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Comparison of PCR and Conventional Cultural Method for Detection of *Salmonella* from Poultry Blood and Faeces

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ABSTRACT

The aim of the study was to compare Polymerase Chain Reaction (PCR) and conventional method for detection of *Salmonella* from field poultry samples (n = 510, poultry blood and faeces 255 each). The prevalence rate of *Salmonella* in chicken was found to be 5.09% using conventional method and 5.88% by PCR assay. Serotyping of 26 *Salmonella* isolates revealed 57.69% *Salmonella* Typhimurium, 19.23% rough type, 15.38% *Salmonella* Enteritidis and 7.69% untypable. Among *Salmonella* Typhimurium isolates, 73.33% were from poultry blood and 26.66% from faeces samples. All isolates belonging to Typhimurium and Enteritidis serotypes were confirmed by PCR targeting of *Salmonella* Typhimurium (*typh*) and *Salmonella* Enteritidis (*ent*) specific genes. However, 4 isolates found to be rough type also turned out to be positive for *ent* gene. The PCR employed for detection of *Salmonella* was found 100% sensitive for poultry blood but its sensitivity was very less (77.77%) for faeces samples as compared with culture method. However, PCR was 100% specific with regard to faeces samples. The specificity from blood samples was 97.89% by PCR. The positive predictive values of PCR from blood and faecal samples were 77.27 and 100% with a concordance of 98.03 and 99.21%, respectively. The negative predictive values from blood and faecal samples were 100 and 99.19%. The study demonstrated usefulness of genus specific PCR for detection of *Salmonella* in poultry clinical samples. Owing to its robustness and rapidity it can be used for wide epidemiological studies. Serotype specific PCR detection of Typhimurium and Enteritidis serotypes has added advantage in identifying them even where there is loss of O antigen.

Key words: *Salmonella*, isolation, PCR, serotyping, poultry

INTRODUCTION

Salmonella organisms are responsible for a variety of acute and chronic diseases in poultry, animals and humans (Barrow *et al*., 2012). Every year, about one third of the food-borne disease outbreaks in human beings are attributed to *Salmonella* alone (Daniels *et al*., 2002; Dhama *et al*., 2013). Transmission of salmonellosis is often associated with animal (CDC, 2007; Dhama *et al*.,

2008a) and plant products (CDC, 2009). Contaminated poultry products are among the most important sources for food-borne outbreaks in humans and *Salmonella* are isolated more often from poultry and poultry products than from any other food animals (Myint, 2004; Braden, 2006; Linam and Gerber, 2007; Kabir, 2010). Infections of domestic poultry with *Salmonella* are expensive both for the poultry industry and for society as a whole (Mead *et al*., 1999; Dey *et al*., 2005; Dhama *et al*., 2008b; Kabir, 2010). The total costs of food-borne *Salmonella* infections of humans in the US have been estimated to 3.3 billion dollars per year (Erol *et al*., 2013). More than 2,600 serotypes of *Salmonella* are known and among them serotypes Enteritidis and Typhimurium accounted for the majority of cases of human salmonellosis (O'Regan *et al*., 2008).

Traditional culture methods for *Salmonella* detection in foods consist of a series of steps that include nonselective enrichment, selective enrichment and selective/differential plating and finally, biochemical and serological confirmation. The traditional microbiological method for *Salmonella* isolation is labor-intensive and requires a minimum of 5 days to complete the analysis (Hammack *et al*., 2004). Consequently, there is a need to develop and validate faster screening and detection methods for this pathogen. Attention is now being focused on molecular based detection methods due to their high sensitivity, specificity and reduced assay time (Kataria *et al*., 2005; Menghistu *et al*., 2011; Batista *et al*., 2013; Sokolov and Sokolov, 2013). In this study, the effectiveness of Polymerase Chain Reaction (PCR) was evaluated for *Salmonella* detection in field samples of poultry by targeting genus specific *inv*A gene and Typhimurium and Enteritidis serotype specific *typh* and *ent* genes, respectively.

MATERIALS AND METHODS

Sample collection: A total of 510 samples comprising poultry blood (255) and faeces (255) from 255 birds were collected from retail outlets of Bareilly city, Uttar Pradesh, India. Ten milliliter of poultry blood was collected aseptically in sterile tubes containing anticoagulant (1.5 mg EDTA mL^{-1} blood) and transported to the laboratory under chilled condition (±4°C). The caecum from the same birds were aseptically collected into sterile polythene bags, transported under chilled condition (±4°C) and processed immediately.

