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Identification and Discrimination of *Salmonella Enteritidis*, *S. Pullorum*, *S. Gallinarum* and *S. Dublin* Using Salmonella Specific Genomic Regions Amplification Assay

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Abstract:

BACKGROUND: DNA amplification method has been developed for identifying and discriminating Salmonella serovars, using specific primers at the genus and serovar levels and to identify the *S. Enteritidis*, *S. Dublin*, *S. Gallinarum* and *S. Pullorum*.

OBJECTIVES: This study was conducted for molecular identification and discrimination among some important Salmonella serovars.

METHODS: Fifty isolates of Salmonella were assayed. The PCR assay was designed to amplify DNA fragments from six Salmonella genes, invA (284 bp), tcpS (882 bp), lygD (339 bp), flhB (155 bp), SlgC (252 bp), and speC (174 bp).

RESULTS: The results showed invA and tcpS genes presence in all four Salmonella serovars, whereas the lygD gene only exists in *S. Enteritidis* and is not found in *S. Dublin*, *S. Gallinarum* and *S. Pullorum*. The flhB gene is only present in *S. Enteritidis* and *S. Dublin* whereas it does not exist in *S. Gallinarum* and *S. Pullorum*. The SlgC gene exists in both *S. Gallinarum* and *S. Pullorum*, the SpeC gene is specifically present in *S. Gallinarum*, whereas SlgC and SpeC genes are not found in *S. Enteritidis* and *S. Dublin*. *Salmonella Dublin* serovar amplification assay successfully identified three selected serovar specific genomics regions (SSGRs) and hut gene. The results identify hut gene (495 bp), DSR1 (Dublin-specific genomics region1) (105 bp), DSR2 (Dublin-specific genomics region2) (203 bp), and DSR3 (Dublin-specific genomics region3) (296 bp).

CONCLUSIONS: Amplification techniques on Salmonella serovars specific genomics regions are able to identify and discriminate clinically significant Salmonella serovars, and therefore, have the possibility to be used as a useful and rapid screening assay and support conventional biochemical and serological examinations.

Keywords:

Salmonella Dublin, *Salmonella Enteritidis*, *Salmonella Gallinarum*, *Salmonella Pullorum*, SSGRs

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Introduction

Salmonellosis is a considerable public health concern and important disease resulting in increased morbidity and mortality of affected poultry, animals and foodstuff as well as high cost of treatment, cause the majority of cases universally each year. Salmonella is a genus of Gram-negative, facultative anaerobic, rod-shaped bacteria of the family Enterobacteriaceae. Members of this genus are motile by peritrichous flagella except *Salmonella Pullorum* and *Salmonella Gallinarum*. Presently there are two recognized species, Salmonella bongori and *Salmonella enterica* which consist of six subspecies (Grimont and Weill, 2007; Issenhuth-Jeanjea et al., 2014).

Salmonella serovars are of particular concern to the poultry, animals and foodstuff. The most frequent and epidemiological important serovars are *S. Enteritidis*, *S. Dublin*, *S. Gallinarum* and *S. Pullorum*. *Salmonella Enteritidis* is one of the two most frequent etiological agents of human food borne salmonellosis. Contaminated poultry products are the main vehicle of *S. Enteritidis* ranging from 29% to 34% of all Salmonella infections (Henriques et al., 2013; Linam and Gerber, 2007) and are responsible for causing the highest number of bacterial foodborne infections in the United States (Scallan et al., 2011). *Salmonella Enteritidis* is a major serovar associated with human salmonellosis and is related to consumption or handling of contaminated poultry products, including eggs (Much et al., 2009). Salmonella infections are widespread internationally in both developed and developing countries and are effective reasons of the morbidity and economic loss (Zahraei Salehi et al., 2007).

