African Journal of Biotechnology Vol. 11(14), pp. 3452-3458, 16 February, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1353
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Rapid detection and characterization of *Salmonella enterica* serovars by multiplex polymerase chain reaction assay

Moussa, I. M.[1]*, Ashgan MH[2,5], Mahmoud MH[3] and Al-Doss, A. A.[4]

[1]Center of Excellence in Biotechnology, King Saud University, P. O. 2460 Riyadh, King Saudi Arabia.
[2]College of Applied Studies and Community Service, King Saud University, Riyadh, King Saudi Arabia.
[3]Deanship of Scientific Research, King Saud University, Riyadh, King Saudi Arabia.
[4]College of Food and Agriculture Research, King Saud University, Riyadh, King Saudi Arabia.
[5]College of Veterinary Medicine, Cairo University, Egypt.

Accepted 20 January, 2012

Multiplex polymerase chain reaction (PCR) was used for molecular typing of *Salmonella enterica* serovars in Egypt. During the summer of 2010, a total of 1075 samples were collected from cattle, sheep and poultry farms to be subjected for isolation of *Salmonella* (290 rectal swabs from cattle, 335 rectal swabs from sheep and 450 cloacal swabs from poultry). Bacteriological examination revealed the isolation of 68 *Salmonella* belonging to 13 different *Salmonella* serovars. The most common serovars were *Salmonella* Typhimurium (16 isolates), *Salmonella* Enteritidis (13 isolates), *Salmonella* Kentucky (eight isolates) and *Salmonella* Arizona (seven isolates). Other serovars typed were *Salmonella* Heidelberg (four), *Salmonella* Cerro (four), *Salmonella* Gallinarum (three), *Salmonella* Virginia (three), *Salmonella* Paratyphi-*A* (three), *Salmonella* Dublin (two), *Salmonella* Agona (two), *Salmonella* Hadar (two) and *Salmonella* Bardo (one). The results of molecular techniques highlight the usefulness of the multiplex PCR for the rapid detection of the two serotypes of *Salmonella* from field samples especially after pre-enrichment on Rappaport-Vassiliadis (RV) media. Moreover, detecting *S.* Typhimurium and *S.* Enteritidis by this assay was carried out within two days as opposed to five to six days by the bacteriological and serological methods.

Key words: Multiplex polymerase chain reaction (PCR), *Salmonella* Enteritidis, *Salmonella* Typhimurium, Egypt.

INTRODUCTION

Salmonellosis is one of the major foodborne diseases. Due to its endemic nature, high morbidity and association with a wide range of foods, this zoonotic disease is of high public health concern (Aarestrup et al., 2007; Alizadeh et al., 2007; Kottwitz et al., 2008).

Salmonella infection has been associated with the consumption of raw and undercooked poultry and other meat products; however, many outbreaks have also been associated with contaminated fresh fruits and vegetables (Gallegos-Robles et al., 2008). *Salmonella* infections occur worldwide in both developed and developing countries and are a major contributor to morbidity and economic costs (Antoine et al., 2008).

Salmonella Typhimurium and *Salmonella* Enteritidis are the most frequently isolated serovars from foodborne outbreaks throughout the world (Herikstad et al., 2002). *S.* Enteritidis is a major cause of foodborne diseases (Brenner et al., 2000), and during the last two decades, it has been isolated worldwide in increasing numbers (Rodrigue et al., 1990; Herikstad et al., 2002).

Salmonella is detected by standard bacteriological, biochemical and serological tests. These tests are generally time-consuming, tedious and costly as they require hundreds of antisera as well as well-trained technicians (Echeita et al., 2002; Nori and Thong, 2010).

Several rapid and sensitive methods have been

*Corresponding author. E-mail: imoussa1@ksu.edu.sa. or moussaihab@gmail.com. Tel: 00966560749553. Fax: 00966 14678456.

Table 1. Reference standard strains used for evaluation of the specificity of PCR.