Conventional cultural method: Isolation and identification of *Salmonella* spp. from the 510 samples was carried out by following standard protocol (Agarwal *et al*., 2003). Samples were pre-enriched in Buffered Peptone Water (BPW) in 1:10 ratio (37°C, 16 h). Selective enrichment (42°C, 24 h) was done in tetrathionate broth (TT) and selective plating was performed on Hektoen Enteric Agar (HEA) at 37°C for 24 h. Smooth, transparent colonies with greenish periphery with or without black centre on HEA were picked up and confirmed biochemically (Barrow and Feltham, 1993). Biochemical identification was carried out on 3-5 isolated colonies. Initially, the isolates were inoculated in Triple Sugar Iron (TSI) agar and urea broth at 37°C for 24 h. The colonies showing a TSI result of alkaline slant (pink) and acidic butt (yellow) with or without H_2S production (blackening) and urease negative were considered as positive for *Salmonella* presumptively. The isolates which were positive for *Salmonella* by these tests were further subjected to the primary and secondary biochemical identification tests (Table 1).

Serotype confirmation of the biochemically positive isolates was carried out by tube agglutination test using polyvalent antiserum and tube agglutination test using somatic and flagellar group specific and factor antiserum available in the laboratory.

Table 1: Biochemical characterization of *Salmonella* isolates

Test	Typical reaction of *Salmonella*
Primary identification	
Urease	Negative
Motility	Positive
Indole	Negative
TSI	Acid butt, alkaline slant, $H_2S\pm$, Gas+
Gas from glucose	Positive
Glucose to acid	Positive
Lactose to acid	Negative
Sucrose to acid	Negative
Manitol to acid	Negative
Secondary identification	
Citrate utilization	Positive
Lysine decarboxylase	Positive
ONPG reaction	Negative

Table 2: Oligonucleotide primers used in the study

Primer sequences	Target gene	Expected product size (bp)	Reference
F: GTG AAA TTA TCG CCA CGT TCG GGC AA	*inv*A	284	Galan *et al*. (1992)
R: TCATCGCACCGTCAAAGGAACC			
F: TGT GTT TTA TCT GAT GCA AGA GG	*ent*	304	Alvarez *et al*. (2004)
R: TGA ACT ACG TTC GTT CTT CTG G			
F: TTG TTC ACT TTT TAC CCC TGA A			
R: CCC TGA CAG CCG TTA GAT ATT	*Typh*	401	Alvarez *et al*. (2004)

PCR method

Oligonucleotide primers: Primers used in the study (Table 2) were custom synthesized from Genuine Chemical Corporation (GCC), New Delhi, India.

Template preparation: DNA was isolated from pre-enriched samples (in buffered peptone water) by Snap-chill method. Briefly, 2 mL of the pre-enriched samples were centrifuged at 6,000 rpm for 10 min to pellet the bacterial cells and washed with sterile Phosphate Buffered Saline (PBS) (10 mM; pH 7.4) once. The pellet was resuspended in 50 µL of nuclease free water and kept in a boiling water bath (100°C) for 10 min. This was then transferred immediately to -20°C for 10 min. After incubation, the suspension was centrifuged again at 6,000 rpm for 10 min and the supernatant collected which was used as template for PCR assay.

Genus specific PCR: The PCR was standardized for genus specific detection of *Salmonella* from poultry blood and faecal samples (510) targeting *inv*A gene (Galan *et al*., 1992). The PCR reaction mixture was finally optimized to contain 2.5 µL of 10X PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTP mix, 10 pmol of each forward and reverse primers, 1 U of Taq DNA polymerase, 4 µL of the template prepared by boiling and snap chilling method and sterile deionised water upto 25 µL.

The reaction was performed in Eppendorf gradient thermocycler with preheated lid (lid temperature 105°C). The cycling condition comprised of initial denaturation at 94°C for 5 min, followed by 34 cycles each of denaturation at 94°C for 1 min, primer annealing at 50°C for 1 min,

elongation at 72°C for 1 min and finally a single extension step at 72°C for 7 min. The bands were visualized on 1.5% agarose gel electrophoresis and photographed by the gel documentation system. All the samples were screened for the presence of *inv*A gene.

Serotype specific PCR: All the isolates recovered by cultural method were subjected for serotypic identification of *Salmonella* Typhimurium and *Salmonella* Enteritidis by targetting *typh* and *ent* genes, respectively, as per Alvarez *et al.* (2004). The PCR mixture and cyclic condition were same as that for *inv*A gene except that the annealing temperature for *typh* gene was 57°C for 1 min. Electrophoretic analysis of PCR amplified product was performed on 1.5% agarose gel and results were recorded.

Statistical analysis: Evaluation sensitivity, specificity, positive predictive value and negative predictive value and concordance of diagnostic tests, viz., PCR and cultural method were done as per Thrusfield (2005).