Salmonella Dublin is the most commonly encountered Salmonella serovar in cattle and cattle products in many parts of the world (Uzzau et al. 2000). The prevalence of Salmonella in beef and milk products in Iran is low compared to products of other animal food sources such as poultry. Acquiring an infection in human from bovine origin foods is much lower than poultry products. *Salmonella Dublin* is one of the few hosts-adapted serovars also known to occasionally cause severe infections in humans (Uzzau et al. 2000). *Salmonella Dublin* infections have been demonstrated to lead to a significantly higher mortality in humans compared to infections caused by *S. Typhimurium* and *S. Enteritidis* (Jones et al. 2008), therefore considerable attention must be paid to control of *S. Dublin* infections of cattle.

Salmonella Pullorum and *S. Gallinarum* are very similar phenotypically. They are the agents of Pullorum disease which causes white diarrhea in young chickens and fowl typhoid, respectively. These two diseases are responsible for economic losses in the poultry production industry (Shivaprasad et al., 2013). Although *S. Pullorum* and *S. Gallinarum* are difficult to differentiate in routine laboratory procedures due to their high level similarities in their antigenic characteristics, the differentiation among four Salmonella serovars is very significant both from an epidemiological viewpoint and in relation to controlling programs (Soria et al., 2012).

White–Kauffmann–Le Minor scheme enables the classification of the genus Salmonella in more than 2,600 serovars (Ranieri et al. 2013) using the combination of somatic (O) and flagellar (H) antigens (Majchr-

zak et al. 2014). *Salmonella Enteritidis*, *S. Dublin*, *S. Gallinarum* and *S. Pullorum* are characterized as *Salmonella enterica* sub species enterica serovars, which are known as group D (somatic antigens 1, 9, 12) and show antigenic similarities. Both *S. Enteritidis* and *S. Dublin* have flagellar antigen (H1) and do not have flagellar antigen (H2). However, the differentiation between *S. Gallinarum* and *S. Pullorum* is still not possible serologically; they do not have flagella and flagellar antigens. Conventional methods are laborious, expensive and time-consuming, so alternative methods have been developed, such as a simple, inexpensive accurate and fast PCR assay to identify and discriminate among Salmonella serovars (Karns et al. 2015).

In the present study, Salmonella serovars were identified based on their specific genomic region amplification using distinct target DNA sequences determined by polymerase chain reaction (PCR), *Salmonella enterica* serovars *S. Enteritidis*, *S. Dublin S. Pullorum* and *S. Gallinarum* were specifically identified and discriminated by amplification PCR according to the presence of invA, tcpS, lygD, flhB, SlgC and speC genes, as well as hut gene and also 3 Dublin specific genomic regions: DSR1, DSR2 and DSR3 were employed to identify *S. Dublin* which were located on serovars specific genomic regions (SSGRs) (Akiba et al. 2011).

The invA has been recognized as a universal standard for detection of Salmonella genus and has exemplified as adequate target with possible diagnostic applications (Salehi et al. 2005), lygD gene has been found only in *S. Enteritidis* and could be used to distinguish these serovars, specifically (Zhu et al. 2015). flhB gene can be used to detect *S. Gallinarum* and *S. Pullorum*, which lack this specific region compared with *S. Enteritidis* and *S. Dublin* which have flhB gene (Xiong et al. 2016). The tcpS gene has been found in *S. Enteritidis*, *S. Dublin*, *S. Gallinarum* and *S. Pullorum* (Xiong et al. 2017).

Polymerase chain reaction (PCR) assay is a reliable method to identify and differentiate between biovars *S. Gallinarum* and *S. Pullorum* by means of target regions of SlgC and speC genes (Barrow and Neto 2011; Ribeiro et al. 2009).

The main goal of this study was to identify and discriminate of *S. Enteritidis*, *S. Dublin*, *S. Gallinarum* and *S. Pullorum* using invA, tcpS, lygD, flhB, SlgC and speC genes. Also, hut gene and a serovar specific genomic region (SSGRs) for *S. Dublin* were employed in multiplex PCR to detect this serovar specifically. This study was conducted in 2017 for molecular identification and discrimination among some important Salmonella serovars.