No.	Bacterial species	Source
1	*Salmonella* Typhimurium	ATCC-14028
2	*Salmonella* Typhimurium	NCIMB-50076
3	*Salmonella typhi*	ATCC-9992
4	*Salmonella* Heidelberg	WHO
5	*Salmonella* Enteritidis	WHO***
6	*Salmonella* Enteritidis	ATCC**-13076
7	*Escherichia coli* (O157:H7)	ATCC-35150
8	*Escherichia coli*	NCIMB*-50034
9	*Enterococcus fecalis*	NCIMB-50029

*NCIMB, National Collection for Industerial and Marine Bacteria; **ATCC, American Type Culture Collection; ***WHO, World Health Organization. PCR, Polymerase chain reaction.

developed for identification of *Salmonella* serovars from clinical samples (Zahraei et al., 2007). These methods, however, still lack the necessary sensitivity and specificity (Widjojoatmodjo et al., 1992; Aabo et al., 1993). *In vitro* amplification of DNA by the polymerase chain reaction (PCR) method is a powerful tool in microbiological diagnostics (Malorny et al., 2003). Multiplex PCR provides us with a specific method and superior ability to detect *Salmonella enterica* and the serovar *S.* Enteritidis and/or *S.* Typhimurium in the presence of other bacteria simultaneously (Malkawi and Gharaibeh, 2004; Yan and Sekaran, 2010). In this method, several genes were used to detect *Salmonella* genus or serovars including virulent chromosomal genes such as *invA* (Malorny et al., 2003; Zahraei et al., 2007), and plasmid genes such as *spv* (Soumet et al., 1999). Therefore, the objective of the present study was to study the incidences of *Salmonella* species from different sources collected from Egypt on molecular basis.

MATERIALS AND METHODS

Bacteria and reagents

The bacterial reference strains used in this study are illustrated in Table 1. The materials, chemicals and reagents used in this study were purchased from Sigma Chemicals Co. (St. Louis, MO. USA) unless otherwise specified. PCR reagents were purchased from Promega (Madison, WI, USA).

Samples

During the summer of 2010, a total of 1075 samples were collected from cattle, sheep and poultry farms to be subjected for isolation of *Salmonella* (290 rectal swabs from cattle, 335 rectal swabs from sheep and 450 cloacal swabs from poultry).

Isolation and identification of *Salmonella*

For the isolation and identification of *Salmonella*, the technique was recommended by the International Organization for Standardization (ISO) 6579 (1998). Briefly, each sample was enriched by inoculation in 225 ml of buffered peptone water (BPW), after incubation at 37°C for 16 to 20 h, 0.1 ml was inoculated into a tube containing 10 ml of Rappaport-Vassiliadis (RV) magnesium chloride-malachite green broth (Oxoid, England) and was incubated at 42°C for 18 to 24 h. Another 1 ml from same pre-enrichment culture was inoculated into 10 ml of selenite cystine (SC) broth (Difco, USA) and was incubated at 37°C for 18 to 24 h. Each selective enrichment broths were streaked onto brilliant green-phenol red-lactose-sucrose (BPLS) agar (Merck, Darmstadt, Germany) and xylose lysine deoxycholate (XLD) agar (Merck, Darmstadt, Germany). Presumptive positive colonies (non lactose fermentative with suitable colony morphology) were identified morphologically, biochemically and serologically by slide agglutination test using polyvalent and monovalent somatic (O), virulence (Vi) and tube agglutination test for flageller (H) antigens (Difco Laboratories, Detroit, Michigan, USA) and enzyme linked immuno-sorbant assay (ELISA) (Reveal Salmonella test kits systems, Neogen Corporation). 1 ml of BPW which had been incubated at 37°C was saved for the PCR-non selective test (PCR-NS) and 1 ml of the 37°C RV broth for the PCR-RV test.

Extraction of DNA

The standard and bacteriollogically positive strains were grown in 10 ml tryptic soya broth (TSB) at 37°C for 24 h. The overnight cultures were centrifuged at 3000 rpm for 5 min and the supernatant were decanted carefully. The bacterial pellets were washed three times with phosphate buffer saline pH 7.2 and resuspended in 400 µl Tris- ethylene diaminetetra acetic acid (EDTA buffer (pH 8.0) and heated in water bath at 100°C for 20

Table 2. Primers used for molecular identification of *Salmonella* species.

Target sequence	Primer set	Primer sequence	Size
Random sequence	ST11	GCCAACCATTGCTAAATTGGCGCA	429
	ST15	GGTAGAAATTCCCAGCGGGTACTGG	
fliC gene	Fli15	CGGTGTTGCCCAGGTTGGTAAT	559
	Tym	ACTCTTGCTGGCGGTGCGACTT	
sefA gene	Sef167	AGGTTCAGGCAGCGGTTACT	312
	Sef478	GGGACATTTAGCGTTTCTTG	

min. There were left to cool at room temperature and centrifuged at 14,000 rpm for 10 min. An aliquot of 5 µl of the supernatant was used as template DNA in the PCR. While the extraction of DNA from the field samples enriched in RV broth was carried out by the same method reported by Oliveira et al. (2003).