RESULTS

A total of 510 field samples (poultry blood and faeces) were subjected to conventional cultural isolation and PCR methods for detection of *Salmonella* with special reference to Typhimurium and Enteritidis serotypes. Samples were pre-enriched in BPW, followed by enrichment in TT broth and selective plating on HEA plates. The suspected colonies were confirmed by biochemical reactions. Out of 510 samples, *Salmonella* spp. was isolated from a total of 26 (5.09%) samples, of which 17 (6.66%) were from blood samples and 9 (3.52%) from faecal samples. On serotyping, 15 isolates (57.69%) were recognized as *Salmonella* Typhimurium, 5 isolates (19.23%) as *Salmonella* rough type, 4 isolates (15.38%) as *Salmonella* Enteritidis and 2 (7.69%) isolates were untypable. Out of 15 *Salmonella* Typhimurium isolates, 11 isolates (73.33%) were from poultry blood samples and remaining 4 isolates (26.66%) were from poultry faeces samples. Four isolates of *Salmonella* Enteritidis isolates were from blood and out of 5 rough strains 4 were isolated from faeces samples and the remaining one was from blood sample. The untypable isolates (1 each) were from blood and faeces (Table 3).

PCR assay for the detection of *Salmonella* from pre-enriched samples was standardized employing a set of primer of *inv*A gene. Electrophoretic analysis of the PCR product revealed the specific amplification of a 284 bp fragment (Fig. 1). No non-specific products were detected on agarose gel electrophoresis. Similarly, serotype specific PCR for detection of Typhimurium and Eneteritidis were successfully standardized, resulting amplification of 401 bp (Fig. 2) and 304 bp (Fig. 3) products, respectively. Analysis of 510 poultry samples (255 each of blood and faeces) by

Table 3: Serotypes of *Salmonella* recovered from poultry samples

				Total No. of isolates	
Serotype	Antigenic formula	From blood	From faeces	No.	%
Salmonella Typhimurium	1, 4, [5], 12: i: 1, 2	11	4	15	57.69
Salmonella Enteritidis	1, 9, 12: [f], g, m, [p]: [1,7]	4		4	15.38
Rough	-	1	-	5	19.23
Untypable	-	1	1	2	7.69
Total		17 (6.66%)	9 (3.52%)	26	5.09

Fig. 1: PCR amplification of *inv*A gene, Lane M: 1.5+100 bp DNA ladder, Lane 1: *Salmonella* Typhimurium and Lane 2: *Salmonella* Enteritidis

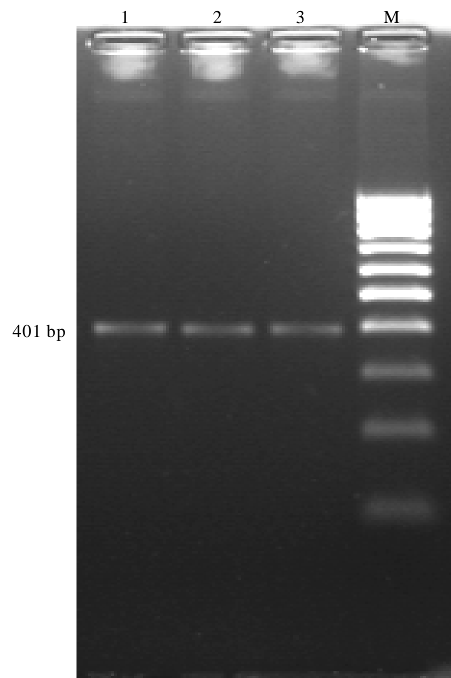

Fig. 2: PCR amplification of *typh* gene, Lane M: 100 bp DNA ladder and Lane 1, 2, 3: *Salmonella* Typhimurium

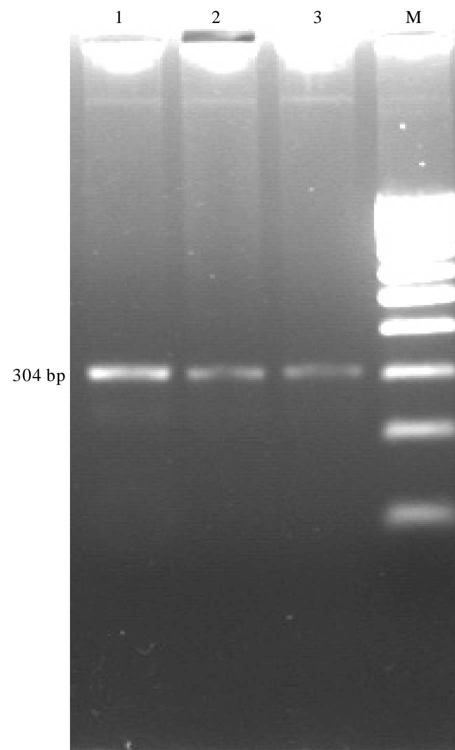

Fig. 3: PCR amplification of *ent* gene, Lane M: 100 bp DNA ladder and Lane 1, 2, 3: *Salmonella* Enteritidis

PCR employing *inv*A gene revealed that, of the 255 blood samples, 22 (8.62%) were positive but only 7 (2.74%) of the 255 faecal samples gave positive amplification. In all 29 samples were positive by PCR, whereas only 26 samples were positive by cultural method.