Materials and Methods

Sample collection: Obtained out on fifty Salmonella isolates from the collection of Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran, these samples were isolated from chicken (37 samples), calves (10 samples) and foodstuffs (5 samples).

Isolation and Identification: All isolates were confirmed as Salmonella using both morphological and biochemical assays (Quinn et al. 2011). Afterwards the samples were inoculated in the brain heart infusion broth and incubated at 37 °C for 24 h, then were transferred to Luria Bertani (LB) agar and after 24 h bacterial colony was harvested.

Serotyping: Fifty biochemically identi-

Table 1. Primers used in PCR for detecting *S. Enteritidis*, *S. Pullorum*, *S. Gallinarum* and *S. Dublin* with amplification protocol (Fig. 1 and 2).

Primers	Target gene	Length	Sequence(5′-3′)	Product size(bp)	References
tcpS-F	tcpS	21	ATGTCTATAAGCACCACAATG	882	(Xiong et al. 2017)
tcpS-R	tcpS	22	TCATTTCAATAATGATTCAAGC	882	
lygD-F	lygD	28	CATTCTGACCTTTAAG-CCGGTCAATGAG	339	(Xiong et al. 2017)
lygD-R	lygD	29	CCAAAAAGCGAGACCT-CAAACTTACTCAG	339	
ST139-F	invA	26	GTGAAATTATCGCCAC-GTTCGGGCAA	284	(Rahn et al. 1992)
ST141-R	invA	22	TCATCGCACCGTCAAAGGAACC	284	
FlhBin-ner-F	flhB	27	GCGGACGTCATTGT-CACTAACCCGACG	155	(Xiong et al. 2016)
FlhBin-ner-R	flhB	27	TCTAAAGTGGGAAC-CCGATGTTCAGCG	155	
SGP-F	SlgC	18	CGGTGTACTGCCCGCTAT	252	(Kang MS 2011)
SGP-R	SlgC	17	CTGGGCATTGACGCAAA	252	
SG-F	speC	18	GATCTGCTGCCAGCTCAA	174	(Kang MS 2011)
SG-R	speC	19	GCGCCCTTTTCAAAACATA	174	
			Amplification protocol (1) for (Fig.1)		
Primary de-naturation	denatur-ation	annealing	elongation	Latest elongation	No .of cycles
94°C	94°C	55°C	72°C	72°C	29
5 min	45 s	45 s	1 min	10 min	
			Amplification Protocol (2) for (Fig.2)		
Primary de-naturation	denatur-ation	annealing	elongation	Latest elongation	No .of cycles
94 °C	94 °C	56°C	72°C	72°C	32
4min	45s	30s	45s	10min	

fied Salmonella isolates were identified serologically according to the White–Kauffmann–Le Minor scheme (Swayne, 1998). Salmonella isolates were serotyped by slide agglutination test for determining somatic antigen (O) and tube agglutination test for determining the flagellar antigens (H) using Salmonella polyvalent and monovalent antisera (BIORAD, Difco, USA).

DNA extraction: After 24 h incubation in LB media, bacterial colony was harvested and DNA was extracted using the boiling method as described before(Swayne 1998). The DNA extract was divided into aliquots and kept at −20 °C until using as PCR template.

Optimization of primers and DNA amplification: Protocols of each PCR reaction were designed and programmed for each pair of primers according to annealing temperatur es using Thermal Cycler (Bio-Rad, Hercules, California, USA) as mentioned

Table 2. Primers used in PCR for detecting *Salmonella Dublin* and amplification Protocol (Fig.3).