Polymerase chain reaction

Oligonucleotide primers

For multiplex PCR, three primer pairs were tested. ST11 to ST15 primers were selected from a randomly cloned chromosomal fragment and have been shown to be specific for *Salmonella* species (Aabo et al., 1993; Soumet et al., 1999). Sef167 to Sef478 primers were chosen from the fimbrial antigen SEF14 encoded by the *sefA* gene (Turcotte and Woodward, 1993; Soumet et al., 1999) and are specific for *S.* Enteritidis. The occurrence of this gene sequence among other *Salmonella* serotypes is restricted to the serotypes Enteritidis, Blegdam, Dublin, Gallinarum, Pullorum, Rostock, Seremban and Typhi (Turcotte and Woodward, 1993; Soumet et al., 1999). For *S.* Typhimurium, Fli15-Tym primers were selected from the *fliC* gene sequence (Joys, 1985, Soumet et al., 1999) encoding flagellin H1. All primer sequences used in this study are given in Table 2.

DNA amplification

PCR amplifications were performed in a final volume of 50 µl in micro-amplification tubes (PCR tubes). The reaction mixtures consisted of 5 µl of the DNA template, 5 µl 10x PCR buffer (75 mM Tris-HCl, pH 9.0, 2 mM MgCl2, 50 mM KCl, 20 mM (NH4)2SO4), 1 µl dNTPs (40µM), 1 µl (1U Ampli Taq DNA polymerase), 1 µl (25 pmol) from the forward and reverse primers of both primer pairs and the volume of the reaction mixture was completed to 50 µl using deionised distilled water (DDW). The thermal cycler was adjusted as follows: Initial denaturation at 94°C for 5 min, followed by 35 cycles of (denaturation at 94°C for 1 min, annealing at 56°C for 1 min. and extension at 72°C for 1 min). Final extension was carried out at 72°C for 10 min and the PCR products were stored in the thermal cycler at 4°C until they were collected.

Agarose gel electrophoresis

The PCR products were tested for positive amplification by agarose gel electrophoresis previously reported by Sambrook et al. (1989) using suitable molecular weight markers.

RESULTS

Bacteriological examination

Bacteriological examination of 1075 samples (290 rectal swabs from cattle, 335 rectal swabs from sheep and 450 cloacal swabs from poultry) from apparently health animals showed that 68 samples harbored various *Salmonella* serovars. As shown in Table 3, it is clear that 6.3% of the samples were positive for *Salmonella*. The highest rate of isolation 8% was obtained from cloacal swabs from poultry, followed by rectal swabs from sheep 5.7% and finally the rectal swabs from cattle 4.5%.

As shown in Tables 3 and 4, it is evident that five serovars were isolated from cattle, six from sheep and eight from poultry. The cattle *Salmonella i*solates were *S.* Typhimurium and *S.* Enteritidis (four each), *Salmonella* Agona and *Salmonella* Dublin (two each) and *Salmonella* Paratyphi A (one isolate). The sheep isolates were typed as *S.* Typhimurium (five), *S.* Enteritidis (four), *Salmonella* Heidelberg (four), *Salmonella* Kentucky (three), *S.* Paratyphi A (two) and *Salmonella* Bardo (one). The most common serovars of poultry were *S.* Typhimurium and *Salmonella* Arizona (seven isolates each), followed by *S.* Enteritidis and *S.* Kentucky (five isolates each), *Salmonella* Cerro (four), *Salmonella* Virginia and *Salmonella* Gallinarum (3 each) and *Salmonella* Hadar (2).

The results presented in Table 4 and Figures 1 and 2 reveal the incidence of *S.* Enteritidis and *S.* Typhimurium in cattle, sheep and poultry. It is evident that *S.* Typhimurium was more frequent 23.5% than *S.* Enteritidis 20.6% among the total *Salmonella* isolates. On the other hand, the rate of *S.* Enteritidis was higher 38.5% than that of *S.* Typhimurium 30.8% in cattle, while *S.* Typhimurium was more common than *S.* Enteritidis in sheep and poultry 26.3 and19.4% versus 21.1 and 13.8%, respectively.