All the 26 isolates of *Salmonella* were also checked by serotype specific PCR to confirm the presence of *Salmonella* Typhimurium and *Salmonella* Enteritidis. The analysis revealed that the 15 (57.69%) isolates found positive for *Salmonella* Typhimurium by serotyping were also found positive for *typh* gene indicating them to be *Salmonella* Typhimurium. However, 8 (30.76%) isolates were found to be positive for *ent* gene (Table 3) but by serotyping only 4 isolates were found to be Eneritidis and remaining 4 were recognized as rough type. On further probing of these 4 rough isolates, it was found that although they lacked O antigen but all of them were positive for g, m flageller antigen. Interestingly, *Salmonella* Enteritidis serotype also has g, m as flageller antigen.

The results of the PCR were used to estimate diagnostic test-characteristics, viz., sensitivity, specificity, positive predictive value, negative predictive value and concordance (Thrusfield, 2005) with respect to conventional cultural method which was considered as standard method (Table 4).

Diagnostic sensitivity of PCR was 100% and specificity was 97.89% for poultry blood samples. The diagnostic sensitivity from faecal samples was 77.77%, while specificity was found to be 100%. The positive predictive values of PCR from blood and faecal samples were found to be 77.27 and 100% with a concordance of 98.03 and 99.21%, respectively. The negative predictive values from blood and faecal samples were found to be 100 and 99.19% (Table 5).

Table 4: Comparative efficacy of PCR and conventional isolation for the detection of *Salmonella* from poultry blood and faeces samples

	Conventional isolation	
PCR	Positive	Negative
Blood		
Positive (22)	17	5
Negative (233)	0	233
Total (255)	17	238
Faeces		
Positive (7)	7	0
Negative (248)	2	246
Total (255)	9	246

Table 5: Diagnostic test characteristics of PCR from poultry blood and faeces samples

Test characteristics (%)	Blood	Faeces
Sensitivity	100.00	77.77
Specificity	97.89	100.00
Positive predictive value	77.27	100.00
Negative predictive values	100.00	99.19
Concordance	98.03	99.21

DISCUSSION

Salmonellosis has remained a significant public health problem causing food poisoning in humans. Poultry, its products and eggs, represents an important source of *Salmonella* organism for consumer health (Altekruse *et al*., 2006; Kabir, 2010; Barrow *et al*., 2012; Dhama *et al*., 2013). The conventional method of cultivation used in the detection of *Salmonella* is reliable but slow as it includes stages of pre-enrichment, selective enrichment, cultivation in selective agars, biochemical characterisation of suspected isolates and a final serological confirmation (OIE, 2008). Moreover, it may not identify all *Salmonella* infected flocks because of the intermittent nature of *Salmonella* excretion (Hassan *et al*., 1990; Nicholas and Cullen, 1991; Van Zijderveld *et al*., 1992). Skov *et al*. (2002) demonstrated that the number of fecal excretors declined rapidly with time in experimental chickens, down to 6% in 16 weeks for *Salmonella* Typhimurium and down to a similar level within the first 8 weeks for *Salmonella* Enteritidis. The relatively long time required to carry out analysis (4-7 days) as well as labour intensive nature of conventional identification procedure have stimulated the development of faster detection methods. Recent advances in biotechnology and molecular biology have provided molecular detection methods like of Polymerase Chain Reaction (PCR) and its allied versions which have proven to be highly sensitive, specific and provide a rapid and confirmatory diagnosis of *Salmonella* (Kataria *et al*., 2005; Menghistu *et al*., 2011; Batista *et al*., 2013; Sokolov and Sokolov, 2013). The important criteria in the development of a nucleic acid based detection assay for *Salmonella* is the ability to detect all the diverse serotypes of the organism and PCR has been employed to replace conventional serotyping methods. PCR-based serotypings depend on specific virulence genes and have provided high specificity (Jarquin *et al*., 2009).

In this study, PCR method was compared with that of conventional cultural isolation. For the purpose, 255 samples each of poultry blood and faeces, were analysed. *Salmonella* spp. was isolated by conventional cultural methods from 17 (6.66%) blood samples and 9 (3.52%) faecal samples. The low rate of faeces culture positive birds compared to blood was in agreement with the findings by earlier researchers (Hassan *et al*., 1990; Nicholas and Cullen, 1991; Van Zijderveld *et al*., 1992).

The low isolation rate from the faecal samples may be because of the intermittent nature of *Salmonella* excretion (Barrow, 1992). A negative faecal culture result may not necessarily indicate that a bird is not infected (OIE, 2008). In this investigation, no records were available about antibiotic treatments of flocks which may also reduce the likelihood of cultivating *Salmonella* from seropositive birds as reported by Feld *et al.* (2000). Wide variation in prevalence rate of *Salmonella* in poultry has been reported. Peplow *et al.* (1999) reported a *Salmonella* prevalence of 43% from fresh preplacement samples and 61% from fresh preslaughter samples of poultry environmental samples. Freezing of samples resulted in drop of prevalence to 13% for preplacement samples and to 23% for preslaughter samples. Leon-Velarde *et al.* (2004) reported 5.5% *Salmonella* prevalence from poultry house environmental samples. It indicates that the nature and condition of sample plays an important role in recovery of organism.