Primers	Target gene	Length	Sequence (5´-3´)	Product size(bp)	References
Hut-F	hut	25	ATGTTGTCCTGCCCCTGGTAAGAGA	495	(Cohen et al. 1993)
Hut-R	hut	24	ACTGGCGTTATCCCTTTCTCTGCTG	495	
DMP3-F	DSR3	20	ATCACCCTCGCAAACTTGTC	296	(Akiba et al. 2011)
DMP3-R	DSR3	20	TCGGGCAATCAGGTCGCCGA	296	
DMP2-F	DSR2	20	ACGCGAAATCTGATGGTCTT	203	(Akiba et al. 2011)
DMP2-R	DSR2	20	GCCCACCAGTTGTGAAAGGC	203	
DMP1-F	DSR1	20	ATCGGTGCTGGGTAATTTTG	105	(Akiba et al. 2011)
DMP1-R	DSR1	20	AGGAACGAGAGAAACTGCTT	105	
			Amplification protocol (Fig3)		
Primary denaturation	denaturation	annealing	elongation	No .of cycles	
94 °C	98°C	60°C	68°C	35	
2 min	10s	30s	30s		

in the Tables (1 and 2). The PCR was performed with a total volume of 25 µl that consists of a 10 µl master mix (Sinaclon, Bioscience, Iran), 1 µl (100 pmol) of each forward and reverse primer, 2 µl of template DNA and nuclease-free water up to 25 µl. Sterile nuclease free water was used instead of DNA as a negative PCR control. The PCR products were electrophoresed in 1.5% agarose gel (Fermentas) for 1 h at 100 V; the gels were stained with ethidium bromide (2 µg per ml) for 15 min. The product size was measured using 100 bp DNA Ladder (Sinaclon, Bioscience, Iran). The gel was photographed by a gel documentation system for visualized fluorescent bands.

Results

All fifty Salmonella samples isolated from chicken, calves and foodstuff were identified biochemically and serologically. All of the isolates revealed the expected biochemical characteristics. Serotyping was used to identify the serogroup and serotype based on the somatic and flagellar antigens as both *S. Enteritidis* and *S. Dublin* have flagellar antigen (H1) and do not have flagellar antigen (H2). *Salmonella Gallinarum* and *S. Pullorum* do not have flagella and flagellar antigens (H1, H2). Thirty-seven samples were identified as *Salmonella Enteritidis*, 9 samples were identified as *S. Dublin*, while 2 samples were identified as *S. Gallinarum* and 2 samples were identified as *S. Pullorum*. Identification was confirmed by PCR assay. All Salmonella serovars showed positive results using primers related to invA and tcpS genes and showed positive bands at 284 and 882bp respectively, the PCR results indicated that *S. Enteritidis* revealed four specific bands for tcpS, invA, lygD and flhB genes in 882, 284, 339 and 155 bp respectively, *S. Dublin* revealed three specific bands for tcpS, invA and flhB genes at 882, 284 and155 bp respectively and was negative for lygD gene. *Salmonella Pullorum*

Table 3. Amplification results for *S. Enteritidis*, *S. Dublin*, *S. Gallinarum* and *S. Pullorum*.

Primers		Target gene	Size(bp)	S.E	S.D	S. G	S. P
tcpS-F							
tcpS- R		tcpS	882	+	+	+	+
lygD-F	lygD- R	lygD	339	+	-	-	-
ST139-F	ST141-R	invA	284	+	+	+	+
FlhBinner-F	FlhBinner R	flhB	155	+	+	-	-
SGP-F	SGP-R	SlgC	252	-	-	+	+
SG-F	SG-R	speC	174	-	-	+	-

