ISSN 0975-6299 Vol 3/Issue 2/April – June 2012

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

MOLECULAR BIOLOGY

DETECTION OF *SALMONELLA SPP.* AND *SALMONELLA ENTERITIDIS* IN TURKEY SAMPLES BY PCR METHOD

RAMYA PUTTURU*, KRISHNAIAH NELAPATI[1], TIRUPATIREDDY EVURI[2] AND BINDUKIRANMAYI CH[3]

[1]Professor, Dept. of Veterinary Public Health,College of Veterinary Science, Rajendranagar,
[2]Ph.d. 1st year, Dept.of Poultry Science,College of Veterinary Science, Rajendranagar.
[3]Ph.d. 2nd year, Dept. of Veterinary Public Health,College of Veterinary Science, Rajendranagar.

RAMYA PUTTURU
Ph.D Scholor, Dept. of Veterinary Public Health,College of Veterinary Science, Rajendranagar,

*Corresponding author

ABSTRACT

A total of 60 samples (20 each of turkey meat, faeces and cloacal swab samples) collected from different sources were subjected to cultural and PCR methods for the presence of *Salmonella* and *Salmonella enteritidis*. Primers for *invA* and *sefA* gene were used for *Salmonella* and *S.enteritidis* respectively. Out of 60 samples, 49 were positive for *Salmonella* spp. i.e. turkey meat-14(70%), turkey cloacal swabs-17(85%), turkey faeces-18(90%) by PCR methods, whereas 43 were positive by cultural method i.e. turkey meat-12(60%), turkey cloacal swabs-15(75%), turkey faeces-16(80%). Out of 49 positive for *Salmonella* by PCR method, 36 (turkey meat-11, turkey cloacal swabs-13, turkey faeces-12) were positive for *S.enteritidis*. Selective enrichment with Rappaport-Vassilidias (RV) broth and Tetrathionate (TT) broth were superior over Selenite-F (SF) and Selenite cysteine (SC) broths.

KEYWORDS

Cultural, PCR, Prevalence, *Salmonella*, *Salmonella enteritidis*, Turkey.

INTRODUCTION

Salmonellosis is a major economic problem for food industry and public health hazard in many countries[18]. In India, Salmonellosis is an endemic and important food borne zoonoses. Animal and their products appear to be the main source of human infections, the majority of originate from birds and eggs[4]. *Salmonella enteritidis* is an important cause of human salmonellosis and food poisoning[15] and it is prevalent in poultry farms as a result of vertical and horizontal transmission in and between large poultry organizations[11].

Several gastroenteritis outbreaks have been reported in all parts of the world, many of which were owing to *S.enteritidis* as a causative agent, as a result of consumption of contaminated food products[19]. *S.enteritidis* has emerged as a pathogen of poultry in mid 1970's, but later became an important human pathogen[12]. *Salmonella* infected samples must be quickly identified so they can be isolated and spread of contamination can be controlled[16]. The symptoms in human beings includes diarrhea, nausea, abdominal pain, mild fever, chills, vomition, prostration, headache, malaise etc. and the diarrhea varies from thin vegetable soup like stools to a massive evacuation with accompanying dehydration[7]. The number of *Salmonella*, to be swallowed inorder to cause infection is rather small that is fewer than ten[10] and it is a must for the livestock products to be tested for the presence of *Salmonella* due to it's potentially low infective dose[3].

The detection of *Salmonella* in foods is problematic due to presence of fewer no.of organisms, together with larger no.of competing microflora and also due to injured organisms by different food processing methods[14]. The conventional culture method, which is routinely used for isolation of *Salmonella* is time consuming, laborious and may not be suitable for viable but non culturable (VBNC) state of the organisms[3]. Recently developed methods like DNA hybridization, Enzyme Immunoassays etc. lacks specificity and sensitivity. PCR is rapid, specific and sensitive method for the detection of food borne pathogens[13]. Keeping inview the rapid and accurate identification of *Salmonella spp.* and *S.enteritidis* in turkey products the present study was conducted