Kumar (2009) reported a 2% isolation of *Salmonella* from poultry faeces and 1% from chicken samples. This value was lower than the isolation rate obtained in this study. However, Oscar (2004) reported a higher isolation rate of 22.2% from chicken feacal samples. Similarly, about 16-21% prevalence rate of *Salmonella* have been reported from chicken meat (Roberts, 1991; Plummer *et al.*, 1995). The percentage of *Salmonella*-positive birds and feacal samples on farms has ranged from 5 to 100% in U.S. (Carraminana *et al.*, 1997; Bailey *et al.*, 2002). Tapchaisri *et al.* (1999) reported the prevalence rate from chicken samples by 7% with the DNA amplification method and conventional culture method.

The PCR results revealed that 22 (8.62%) blood samples and 7 (2.74%) faecal samples were positive for *inv*A gene. Eyigor *et al.* (2005) reported 5.87 and 4.10% *Salmonella* prevalence rate in Turkey between 2000 and 2001 by real-time polymerase chain reaction and bacteriology, respectively, out of a total of 1242 samples. This prevalence rate was considerably higher than the present findings, especially with reference to faecal samples. This may be due to the presence of inhibitory substances present in the faecal matter or due to the overgrowth of the competitive natural microflora present in the faeces than *Salmonella* (Schrank *et al.*, 2001).

The PCR employed for detection of *Salmonella* from poultry blood samples was found to be 100% sensitive, giving a positive predictive value of 77.27%, when compared with the culture method. The specificity from blood samples was found to be 97.89% by PCR. PCR was found to be 100% specific and positively predictive with regard to faeces samples, giving a concordance of 99.21%. Whereas, the two false negative results given by the PCR test from faeces samples (0.78%) directly influenced the sensitivity (77.77%). One of the major problems in using PCR for detection of pathogenic organisms from clinical and environmental samples is the presence of inhibitory substances to the polymerase reaction (Schrank *et al.*, 2001). The negative predictive values from blood and faecal samples were found to be 100 and 99.19%. Eyigor *et al.* (2007) reported a PCR-ELISA to detect *Salmonella* DNA from selective primary enrichment culture of chicken intestinal samples using *inv*A primers and reported 100% relative sensitivity and specificity when compared to bacteriology. Perelle *et al.* (2004) compared PCR-ELISA and real-time PCR assays for detecting *Salmonella* species in milk and meat samples by amplifying *inv*A gene and reported 100% concordance with the bacteriological reference method. Tapchaisri *et al.* (1999) reported a sensitivity, specificity, efficacy and positive and negative predictive values of 100, 91.58, 92, 65.21 and 100%, respectively, when they compared the DNA amplification and the culture method. Compared to their observation, the present study values were higher, especially with respect to specificity and positive predictive values from both the blood and faeces samples.

Serotyping of the positive isolates in this study revealed 15 isolates (57.69%) of *Salmonella* Typhimurium, 4 isolates (15.38.76%) of *Salmonella* Enteritidis and 5 rough isolates (3.84%) of *Salmonella* rough type. Among *Salmonella* Typhimurium isolates, 73.33% were from poultry blood samples and 26.66% were from poultry faeces samples. *Salmonella* Enteritidis isolates (4) were obtained from blood samples. *Salmonella* Typhimurium has been reported to be most frequently associated with food poisoning, followed by *Salmonella* Enteritidis in Uruguay (Betancor *et al*., 2004, 2010). *Salmonella* Typhimurium was also most frequently identified from human cases in Great Britain (Cook, 2003). The present study also correlate with these reports, where *Salmonella* Typhimurium was the most common. However, Schneid *et al*. (2006) reported 88.6% of *Salmonella* Enteritidis isolates, out of a total of 35 *Salmonella* positive samples. The prevalence of *Salmonella* on chicken farms and colonizing birds varied considerably with the interflock studies. Poppe *et al*. (1992) isolated 45 and 35% of *Salmonella* Enteritidis from pooled feacal samples of two flocks. Culture results of individual organs from the two flocks indicated that the ceca were the predominant site of *Salmonella* Enteritidis infection in birds. Since clusters of *Salmonella* are not evenly distributed within an affected faecal mass, subsamples may not always have *Salmonella* present. This variation could have been reduced by homogenizing the faecal mass before obtaining subsamples (Cannon and Nicholls, 2002). In the present study, the faeces was also collected directly from the caecum and homogenised before using. Croci *et al*. (2004) also reported the presence of various *Salmonella* serotypes, viz., *Salmonella* Enteritidis, *Salmonella* Give and *Salmonella* Newrochelle from poultry samples. Eyigor *et al*. (2005) reported *Salmonella* Enteritidis as dominant *Salmonella* serovar from poultry and environmental samples.