Table 3. *Salmonella* serovars isolated from different sources.

Animal species	Sample			Salmonella isolate		
	No. tested	No. positive	%	Serovar	No.	%
Cattle	290	13	4.4	Typhimurium	4	30.7
				Enteritidis	4	30.7
				Agona	2	15.3
				Dublin	2	15.3
				Paratyphi *A*	1	07.6
Sheep	335	19	5.6	Typhimurium	5	26.3
				Enteritidis	4	21.0
				Heidelberg	4	21.0
				Kentucky	3	15.7
				Paratyphi *A*	2	10.5
				Bardo	1	5.2
Poultry	450	36	8	Typhimurium	7	19.4
				Arizona	7	19.4
				Enteritidis	5	13.8
				Kentucky	5	13.8
				Cerro	4	11.1
				Verginia	3	08.3
				Gallinarum	3	08.3
				Hadar	2	05.5
Total	1075	68	6.3	-	-	-

Table 4. Incidence of *Salmonella* Enteritidis and *Salmonella* Typhimurium.

Species	No. of isolate	Incidence of *Salmonella* Enteritidis		Incidence of *Salmonella* Typhimurium	
		No.	%	No.	%
Cattle	13	5	38.5	4	30.8
Sheep	19	4	21.1	5	26.3
Poultry	36	5	13.8	7	19.4
Total	68	14	20.6	16	23.5

Molecular typing using polymerase chain reaction (PCR)

The specificity of the oligonucleotid primers were carried out by testing all the recovered *Salmonella* strains in addition to the standard positive and standard negative strains with PCR using the primer pairs (ST11 and ST15) targeting a randomly cloned chromosomal fragment previously identified as a specific fragments for *Salmonella* species. Moreover, Fli15 and Tym primer pair targeting *fliC* gene specific for *S.* Typhimurium and Sef167-Sef478 primer pair encoded by the *sefA* gene and specific for *S.* Enteritidis will be used for molecular characterization of both *S. Typhimurium* and *S. Enteritidis.* All the examined field samples with bacteriological examination were tested by PCR using the same primer pair after selective enrichment on RV broth.

All *Salmonella* serovars were positive for amplification of 429 bp fragments, while all non *Salmonella* serovars were negative (Figure 1). Positive samples for *S.* Typhimurium showed amplification of 429 and 559 bp fragments, while all the bacteriologically positive samples for *S.* Enteritidis showed amplification of 429 and 312 bp

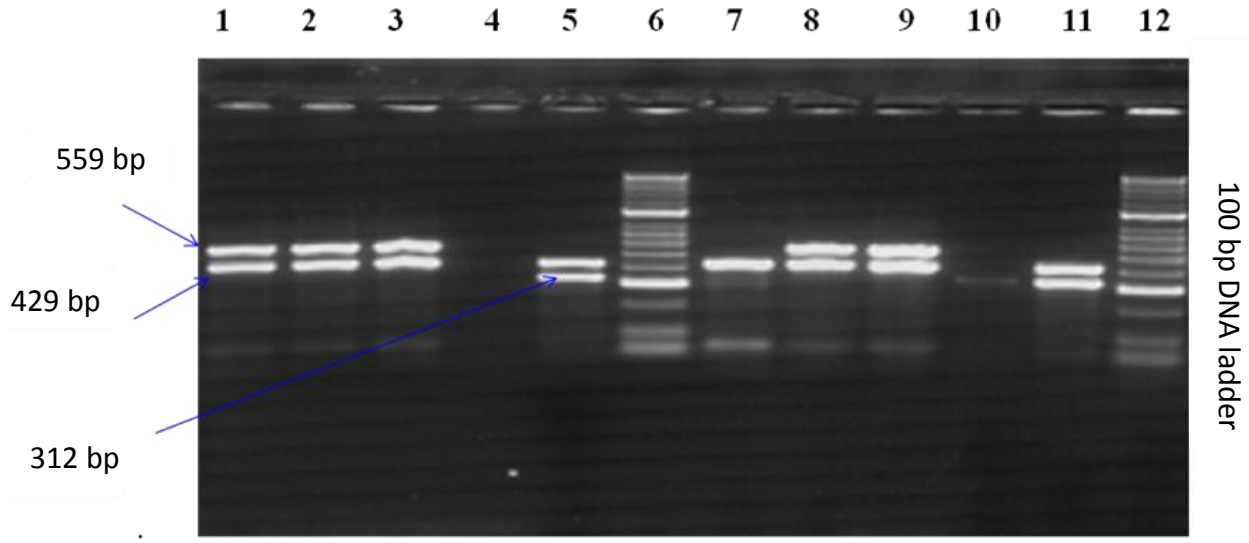

Figure 1. Agarose gel electrophoresis showing amplification of 429 bp fragments of *Salmonella* species, 312 bp fragments of *Salmonella enteriditis* and 559 bp fragments of *Salmonella typhimyrium*.