and *S. Gallinarum* serovars revealed two specific bands for tcpS and invA at 882 and 284 bp and were negative for lygD and flhB genes (Fig.1). It is not possible to differentiate between *S. Pullorum* and *S. Gallinarum* by lygD and flhB related primers and these primers only are able to differentiate between *S. Enteritidis* and *S. Dublin* (Fig.1). The PCR assay could differentiate between *S. Enteritidis* and the other three serovars by revealing a specific band for lygD gene at 339 bp which exists only in *S. Enteritidis* and is not found in the others. *S. Enteritidis* and *S. Dublin* PCR results revealed a specific band at 155 bp for flhB gene, which is not present in *S. Gallinarum* and *S. Pullorum* PCR products (Fig. 1). *Salmonella Gallinarum* and *S. Pullorum* PCR result in (Fig. 2) indicates a specific band at 252 bp for SlgC gene, which exists in *S. Gallinarum* and *S. Pullorum* and is not present in *S. Enteritidis* and *S. Dublin*. A specific primer pair for speC gene was employed, which showed the specific band at 174 bp exists only in *S. Gallinarum* and is not found in the three other serovars (Fig.2). Therefore, the PCR assay detected fifty Salmonella serovars, the results showed that *S. Enteritidis* indicated four specific bands for tcpS, invA, lygD and flhB genes (882, 284, 339 and 155 bp). *S. Dublin* indicated three specific bands for tcpS, invA and flhB genes (882, 284 and 155 bp), *S. Pullorum* indicated three specific bands for tcpS, invA and SlgC genes (882, 284 and 252 bp), whereas, *S. Gallinarum* showed four specific bands for tcpS, invA, SlgC and speC genes (882, 284, 252 and 174 bp) respectively, differentiating between *S. Gallinarum* and *S. Pullorum* (Table 3). In the present study, three SSGRs from *S. Dublin* and also hut gene were selected as target regions for the m-PCR assay based on their predominance in dairy cows and calves, all target regions indicated positive bands of SSGRs for DSR1, DSR2 and DSR3 (105, 203 and 296 bp) and hut gene (495 bp) which are shown in (Fig.3).

Discussion

Salmonella has been identified as an important threat to the public health throughout the world, Salmonella continues to exist in the most predominant serious causes of food borne pathogens (Crim et al. 2014). There are several techniques defined globally as standard procedures for detecting and monitoring different Salmonella serovars (Whyte et al. 2002). These procedures are time-consuming and expensive and require specialized technicians. These procedures such as morphological characterization, biochemical tests and serological examinations may not produce completely acceptable results (Persson et al. 2012) and have much lower sensitivity compared to molecular assays (Oliveira et al. 2002).

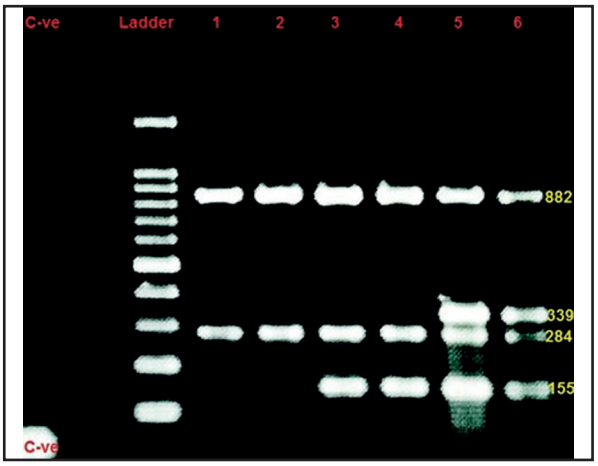

Figure 1. Gel electrophoresis of PCR products of *Salmonella Enteritidis*, *S. Dublin*, *S. Pullorum* and *S. Gallinarum*. Lane M: 100 bp DNA Ladder, Lane1, 2: *S. Pullorum* and *S. Gallinarum* showing bands at 882, 284 bp of tcpS and invA genes Lane 3, 4: *S. Dublin* showing bands at 882,284 and 155 bp of tcpS, invA and flhB genes. Lane 5, 6: *S. Enteritidis* showing bands at 882,339,284 and 155 bp of tcpS, lygD, invA and flhB genes respectively. C-ve: negative control.