On PCR analysis of 26 isolates for Typhimurium and Enteritidis specific genes (*typh* and *ent*, respectively), it was observed that 15 Typhimurium isolates were positive for *typh* gene. However, four of the rough isolates showed the presence of *ent* gene, indicating them to be *Salmonella* Enteritidis. Interestingly, these four rough isolated possessed g, m flagller factor. The rough strains lack immunoreactive O-chain (Guard-Petter *et al*., 1999) which may happen due to mutagenesis of the O-antigen gene cluster involved in O-antigen synthesis (Ochoa-Reparaz *et al*., 2005). Thus, serotype specific PCR has advantage in identifying serotypes among rough strains.

CONCLUSION

It may be concluded from the study that PCR is reliable and fast technique in detecting *Salmonella* in blood and poultry samples and thus can be used in prevalence studies.

REFERENCES

Agarwal, R.K., K.N. Bhilegaonkar, D.K. Singh, A. Kumar and R.S. Rathore, 2003. Laboratory Manual for the Isolation and Identification of Foodborne Pathogens. Indian Veterinary Research Institute, Izatnagar, Bareilly, India, pp: 35-37.

Altekruse, S.F., N. Bauer, A. Chanlongbutra, R. DeSagun and A. Naugle *et al*., 2006. *Salmonella* Enteritidis in broiler chickens, United States, 2000-2005. Emerg. Infect. Dis., 12: 1848-1852.

Alvarez, J., M. Sota, A.B. Vivanco, I. Perales, R. Cisterna, A. Rementeria and J. Garaizar, 2004. Development of a multiplex PCR technique for detection and epidemiological typing of *Salmonella* in human clinical samples. J. Clin. Microbiol., 42: 1734-1738.

Bailey, J.S., N.A. Cox, S.E. Craven and D.E. Cosby, 2002. Serotype tracking of *Salmonella* through integrated broiler chicken operations. J. Food Prot., 65: 742-745.

Barrow, C.J. and R.K.A. Feltham, 1993. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd Edn., Cambridge Press, London Pages: 238.

Barrow, P.A., 1992. Further observations on the serological response to experimental *Salmonella* Typhimurium in chickens measured by ELISA. Epidemiol. Infect., 108: 231-242.

Barrow, P.A., M.A. Jones, A.L. Smith and P. Wigley, 2012. The long view: *Salmonella*-the last forty years. Avian Pathol., 41: 413-420.

Batista, D.F.A., O.C. de Freitas Neto, P.D. Lopes, A.M. de Almeida, P.A. Barrow and A. Berchieri Jr., 2013. Polymerase chain reaction assay based on *ratA* gene allows differentiation between *Salmonella enterica* subsp. *enterica* serovar Gallinarum biovars Gallinarum and Pullorum. J. Vet. Diagn. Invest., 25: 259-262.

Betancor, L., F. Schelotto, A. Martinez, M. Pereira and G. Algorta *et al.*, 2004. Random amplified polymorphic DNA and phenotyping analysis of *Salmonella enterica* serovar enteritidis isolates collected from humans and poultry in Uruguay from 1995 to 2002. J. Clin. Microbiol., 42: 1155-1162.

Betancor, L., M. Pereira, A. Martinez, G. Giossa and M. Fookes *et al.*, 2010. Prevalence of *Salmonella enterica* in poultry and eggs in Uruguay during an epidemic due to *Salmonella enterica* serovar enteritidis. J. Clin. Microbiol., 48: 2413-2423.

Braden, C.R., 2006. *Salmonella enterica* serotype enteritidis and eggs: A national epidemic in the United States. Clin. Infect. Dis., 43: 512-517.

CDC, 2007. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food-10 States, 2006. Morbidity Mortality Weekly Rep., 56: 336-339.

CDC, 2009. Multistate outbreak of salmonella infections associated with peanut butter and peanut butter-containing products-United States, 2008-2009. Morbidity Mortality Weekly Rep., 58: 85-90.

Cannon, R.M. and T.J. Nicholls, 2002. Relationship between sample weight, homogeneity and sensitivity of fecal culture for *Salmonella enterica*. J. Vet. Diagn. Invest., 14: 60-62.

Carraminana, J.J., J. Yanguela, D. Blanco, C. Rota, A.I. Agustin, A. Arino and A. Herrera, 1997. *Salmonella* incidence and distribution of serotypes throughout processing in a Spanish poultry slaughterhouse. J. Food Prot., 60: 1312-1317.

Cook, N., 2003. The use of NASBA for the detection of microbial pathogens in food and environmental samples. J. Microbiol. Methods, 53: 165-174.