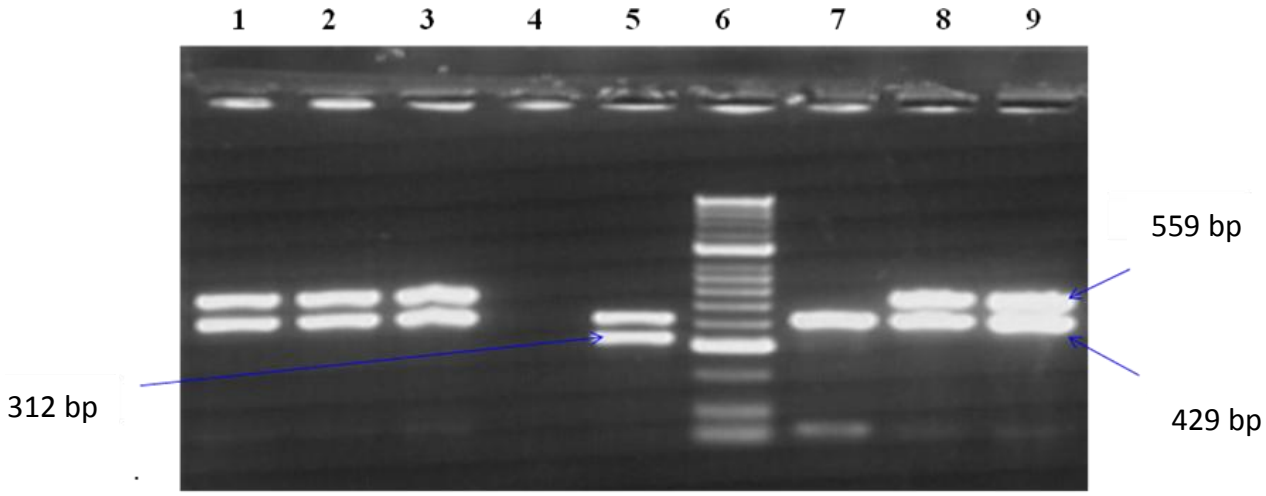

Figure 2. Multiplex PCR showing amplification of 429 and 312 bp fragments of *Salmonella enteriditis* in lane 5 while lanes 1, 2, 3, 8 and 9 showing amplification of 429 and 559 bp fragments of *Salmonella typhimyrium*. Lane 7 showing amplification 429 bp fragments of *Salmonella* species.

fragments as shown in Figures 1 and 2.

DISCUSSION

Salmonella infections occur worldwide in both developed and developing countries and are a major contributor to morbidity and economic costs (Antoine et al., 2008). The contamination of food products with *Salmonella* generates serious health and economic consequences, which have stimulated numerous studies designed to investigate the survival capacity and the transmission routes of these organisms in different farm animals and the environment (Winfield and Groisman, 2003). *Salmonella* is frequently isolated from environmental sources that serve as a relay for the bacteria and play a major role in its spread between different hosts (Liljebjelke et al., 2005).

In the present work, bacteriological examination of 1075 samples (290 rectal swabs from cattle, 335 rectal swabs from sheep and 450 cloacal swabs from poultry) from apparently healthy animals show that 68 samples harbored various *Salmonella* species. The highest rate of isolation 8% was obtained from cloacal swabs in poultry, followed by rectal swabs in sheep 5.6% and finally the rectal swabs in cattle 4.4%.

Similar results have been reported in Egypt (Moussa et al., 2010) and other countries of the world (Davies et al., 2004; Threlfal et al., 2006; Nunes et al., 2010).

The obtained percentage was lower when compared with that recorded by Cortez et al. (2006), who reported that the percentage of positive *Salmonella* samples was 10%. Differences in prevalence may be the result of using different sample types, or different methods for detection of *Salmonellae*. The incidence of various *Salmonella* species seems to vary with geographic location and the types of food consumed.

