

Food safety hazards caused by food-borne pathogens such as Salmonella Typhimurium remain a major problem for the food industry, particularly poultry processors (USDA-2003). It is the major causes of food-borne disease throughout the world (Wang *et al*., 2008). *Salmonella* infected chickens represent a source of pathogens for humans, causing severe illness and even death. *Salmonella Typhimurium* is also the most frequently isolated serovar from global food-borne outbreaks. Poultry are one of the most important reservoirs of *Salmonellae* that can be transmitted to humans through the food-chain. The commonest serotypes causing disease in humans are *S. enteritidis* and *S. Typhimurium* (Aktas *et al.*, 2007). Kotova *et. al*. (1988) observed that humans develop the salmonella carrier state after acute salmonellosis and it is due to result of occupational exposure to poultry (6.1% -8.8%). There has long been an association between the contamination of eggs and egg products with salmonella and human infection (Humphrey, 2000). Poultry products can be major vehicles of food borne salmonellosis because the raw products are initially contaminated with salmonella cells (Bryan and Doyle 1995).

Salmonella enterica is mainly transmitted to humans following consumption of contaminated eggs and poultry products. Hence, a rapid detection and identification method of this serovar is necessary in the food industry (Lim *et.al.* 2003). 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. The test supplies the growing demand for validated diagnostic PCR methods for screening of samples in meat production chain to assure safe food Lofstrom *et. al*. (2010).

Materials and Methods

Sample collection: 20 each cloacal swabs and egg swabs, 12 poultry faecal samples and 10 feed samples from poultry farm were aseptically collected from All India Coordinated Research (AICRP) on poultry for eggs, Hyderabad. Fifty

chicken samples (50g) were collected aseptically from local shops of Rajendranagar.

Enrichment of samples: Cloacal and egg swabs, faecal samples and feed samples were inoculated in buffered peptone water in test tubes (50ml) and incubated at 37°C, 16h. About 10g of each chicken samples were inoculated into 90ml buffered peptone water (BPW) in individual sterile polythene bags homogenized thoroughly in a stomacher for 3 to 5 min and incubated at 37°C for 16h. Selective enrichment was done in Tetra thionate (TT) broth for which 1ml of pre-enrichment inoculum was transferred to TT (10ml) and were incubated at 42°C for 18h.

Bacterial strains: The bacterial strains *Salmonella Typhimurium* and *Salmonella Enteritidis* were obtained from Department of Veterinary Microbiology, College of Veterinary Science, Rajendranagar

DNA isolation: The genomic DNA isolation was carried out by phenol: chloroform: iso amyl alcohol method from the bacterial strain *Salmonella Typhimurium* to standardize PCR assay for detection of *Salmonella Typhimurium.* DNA templates were prepared from samples by boiling and snap chilling method. In this method, about 1000 µl of the 24h inoculums from the selective enrichment was centrifuged at 6000rpm 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, later it was centrifuged at 13000rpm for 5min. for PCR technique, five µl of supernatant was used as template.

Standardization of PCR: The primers derived from fli C gene for detection of *Salmonella Typhimurium* were custom synthesized by integrated DNA technologies. The nucleotide sequence of the primers (Olivera et.al. 2002) used in this study was Fli 15 (22): 5'- CGG TGT TGC CCA GGT TGG TAA T-3' and Typ 04 (16): 5'- ACT GGT AAA GAT GGC T-3'. The PCR protocol was initially standardized by optimizing the concentration of the components of the reaction mixture in the PCR assay and by varying annealing temperatures and cycling conditions. The reaction mixture consisted of 5ul of the template, 2.5 µl of 10x assay buffer for Taq polymerase containing 1.5 mM $MgCl_2$, 1 µl of 25 µM each dNTP mix, 1 µl each of forward and reverse primer (4 pmol) and 0.9 U/ µl of Taq DNA polymerase made up to 25 µl using molecular grade water. Routinely, master mix was prepared and 20 µl each was distributed to the PCR tubes, to which 5 µl of the template was added. Amplification was carried out with initial denaturation at 94 °C for 5 min, followed by 35 cycles each of denaturation at 94°C for 1 min, annealing at 45.1°C for 30 sec and extension at 72°C for 38 sec with a final extension period of 72°C at 7 min. 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.

Results and Discussion

In India the *Salmonella* has been isolated from variety of livestock foods including milk and milk products, eggs, fish, crustaceans, meat and meat products and also from various environmental samples collected from slaughter houses and fish processing plants (Nagappa et al 2007; Sharma *et.al.,*1995). There is wide difference in the isolation of *Salmonella* spp. by various scientists from various foods ranging from 0 (Ramasastry *et.al.* 1999) to 100% (Sharma *et.al.* 1989; Kamat *et.al.* 1991). These differences might be due to geographic, seasonal variations and also due to procedures adopted for isolation.

Therefore, there is a significant need for more rapid and/or sensitive methods for the identification of *Salmonella*. Several techniques have been developed to address this need, including DNA hybridization (Chevrier *et.al.* 1995), fluorescent antibodies and enzyme-linked immunosorbent assay (Fadeel *et.al.* 2004). However, sensitivity and specificity problems have limited the application of such methods, and there is still a need for a rapid, sensitive, specific and user-friendly method. One possible approach involves polymerase chain reaction based assay, which has become a powerful and increasingly popular tool

in other areas of microbial detection and identification. As PCR relies on the detection of specific gene fragments, it can be applied in mixed microbial cultures, avoiding problems which may arise using other biochemical and morphological tests (Catarame *et.al.* 2005).

Several workers have used PCR with varied success for detection of *Salmonella* from foods using specific gene sequences for targeting (Bennett *et.al.* 1998; Chao *et.al.* 1998; Tsen and Chen, 2001). Of these, *inv* A gene and *fli* C gene have been the most frequently targeted genes for primer selection in PCR based *Salmonella* spp. detection (Chen and Griffiths, 2001) and at the serovar level, *Salmonella Typhimurium* was very specific because it could amplify 620 bp fragments of *fli C* gene in all standard *S. typhimurium* strains (Lin *et al*., 2007).

Screening of 112 samples revealed a wide variation in detection of *Salmonella Typhimurium* in poultry samples. Out of 50 chicken samples, 3 samples were positive for *Salmonella Typhimurium*. Out of 20 swabs from eggs, 4 swabs were positive. These observations were in accordance with the findings of Moussa *et.al.* (2010). One sample was found positive out of 10 feed samples analyzed. Out of 12 faecal samples and 20 cloacal swabs, one and 3 samples were positive. These findings are more or less similar to the results of Gonclaves *et.al.* (1998).

Acknowledgement

Authors are thankful to the Sri Venkateswara Veterinary University for providing funds under state plan budget to carry out my research work in the scheme entitled "Standardization of diagnostic techniques on food-borne infections with particular reference to Salmonella".

Conflict of interest

Authors declare that they have no conflict of interest.

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