Croci, L., E. Delibato, G. Volpe, D. de Medici and G. Palleschi, 2004. Comparison of PCR, electrochemical enzyme-linked immunosorbent assays and the standard culture method for detecting *Salmonella* in meat products. Applied Environ. Microbiol., 70: 1393-1396.

Daniels, N.A., L. Mackinnon, S.M. Rowe, N.H. Bean, P.M. Griffin and P.S. Mead, 2002. Foodborne disease outbreaks in United States schools. Pediatr. Infect. Dis. J., 21: 623-638.

Dey, S., C.M. Madhan, J.M. Kataria and K. Dhama, 2005. Common disease conditions of ducks. Poult. World, 1: 19-25.

Dhama, K., M. Mahendran and S. Tomar, 2008a. Pathogens transmitted by migratory birds: Threat perceptions to poultry health and production. Int. J. Poult. Sci., 7: 516-525.

Dhama, K., M. Mahendran and S. Tomar, 2008b. Poultry health care and management strategies for socio-economic development of rural farmers. Poult. World, 2: 24-29.

Dhama, K., S. Rajagunalan, S. Chakraborty, A.K. Verma, A. Kumar, R. Tiwari and S. Kapoor, 2013. Food-borne pathogens of animal origin-diagnosis, prevention, control and their zoonotic significance: A review. Pak. J. Biol. Sci., 16: 1076-1085.

Erol, I., M. Goncuoglu, N.D. Ayaz, L. Ellerbroek, F.S.B. Ormanci and O.I. Kangal, 2013. Serotype distribution of *Salmonella* isolates from Turkey ground meat and meat parts. BioMed Res. Int., Vol. 2013. 10.1155/2013/281591

Eyigor, A., G. Goncagul, E. Gunaydin and K.T. Carli, 2005. *Salmonella* profile in chickens determined by real-time polymerase chain reaction and bacteriology from years 2000 to 2003 in Turkey. Avian Pathol., 34: 101-105.

Eyigor, A., G. Goncagul and T. Carli, 2007. A PCR-ELISA for the detection of *Salmonella* from chicken intestine. J. Biol. Environ. Sci., 1: 45-49.

Feld, N.C., L. Ekeroth, K.O. Gradel, S. Kabell and M. Madsen, 2000. Evaluation of a serological *Salmonella* Mix-ELISA for poultry used in a national surveillance programme. Epidemiol. Infect., 125: 263-268.

Galan, J.E., C. Ginocchio and P. Costeas, 1992. Molecular and functional characterization of the *Salmonella* invasion gene i3vA: Homology of invA to members of a new protein family. J. Bacteriol., 174: 4338-4349.

Guard-Petter, J., C.T. Parker, K. Asokan and R.W. Carlson, 1999. Clinical and veterinary isolates of *Salmonella enterica* Serovar enteritidis defective in lipopolysaccharide O-Chain polymerization. Applied Environ. Microbiol., 65: 2195-2201.

Hammack, T.S., I.E. Valentin-Bon, A.P. Jacobson and W.H. Andrews, 2004. Relative effectiveness of the bacteriological analytical manual method for the recovery of *Salmonella* from whole cantaloupes and cantaloupe rinses with selected preenrichment media and rapid methods. J. Food. Prot., 67: 870-877.

Hassan, J.O., P.A. Barrow, A.P.A. Mockett and S. McLeod, 1990. Antibody response to experimental *Salmonella* Typhimurium infection in chickens measured by ELISA. Vet. Rec., 126: 519-522.

Jarquin, R., I. Hanning, S. Ahn and S.C. Ricke, 2009. Development of rapid detection and genetic characterization of *Salmonella* in poultry breeder feeds. Sensors, 9: 5308-5323.

Kabir, S.M.L., 2010. Avian colibacillosis and salmonellosis: A closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. Int. J. Environ. Res. Public Health, 7: 89-114.

Kataria, J.M., C.M. Mohan, S. Dey, B.B. Dash and K. Dhama, 2005. Diagnosis and immunoprophylaxis of economically important poultry diseases: A review. Indian J. Anim. Sci., 75: 555-567.

Kumar, K., 2009. PCR based detection of zoonotic *Salmonella* from foods. Ph.D. Thesis, H.N.B. Garhwal Central University, India.

Leon-Velarde, C.G., H.Y. Cai, C. larkin, P. Bell-Rogers, R.W. Stevens and J.A. Odumeru, 2004. Evaluation of methods for the identification of *Salmonella enterica* serotype Typhimurium DT104 from poultry environmental samples. J. Microbiol. Methods, 58: 79-86.

Linam, W.M. and M.A. Gerber, 2007. Changing epidemiology and prevention of *Salmonella* infections. Pediatric Infect. Dis. J., 26: 747-748.

Mead, P.S., L. Slutsker, V. Dietz, L.F. McCaig and J.S. Bresee *et al.*, 1999. Food-related illness and death in the United States. Emerg. Infect. Dis., 5: 607-625.