The results reveal that *S.* Typhimurium (23.5%) and *S.* Enteritidis (20.6%) were most frequent among the total *Salmonella* isolates. This finding substantiates the report of the CDC (2003), which indicated that the two leading *S. enterica* serovars that cause *Salmonellosis* in the United States are *S.* Typhimurium and *S.* Enteritidis (22 and 17% of all *Salmonella* infections) and Smith-Palmer et al. (2003) who concluded that the two serovars of *Salmonella*, which were currently of particular importance in both human and animal infection in Scotland were *S.* Enteritidis and *S.* Typhimurium. *Salmonella* is detected by standard bacteriological, biochemical and serological tests. These tests are generally time-consuming, tedious and costly as they require hundreds of antisera as well as well-trained technicians (Echeita et al., 2002; Nori and Thong, 2010). Therefore, the current studies were aimed to utilize multiplex PCR for identification of *S.* Enteritidis and *S.* Typhimurium. The result denote that positive samples for *S.* Typhimurium showed amplification of 429 and 559 bp fragments, while all the bacteriologically positive samples for *S.* Enteritidis showed amplification of 429 and 312 bp fragments as shown in Figures 1 and 2. These results highlight the usefulness of the multiplex PCR for the rapid detection of the two serotypes of *Salmonella* from field samples especially after pre-enrichment on RV media. Moreover, detecting *S.* Typhimurium and *S.* Enteritidis by this assay was carried out within two days opposed to five to six days by the bacteriological and serological methods.

ACKNOWLEDGMENT

This work was supported by the National Plane for Science and Technology Program, Vice Rectorate for Graduate Studies and Scientific Research, King Saud University, 10-AGR1316-2.

REFERENCES

Aabo SOR, Rasmussen PD, Olson JE (1993). *Salmonella* identification by polymerase chain reaction. Mol. Cell. Probes, 7: 171-178.

Aarestrup FM, Rene S, Hendriksen J L, Katie G, Kathryn T, Patrick F. McDermott F J, Peter GS (2007). International spread of multidrug-resistant *Salmonella* Schwarzengrund in food products. Emerging Infectious Diseases, 13 (5): 726-731.

Alizadeh AH, Behrouz N, Salmanzadeh S, Ranjbar M, Azimian MH, Habibi E, Jaafari F, Zolfagharian K, Zali MR (2007). *Escherichia coli*, *Shigella* and *Salmonella* species in acute diarrhoea in Hamedan, Islamic Republic of Iran. East. Mediter. Hlth. J. 13. (2): 243-249.

Antoine ST, Annaelle K, Anne B (2008).Epidemiological analysis of *Salmonella* enterica from beef sampled in the slaughter house and retailers in Dakar (Senegal) using pulsed-field gel electrophoresis and antibiotic susceptibility testing.J. Food Microbiol. 123: 191-197.

Brenner FW, Villar RG, Angulo FJ, Tauxe R, Swaminathan B (2000): *Salmonella* nomenclature. Clin. Microbiol., 38: 2465-2467.

Centers of Disease Control and Prevention “CDC” (2003): *Salmonella*-annual summary 2002. Centers for Disease Control and Prevention, Atlanta, Ga.

Cortez AL, Carvalho AB, Ikuno AA, Bu¨rger KP,Vidal-Martins AC (2006).Identification of *Salmonella* spp. isolates from chicken abattoirs by multiplex-PCR. Res. Vet. Sci. 81: 340-344.

Davies RH, Dalziel R, Gibbens JC, Wilesmith JW, Rany JM, Evans SJ, Teale CJ (2004) : National survey for *Salmonella* in pigs , cattle and sheep at slaughter in Great Britainia. Veterinary Laboratories Agency (VLA) Weybridge Newhow Addestone surrey, KT 15 3 N B ,uk .

Echeita MA, Herrera S, Garaizar J, Usera MA (2002).Multiplex PCR based detection an identification of the most common *Salmonella* second-phase flagellar antigen. Res. Microbiol. 153: 107-113.

Gallegos-Robles M, Loredo A, Ojeda G, Vega A, Chew Y, Velarde S, Fratamico P (2008).Identification of *Salmonella* serovars Isolated from Cantaloupe and Chile Pepper Production Systems in Mexico by PCR-Restriction Fragment Length Polymorphism. J. Food Protect. 71 (11): 2217-2222.