MATERIALS AND METHODS

A total of 60 different turkey samples (20 samples each of turkey meat, cloacal swabs and faecal samples) were collected from local markets and slaughter houses in and around Hyderabad. The samples were collected in the sterile containers and transferred under cold conditions (with icepack) to the lab. Turkey meat (10g) samples were preenriched in 90ml of buffered peptone water (BPW) in individual sterile polythene bags homogenized thoroughly in a stomacher for 3-5 mins and incubated at 37^0C for 16h. Faeces and cloacal swabs were inoculated in BPW in test tubes (50ml) and incubated at 37^0C for 16h. After preenrichment 1ml of each inoculum was transferred into selective broths like Tetrathionate (TT) broth, Selenite-F (SF) and Selenite cysteine (SC) broths and 0.1ml to Rappaport-Vassilidias (RV)broth, incubated at 42^0C for 18h (For SC broth, 37^0C, 18h). The broth cultures were spread plated onto selective media agar plates like Xylose Lysine Deoxycholate agar (XLD), Bismuth Sulphite Agar (BSA), Brilliant Green Agar (BGA), Salmonella-Shigella Agar (SSA) and Hektoen Enteric Agar (HEA) and differential agar like Mac Con key Agar. Petridishes were incubated at 37^0C for 24h. The presumptive colonies of *Salmonella* were taken for

furtherconfirmation by biochemical tests like IMViC (Indole, Methyl red, Voges-Prosverker, Citrate), triple sugar iron test, sugar fermentation tests, lysine decarboxylase, ONPG (ortho-nitrophenyl galactosidase).

All the enriched samples were subjected to PCR analysis for the presence of *Salmonella* spp.

Table 1
Details of the primers used

Primer	Target Gene	Primer sequence	Amplification product (bp)	Reference
Salm-3	*invA*	GCT GCG CGC GAA CGG CGA AG	389	Malorny *et. al.* (2003)
Salm-4	*invA*	TCC CGG CAG AGT TCC CAT T	389	Malorny *et. al.* (2003)
SefA2	*sefA*[b]	GCC GTA CAC GAG CTT ATA GA	310	Pan and Liu, (2002)
SefA4	*sefA*[b]	ACC TAC AGG GGC ACA ATA AC	310	Pan and Liu, (2002)

using primers specific to *invA* (Table1). The samples positive for *Salmonella* by PCR method were further examined for the presence of *S.enteritidis* strains using primers specific for *sefA* gene
(Table1).

S.enteritidis strain obtained from Microbial Type Culture Collection (MTCC), Chandigarh was used as known positive strain in PCR analysis. About 1.5 ml of enriched broths were taken in eppendorf tubes and bacteria were pelleted by centrifugation at 8000 rpm for 10 min and the supernatant was discarded. 50 µl of sterile distilled water was added to the tubes and boiled in a water bath at 100^0C for 10 min and immediately snap chilled to release DNA. Then centrifuged at 13,000 rpm for 5 min and the supernatants were used as DNA templates for PCR analysis.

Bacterial DNA amplification was done in 20µl reaction mixture containing 2 µl of 10x Taq DNA polymerase buffer containing 100mM tris with P^H 9.0, 500mMKCl, 15mM $MgCl_2$ and 1% triton X-100), 2 µl of 10 mM dNTP mix, 0.9U/µl of Taq DNA polymerase(Genei), 2 µl each of forward and reverse primer (4pmol/µl) and 5 µl of crude bacterial cell lysate. Make this mixture to 20 µl using molecular grade water. Amplification was done in thermal cycler following standardized conditions
(Table2).

The amplified DNA fragments were resolved by agarose gel electrophoresis, stained with ethidium bromide (0.5 µg/ml) and visualized with an UV transilluminater (Fig. 1).