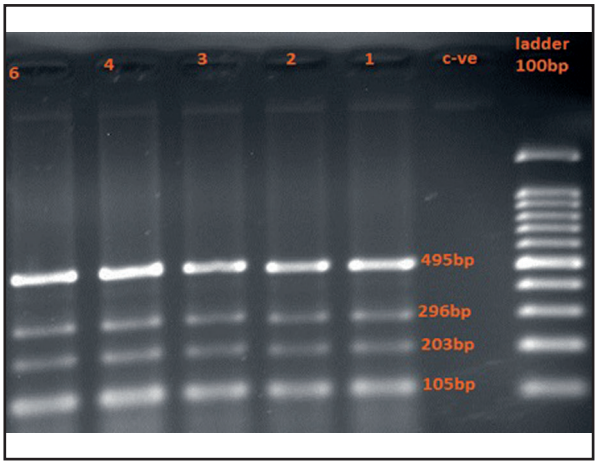

Figure 3. Gel electrophoresis of PCR products of *S. Dublin*. Lane M: 100 bp DNA ladder. C-ve: negative control, Lane: 1, 2, 3, 4 and 5 showing amplicons at 495, 296, 203 and 105bp of the hut, DSR3, DSR2 and DSR1 genes respectively.

Molecular assays are more appropriate, convenient and efficient than conventional techniques. Amplification assay is an easy tool, a rapid and precise device to identify different Salmonella serovars and has been concerned in the last decades (Karns et al. 2015; Persson et al. 2012). In the present study the invA gene is used for diagnosis

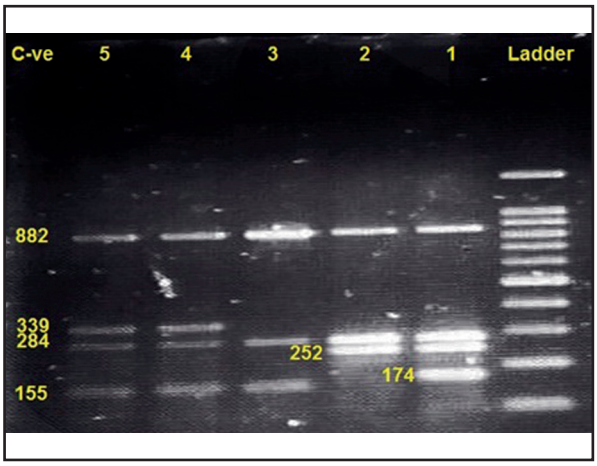

Figure 2. Gel electrophoresis of PCR products of *S. Enteritidis*, *S. Dublin*, *S. Gallinarum* and *S. Pullorum*. Lane M: 100 bp DNA ladder, Lane 1: *S. Gallinarum* showing bands at 882, 284, 252 and 174 bp of tcpS, invA, SlgC and speC genes. Lane 2: *S. Pullorum* shows, bands at 882, 284 and174 bp of tcpS, invA and speC genes. Lane 3: *S. Dublin* shows, bands at 882, 284 and 155 bp of tcpS, invA and flhB genes. Lane 4, 5:S.Enteritidis shows, bands at 882, 339, 284 and 155 bp of tcpS, lygD, invA and flhB genes. C-ve: negative control.

of all Salmonella serovars, the results show specific bands at 284 bp which were in agreement with the previous studies' results (Borges et al. 2013; Salehi et al. 2005). The results also indicated that four understudy Salmonella serovars were positive for tcpS gene specific bands appearing at 882 bp in all Salmonella serovars (Xiong et al. 2017). Also, the results revealed that PCR is able to differentiate among *S. Enteritidis* and other Salmonella serovars *S. Dublin*, *S. Pullorum* and S. Gallinarium showing the specific band at 339 bp due to the lygD gene exists only in *S. Enteritidis* (Xiong et al. 2017; Zhu et al. 2015). lygD gene in SDF locus has been found only in *S. Enteritidis* and could be used to distinguish these serovars specifically, the SDF is located on the chromosomes that were used to screen *S. Enteritidis* genomic library and a unique region was defined. Besides, the m-PCR results revealed specific bands at 155 bp in *S. En-*