Herikstad H, Motarjemi Y,Tauxe RV (2002).*Salmonella* surveillance: a global survey of public health serotyping. Epidemiol. Infect. 129: 1-8.

Joys TM (1985). The covalent structure of the phase-1 flagellar filament protein of *Salmonella* Typhimurium and its comparison with others flagellins. J. Biol. Chem. 260: 15758-15761.

Kottwitz LB M, Back A, Leão JA, Alcocer I, Karan M, Oliveira TM (2008).Contaminação por *Salmonella* spp. emuma cadeia de produção de ovos de uma integração de postura commercial. Arquivos Brasileiro de Med. Vet. Zootecnia, 60 (2): 496-498.

Liljebjelke KA, Hofrace C, Liu T, White D, Ayers S,Young S, Maurer J (2005). Vertical and horizontal transmission of *Salmonella* within integrated broiler production system.J Foodborne Pathog. Dis. 2: 90-102.

Malkawi H, Gharaibeh R (2004). Rapid and simultaneous identification of two *Salmonella* enterica serovars, Enteritidis and Typhimurium from chicken and meat products by Multiplex PCR.Biotechnology, 3 (1): 44-48.

Malorny B, Hoorfar J, Hugas M, Heuvelink, Helmuth R (2003). Interlaboratory diagnostic accuracy of a *Salmonella* specific PCR-based method.Int. J. Food Microbiol. 89: 241-249.

Moussa IM, Ashgan MH, Mohamed MS, Mohamed KH, Al-Doss, A. A (2010). Rapid detection of *Salmonella* species in newborn calves by polymerase chain reaction Int. J. Genet. Mol. Biol. 2 (4): 062-066.

Nori EM,Thong KL (2010).Differentiation of *Salmonella* enterica based on PCR detection of selected somatic and flagellar antigen. Afr. J. Microbiol. Res. 4(9): 871-879.

Nunes JS, Lawhon SD, Rossetti CA, Khare S, Figueiredo JF, Gull T, Burghardt RC, Bäumler AJ, Tsolis, RM, Andrews - Polymenis HL, Adams LG (2010). Morphologic and cytokine profile Characterization of *Salmonella* Typhimurium Infection in calves with bovine leukocyte adhesion deficiency. Vet. Pathol. 47(2): 322-333.

Rodrigue DC, Tauxe RV.Rowe B (1990). International increase in *Salmonella* Enteritidis: a new pandemic. Epidemiol Infect. 105: 21-27.

Sambrook J, Friscgh EF, Meniates T (1989). Molecular Cloning. A laboratory manual. Vol.1., Cold Spring Harbor Laboratory press, New Work.

Soumet C, Ermel G, Rose V, Rose N, Drouin P, Salvat G, Colin P (1999). Identification by a multiplex PCR-based assay of *Salmonella* Typhimurium and *Salmonella* Enteritidis strains from environmental swabs of poultry houses. Letters in Applied Microbiology 1999, 29: 1-6.

Threlfall EJ, Daya M, de Pinna E, Charlett A, Goodyear KL (2006). Assessment of factors contributing to changes in the incidence of Antimicrobial drug resistance in *Salmonella Enteritidis* and Typhimurium from humans in England and Wales in 2000, 2002 and

2004.
Turcotte C, Woodward MJ (1993). Cloning, DNA nucleotide sequence and distribution of the gene coding the SEF14 fimbrial antigen of *Salmonella* Enteritidis. J. Gen. Microbiol. 139: 1477-1485.
Widjojoatmodjo NM, Fluit AC, Torensma R, Verdonk GP, Verhoef J (1992).The magnetic immune polymerase chain reaction assay for direct detection of *Salmonella* in faecal sample. J. Clin. Microbiol. 30: 3195-3199.
Winfield MD, Groisman E (2003).Role of non host environments in the lifestyle of *Salmonella* and *Escherichia coli*. J. Appl. Environ. Microbiol. 69: 3687-3694.
Yan YM, Sekaran SD (2010).Multiplex polymerase chain reaction (M-PCR) assay for the detection of *Enterobactericeae* in clinical samples. Afr. J. Microbiol. Res. 4(11): 1186-1191.
Zahraei T, Tadjbakhsh H, Atashparvar N, Nadalian MG, Mahzounieh M R (2007). Detection and identification of *Salmonella* Typhimurium in bovine diarrhoeic fecal samples by immunomagnetic separation and multiplex PCR assay Zoonosis Public Health, 54: 231-236.