Table 2
Cycling conditions used for two sets of primers

S.No.	Step	*invA (Salmonella spp.)*	*sefA (S. enteritidis)*
1.	Initial denaturation	95^0C/5min	94^0C/5min
2.	Final denaturation	95^0C/1min	94^0C/1min
3.	Annealing	58^0C/80sec	61^0C/1min
4.	Initial extension	72^0C/45sec	72^0C/2min
5.	Final extension	72^0C/7min	72^0C/10min
6.	Hold	4^0C	4^0C

RESULTS AND DISCUSSION

12(60%) turkey meat samples were positive out of 20 samples for *Salmonella* by cultural method and 14(70%) (fig 1) by PCR method. Out of 14 PCR positives, 11 were positive for *S.enteritidis* (fig 2) by PCR which accounts to 55% and 78.57% over total no.of samples and positive samples for *Salmonella* by PCR respectively. The incidence of *Salmonella* (60%) by cultural method in the present study is higher than the incidence (33.33%, 49% and 50%) reported by Jalali *et. al.*[9], Eyigor *et. al.*[5] and Bailey [2] respectively. The incidence by PCR method (70%) in the present study is almost similar to the incidence (66.7%) reported by Fakhr *et. al.*[6] and more than the incidence (56%) reported by Bailey[2]. Results shown in Table3.

Table3
Occurrence of Salmonella and Salmonella enteritidis in turkey samples

Type of sample	No. of samples	Positive result for *Salmonella spp.*					Positive samples for *S.enteritidis*		
		Cultural method		PCR assay		% of cultural method compared to PCR	No.	% over total no.of samples	% over *Salmonella spp.* positive samples
		No	%	No	%				
Turkey meat	20	12	60	14	70	85.71	11	55	78.57

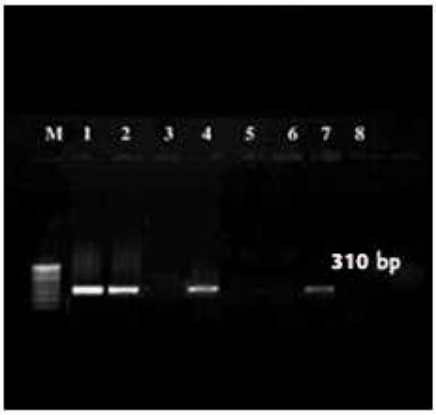
Turkey cloacal swabs	20	15	75	17	85	88.24	13	65	76.47
Turkey faeces	20	16	80	18	90	88.88	12	60	66.66
Total	235	154	65.53	174	74.04	88.50	126	53.62	72.41

Out of 20 turkey cloacal samples, 15(75%) positive for *Salmonella* by cultural method and 17(85%) by PCR method. Out of 17 PCR positives, 13 were positive for *S.enteritidis* by PCR which accounts 65% and 76.47% over total no.of samples and positive samples for *Salmonella* by PCR respectively. The incidence of *Salmonella* (85%) in the present study by PCR method is higher than the incidence (5%, 14.7%, 53.8%) reported by Hoover *et. al.*[8], Turkyilmaz *et. al.*[17], Arora *et. al.*[1] respectively. The incidence of *S.enteritidis* (76.47%) by PCR method in this study is much higher than the incidence (1.9%) reported by Turkyilmaz *et. al.*[17].

Out of 20 turkey faecal samples, 16(80%) were positive for *Salmonella* by cultural method and 18(90%) by PCR method. Out of 18 PCR positives 12 were positive for *S.enteritidis* by PCR which accounts 60% and 66.66% over total no.of samples and positive samples for *Salmonella* by PCR respectively. Low incidence of *Salmonella* by PCR method (5%, 14.7%, 53.8%) was reported by Hoover *et. al.*[8], Turkyilmaz *et. al.*[17], Arora *et. al.*[1] respectively than the incidence (90%) in the present study in turkey faeces. Low incidence (1.9%) of *S.enteritidis* in turkey faecal samples is reported by Turkyilmaz *et. al.*[17] compared to the incidence (66.66%) in the present study.

Figure.1
Results of turkey samples for invA gene
(*Salmonella spp.*)

Figure.2
Results of turkey samples for *sefA* gene
(*Salmonella enteritidis*)