teritidis and *S. Dublin*, which did not exist in *S. Pullorum* and *S. Gallinarum*. The flhB gene could be utilized to identify *S. Gallinarum* and *S. Pullorum* from other serovars *S. Enteritidis* and *S. Dublin* due to individual region being located only in these serovars. The flhB gene is a highly conserved component of the flagellar secretion system (Meshcheryakov et al. 2013), and it plays an important role in the determination of flagellar hook length and regulation of protein export (Hirano et al. 1994). Most Salmonella species possess flagella and exhibit motility. However, *S. Pullorum* and *S. Gallinarum* are two notable exceptions that have shown lack of motility and flagella (Holt and Chaubal 1997). Thus, the flhB gene of *S. Pullorum*/Gallinarum may own some special features that are different from other serovars. This finding was in agreement with previous study (Xiong et al. 2016). SpeC and SlgC genes are pseudogenes and are continually created from ongoing mutational process and are subjected to degradation and removal by further accumulation of mutations, the retention time seems to be extremely short, even in very closely related bacteria. Our multiplex PCR results indicated amplified fragments of the slgC and speC genes and this provides a highly powerful distinction between *S. Gallinarum* and *S. Pullorum*. *Salmonella Pullorum* does not produce amplicon from speC gene and *S. Gallinarum* produces amplicon from slgC and speC genes; moreover, speC gene exists in *S. Gallinarum*, whereas slgC gene exists in both *S. Gallinarum* and *S. Pullorum* genome, but is not found in other Salmonella serovars, and these results were in agreement with the previous studies (Ribeiro et al. 2009; Li et al. 2007;(Kang MS 2011).

The screenings are requested for rapid and suitable assay to identify *S. Pullorum*; just as in the previous studies, it was concluded that traditional DNA based techniques are not convenient due to high similarities in the genome sequence of *S. Gallinarum* and *S. Pullorum* (Batista et al. 2015; Feng et al. 2013). It is difficult to immediately differentiate between biovars by serological and biochemical assays, because they belong to the same serogroup, and they do not have flagella. The diagnostic value of biochemical traits is commonly combined with serological characterization, the whole method requires several days and is likely to be replaced by molecular methods to discriminate between these biovars (Rubio et al. 2017).

In the present study, primers of six Salmonella genes, tcpS, lygD, invA, flhB, SlgC and speC were employed to identify and properly differentiate among Salmonella. Enteritidis, *S. Dublin*, S. Gallinarium and *S. Pullorum* permit the evolution of dependable and rapid m-PCR assay to screen and reveal these important four Salmonella serovars.

On the other hand, the serovar specific genomic regions (SSGRs) were targeted to identify *S. Dublin*, these selected genomic regions are extremely predominant among Salmonella serovars. The best primers utilized to reveal the SSGRs are related to the hut, DSR1, DSR2 and DSR3 genes. The Hut gene is a segment of the knowing histidine transport operon of Salmonella and this gene was selected because it was considered to be highly conserved among Salmonella serovars, and is responsible for regulation of histidine as a source of carbon, energy, and nitrogen. It was concluded that three (SSGRs) perhaps give adequate specificity to the PCR assays used to identify *S.*

Dublin and this finding is in agreement with another study (Akiba et al. 2011). Furthermore, this PCR assay (SSGRs) could further improve the serovar discrimination and the detection limit comparable to solely biochemical and serological tests.

Conclusion: The application of PCR assay was investigated by checking for four prominent Salmonella serovars isolated from chicken, calves and foodstuffs. The results indicated the feasibility of utilizing the amplification assay to rapidly screen in and differentiate among Salmonella serovars and appears to be a suitable technique compared to the conventional methods, furthermore, the integration between conventional techniques and PCR assay would enhance the efficacy of identification and discrimination among Salmonella serovars.