Menghistu, H.T., R. Rathore, K. Dhama and R.K. Agarwal, 2011. Isolation, Identification and Polymerase Chain Reaction (PCR) Detection of Salmonella species from field materials of poultry origin. Int. J. Microbiol. Res., 2: 135-142.

Myint, M.S., 2004. Epidemiology of *Salmonella* contamination of poultry products; Knowledge gaps in the farm to store products. Ph.D. Thesis, Faculty of the Graduate School of the University of Maryland.

Nicholas, R.A. and G.A. Cullen, 1991. Development and application of an ELISA for detecting antibodies to *Salmonella* Enteritidis in chicken flocks. Vet. Rec., 128: 74-76.

O'Regan, E., E. McCabe, C. Burgess, S. McGuinness and T. Barry *et al*., 2008. Development of a real-time multiplex PCR assay for the detection of multiple *Salmonella* serotypes in chicken samples. BMC Microbiol., Vol. 8. 10.1186/1471-2180-8-156

OIE, 2008. Salmonellosis. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, OIE (Ed.). 6th Edn., OIE., Paris, pp: 1267-1283.

Ochoa-Reparaz, J., B. Garcia, C. Solano, I. Lasa, J.M. Irache and C. Gamazo, 2005. Protective ability of subcellular extracts from *Salmonella* Enteritidis and from a rough isogenic mutant against salmonellosis in mice. Vaccine, 23: 1491-1501.

Oscar, T.P., 2004. A quantitative risk assessment model for *Salmonella* and whole chickens. Int. J. Food Microbiol., 93: 231-247.

Peplow, M.O., M. Correa-Prisant, M.E. Stebbins, F. Jones and P. Davies, 1999. Sensitivity, specificity and predictive values of three *Salmonella* rapid detection kits using fresh and frozen poultry environmental samples versus those of standard plating. Applied Environ. Microbial., 65: 1055-1060.

Perelle, S., F. Dilasser, B. Malorny, J. Grout, J. Hoorfar and P. Fach, 2004. Comparison of PCR-ELISA and Light Cycler real-time PCR assays for detecting *Salmonella* spp. in milk and meat samples. Mol. Cell. Probes, 18: 409-420.

Plummer, R.A.S., S.T. Blissett and C.E.R. Dodd, 1995. *Salmonella* contamination of retail chicken products sold in the UK. J. Food Prot., 58: 843-846.

Poppe, C., R.P. Johnson, C.M. Forsberg and R.J. Irwin, 1992. *Salmonella* enteritidis and other *Salmonella* in laying hens and eggs from flocks with *Salmonella* in their environment. Can. J. Vet. Res., 56: 226-232.

Roberts, D., 1991. Source of Infection: Food. In: *Foodborne illness*: A Lancet Review, Waites, W.M. and J.P. Arbuthnott (Eds.). Edward Arnold Publishing, London, pp: 31-37.

Schneid, A.S., K.L. Rodrigues, D. Chemello, E.C. Tondo, M.A.Z. Ayub and J.A.G Aleixo, 2006. Evaluation of an indirect ELISA for the detection of *Salmonella* in chicken meat. Braz. J. Microbiol., 37: 350-355.

Schrank, I.S., M.A.Z. Mores, J.L.A. Costa, A.P.G. Frazzon and R. Sonicini *et al*., 2001. Influence of enrichment media and application of a PCR based method to detect *Salmonella* in poultry industry products and clinical samples. Vet. Microbiol., 82: 45-53.

Skov, M.N., N.C. Feld, B. Carstensen and M. Madsen, 2002. The serologic response to *Salmonella* Enteritidis and *Salmonella* Typhimurium in experimentally infected chickens, followed by an indirect lipopolysaccharide enzyme-linked immunosorbent assay and bacteriologic examinations through a one-year period. Avian Dis., 46: 265-273.

Sokolov, D.M. and M.S. Sokolov, 2013. [Rapid methods for the genus *Salmonella* bacteria detection in food and raw materials]. Voprosy Pitaniia, 82: 33-40, (In Russian).

Tapchaisri, P., P. Wangroongsarb, W. Panbangred, T. Kalambaheti and M. Chongsa-Nguan *et al*., 1999. Detection of *Salmonella* contamination in food samples by dot-ELISA, DNA amplification and bacterial culture. Asian Pac. J. Allergy Immunol., 17: 41-51.

Thrusfield, M., 2005. Veterinary Epidemiology. 3rd Edn., Blackwell Science Ltd., London, pp: 158-329.

Van Zijderveld, F.G., A.M. van Zijderveld-Van Bemmel and J. Anakotta, 1992. Comparison of four different enzyme-linked immunosorbent assays for serological diagnosis of *Salmonella* Enteritidis infections in experimentally infected chickens. J. Clin. Microbiol., 30: 2560-2566.