REFERENCES

1. Arora A.K., Gupta S.C., and Kaushik R.K., Isolation on *Salmonella gallinarum* from turkeys. Indian. Vet. J. 65: 171, (1988).
2. Bailey J.S., Detection of *Salmonella* Cells within 24 to 26 Hours in Poultry Samples with the Polymerase Chain Reaction BAX System. J. Food. Prot. 61(7): 792-795, (1998).
3. Bennett A.R., Greenwood D., Tennant C., Banks J.G., and Betts R.P., Rapid and definitive detection of *Salmonella* in foods by PCR. Lett. Appl. Microbiol. 26: 437-441, (1998).
4. CDC., Preliminary food net data on the incidence of infection with pathogens transmitted commonly through food: selected sites US, 2004. Morbid. Mortal. Wkly. Rep. 54: 338-343, (2005).
5. Eyigor A., Carli K.T., and Unal C.B., Implementation of real-time PCR to tetrathionate broth enrichment step of *Salmonella* detection in poultry. Lett. Appl. Microbiol. 34(1): 37–41, (2002).
6. Fakhr M.K., McEvoy J.M., Sherwood J.S., and Logue C.M., Adding a selective enrichment step to the iQ-Check™ real-time PCR improves the detection of *Salmonella* in naturally contaminated retail turkey meat products. Lett. Appl. Microbiol. 43(1): 78-83, (2006).
7. Forshell L.P., and Wierup M., *Salmonella* contamination: A significant challenge to the global marketing of animal food products. Scientific and Technical Review of the Office International des Epizooties. 25(2): 541-554, (2006).
8. Hoover N.J., Kenney P.B., Amick J.D., and Hypes W.A., Preharvest sources of *Salmonella* colonization in Turkey production. Poultry. Sci. 76: 1232-1238, (1997).
9. Jalali M., Abedi D., Pourbakhsh S.A., and Ghoukasin K., Prevalence of *Salmonellla spp*. In raw and cooked foods in Isfahan-Iran. J. Food. Safety. 28: 442-452, (2008).
10. Matsui T., Suzuuki S., Takahashi H., Ohyama T., kobayashi J., Izumiya H., Watanabe H., Kasuga F., Kijima H., Shibata K., and Okabe N., *Salmonella enteritidis* outbreak associated with a school lunch dissert: cross contamination and a long incubation period, Japan, 2-1. Epidemiol. Infect. 132: 873-879, (2004).
11. O'Brien J.P.D., Aspects of *Salmonella enteritidis* control in poultry. World's Poultry. Sci. Journal. 46: 119-124, (1990).
12. Ochoa-Reparaz J., Sesma B., Alvarez M., Renedo M.J., Irache J.M. and Gamazo C., Humoral immune response in hens naturally infected with *Salmonella enteritidis* against outer membrane proteins and other surface structural antigens. Vet. Res. 35: 291-298, (2004).
13. Olsen J.E., Aabo S., Nielsen E.O., and Nielsen B.B., Isolation of a *Salmonella* specific DNA hybridization probe. APMIS. 99: 114-120, (1991).
14. Prusak-Sochaczewski E., and Luong J.H.T., An improved ELISA method for the detection of *Salmonella typhimurium*. J. Appl. Bacteriol. 66: 127-135, (1989).
15. Seo K.H., Holt P.S., Brackett R.E., Gast R.K., and Stone H.D., Mucosal humoral immunity to experimental *Salmonella enteritidis* infection in chicken crop. Avian. Dis. 46: 1015–1020, (2002).
16. Soumet C., Ermel G., Rose V., Rose N., Drouin P., Salvat G., and Colin P., Identification by a multiplex-PCR-based assay of *Salmonella typhimurium* and *Salmonella enteritidis* strains from environment swabs of poultry houses. Lett. Appl. Microbiol. 29: 1-6, (1999).
17. Turkyilmaz S., Hazimoglu S., and Bozdogan B., Antimicrobial susceptibility and resistance genes in *Salmonella enteric*

serovar enteritidis isolated from turkeys. Israel. J. Vety. Med. 64(3): (2009).

18. Wan J., King K., Carven H., Mcauley C., Tan S.E., and Coventry M.J., Probelia™ PCR system for rapid detection of *Salmonella* in milk powder and ricotta cheese. Lett. Appl. Microbiol. 30: 267-271, (2000).
19. Youssef M., Shurman A., Bougnoux M., Rawashdeh M., Bretagne S., and Strockbine N., Bacterial, viral and parasitic enteric pathogens associated with acute diarrhea in hospitalized children from northen Jordan. FEMS Immunology and Medical Microbiology. 28: 257-263, (2000).