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Conflicts of interest

The author declared no conflict of interest.

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مجله طب دامی ایران، ۱۳۹۸، دوره ۱۳، شماره ۲، ۱۴۲-۱۳۱

شناسایی وتفریق سروارهای سالمونلا انتریتیدیس، سالمونلا پلوروم، سالمونلا گالیناروم و سالمونلا دابلین با استفاده از آزمایش تکثیرنواحی اختصاصی ژنومی

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چکیده

زمینه مطالعه: روش‌های تکثیر DNA برای شناسایی وتفریق سروارهای سالمونلا، با استفاده از پرایمرهای اختصاصی در سطح جنس وسـروار طراحی شـده ومورد مطالعه گرفته اند .از جمله سروارهای مهم سالمونلا، سالمونلا انتریتیدیس، سالمونلا پلوروم، سالمونلا گالیناروم و سالمونلا دابلین می‌باشد.

هدف: این مطالعه به منظور شناسایی مولکولی وتفریق بین برخی سروارهای مهم سالمونلا انجام گرفته است.

روش کار: ۵۰ جدایه‌ی سالمونلا مورد آزمایش قرار گرفت، آزمایش برای PCRتکثیرقطعات ۶ ژن سالمونلا طراحی شد invA (۲۸۴bp)، tcpS (۸۸۲bp)، lygD (۳۳۹bp)، flhB (۱۵۵bp)، SlgC (۲۵۲bp) و speC (۱۷۴bp).

نتایج: نتایج نشانگر حضور ژن هایinvA و tcpS در هر۴ سروار سالمونلا بود. در حالیکه ژن lygD تنها در سالمونلا انتریتیدیس حضور داشت، اما در سالمونلا دابلین، سالمونلا گالیناروم وسالمونلا پلوروم حضور نداشت، ژن flhH تنها در سالمونلا انتریتیدیس وسالمونلا دابلین حضور داشت ودر سالمونلا گالیناروم و سالمونلا پلوروم حضور نداشت، ژن SlgC در هر دو سروار سالمونلا گالیناروم و سـالمونلا پلوروم حضور داشـت، ژن speC به طور اختصاصی در سالمونلا گالیناروم حضور داشت، این در حالی است که ژن‌های SlgC وspeC در سالمونلا انتریتیدیس وسالمونلا دابلین حضور نداشتند. آزمایش تکثیرنواحی ژنومی در سطح سروار برای سالمونلا دابلین به طور موفقیت آمیزی ۳ ناحیه‌ی ژنومی اختصاصی سروار (SSGRs) وهمچنین ژنhut را شناسایی نمود. بر اساس نتایج تحقیق حاضر، ژنhut (۴۹۵bp) وهمچنین ناحیه‌ی ژنومیک اختصاصی دابلین۱ (۱۰۵ bp) DSR۱، ناحیه‌ی ژنومیک اختصاصی دابلین۲ (۲۰۳ bp) DSR۲ و ناحیه‌ی ژنومیک اختصاصی دابلین۳ (۲۹۶ bp) DSR۳ شناسایی شدند.

نتیجه گیری نهایی: تکنیک‌های تکثیرDNA بر روی نواحی ژنومیک اختصاصی سروارهای سالمونلا، قادر به شناسایی وتفریق سـروارهای بالینی سالمونلا مهم می‌باشند، بنابراین می‌توان از آن‌ها به عنوان آزمایش‌های مفید وسریع غربالگری ونیز در جهت تکمیل ویا جایگزین آزمایش‌های بیوشیمیای وسرولوژیکی استفاده نمود.

واژه‌های کلیدی:

سالمونلا دابلین، سالمونلا انتریتیدیس، سالمونلا گالیناروم، سالمونلا پلوروم، تکثیرنواحی اختصاصی ژنومیک

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