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Full Length Research Paper

A survey on the prevalence of poultry salmonellosis and detection of different *Salmonella* serovars isolated from poultry in broiler chicken farms

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Salmonellosis is an important public health problem and food of poultry origin is one of the most common sources of human salmonellosis. The aim of this study was detection of S*almonella* spp and determination of the prevalence of Salmonellosis in broiler poultry farms of Ardebil province, Iran. *Salmonella* detection by both conventional culture and multiplex PCR methods were performed on 400 samples obtained from poultry. The samples were obtained from poultry farms of five different geographic zones (North, South, West, East and Central zone) of Ardebil province and were examined by standard microbiological tests. m-PCR technique was carried out with four and three pairs of specific primers for *Salmonella typhimurium* and *Salmonella enteritidi*s respectively. Out of a total of 400 samples, 37(9.25%) were positive for *Salmonella* by bacteriological tests. The highest prevalence of *Salmonella* was recorded in Central zone (10.43%) while the lowest prevalence was in South zone of Ardebil province (8%). Based on the m-PCR results among 37 isolated *Salmonella*, 11 serovars were S. *typhimurium* and 21 serovars were identified as *S. enteritidis.* Also, there was no significant difference between the prevalence rate of *Salmonella* in five different selected areas (P>0.05). For control and prevention programs of salmonellosis, the results of this study can be used by agriculture and health organizations in Iran.

Key words: m-PCR, ardebil, salmonellosis, poultry.

INTRODUCTION

Salmonella species are gram negative, flagellated, facultatively anaerobic bacilli which are considered as major zoonotic pathogens for both animals and humans (Giannella et al., 1973). Salmonellosis is common throughout the world. The disease in humans usually takes the form of a self-limiting food poisoning but occasionally manifested as a serious systemic infection or enteric fever. Contaminated food is the major mode of transmission for non typhoidal *Salmonella* because salmonellosis is a zoonosis and has an enormous animal reservoir (Doyle and Beuchat, 2007). The most common animal reservoirs are chickens, turkeys, pigs and cows. Other domestic and wild animals also harbor these organisms. *Salmonella enterica* serovar *enteritidis* is a major cause of food borne disease and during last decade it has been isolated worldwide in increasing numbers. Furthermore *S. enterica* serovar Typhimurium is the most frequently isolated serovar worldwide (Madadgar et al., 2008). Dairy products, vegetables, fruits, shellfish, beef, poultry and eggs are the most common sources of human salmonellosis (Doyle and Beuchat, 2007). Poultry are commonly infected with a wide variety of *S. enterica* serovars. The two serovars that have been of most concern in recent years are *S. enteritidis* and *Salmonella typhimurium* (Madadgar et al., 2008). Since 1987, *Salmonella enteritidis* has been the main cause of *Salmonella* poisoning in humans from poultry products (Doyle and Beuchat, 2007). In order to minimize the risk of human salmonellosis, epidemiological studies and microbiological control of the food chain is being increasingly applied. In recent years various molecular techniques have been used to improve the identification and differentiation of *Salmonella* serovars including: PCR-single-strand conformation

Table 1. Sequences of oligonucleotides used as primers in m- PCR for *S. typhimurium* (a) and *S. enteritidis* (b) (Rahn et al., 1992).

Primer	Sequence	Target gene	Amplicon fragment(bp)
RfbJ-s RfbJ-as (a)	5'-CCAJCACCAGTTCCAACTTGATAC 5'-GGCTTCCGGCTTTATTGGTAAGCA	rfbJ	663
FliC-s FliC –as (a)	5'-ATAGCCATCTTTACCAGTTCCCCC 5'-GCTGCAACTGTTACAGGATATGCC	fjlC	183
FljB-s FljB -as (a)	5'-ACGAATGGTACGGCTTCTGTAACC 5'-TACCGTCGATAGTAACGACTTCGG	fljB	526
ST 139-s ST141-as (a)	5'-GTGAAATTATCGCCACGTTCGGGCAA 5'-TCATCGCACCGTCAAAGGAACC	invA	284
ST11 ST14 (b)	5'-GCCAACCATTGCTAAATTGGCGCA 5'-GGTAGAAATTCCCAGCGGGTACTGG	Random sequence	429
S1 S4 (b)	5'-GCCGTACACGAGCTTATAGA 5'-ACCTACAGGGGCACAATAAC	spv	250
SEFA2 SEFA4 (b)	5'-GCAGCGGTTACTATTGCAGC 5'-TGTGACAGGGACATTTAGCG	sefA	310

polymorphism analysis (Satheesh et al., 2002), genomic and phenotyping evaluation (Madadgar et al., 2008), pulsed field gel electrophoresis (Mhand et al., 1999; Thong, 1998), PCR assay (Hoorfar and Ahrens, 2000; Feeder et al., 2001; Kongmuang et al., 1994; Lin and Tsen, 1999; Malorny et al., 2003), RFLP (Aarts et al., 1998). One of the most important used techniques for identification of *Salmonella* serovars is PCR technique using *Salmonella* genes (Kisiela and Kuczkowki, 2005).

In 2004, Alvarez et al. (2004) have desecribed m- PCR as a method for *Salmonella* diagnosis that is simple, inexpensive and sensitive and enables the quick and precise detection of the most prevalent serotypes of *Salmonella* in human clinical samples. In recent years several studies were carried out by different authors in order to determine the prevalence of poultry salmonellosis in Iran. Based on these studies the prevalence of *Salmonella* in poultry were reported in different areas in Iran (Zahraei et al., 2005; Madadgar et al., 2008; Jamshidi et al., 2008; Akbarmehr, 2010). But until now epidemiological study about poultry salmonellosis in Ardebil province which is located in Northwest of Iran has not been widely studied. Therefore in the present study we investigated the poultry salmonellosis in broiler chicken farms of Ardebil province using conventional culture and mPCR assay.

MATERIALS AND METHODS

Sampling and microbiological tests

This study was carried out in Ardebil province, Iran. The province was divided into five different geographic areas as follows: North, South, west, East and Central zone. A total_of 400 samples were collected from broiler poultry farms in the aforementioned areas from January 2010 to June 2011(60, 50, 85, 90, 115 samples were obtained from North, South, west, East and Central zone respectively). The samples were harvested from yolk sac, spleen, intestine and liver of chickens and examined by standard procedures (enrichment 24 h in selenite F (Merk) at 37°C, plated on XLD agar and incubated at 37°C for 24 h, confirmation of suspected colonies by biochemical tests) as described by Quinn et al. (1994).

PCR amplification

The *Salmonella* isolates were grown overnight at 37°C in brain heart infusion broth. 2 ml of the bacteria culture were centrifuged for 10 min at 16000 rpm. Purified DNA was used as a templet for the PCR assay. For the m- PCR, seven primer pairs were used. Four pairs of primers were used for *S. typhimurium* and three pairs of them for *S. enteritidis* (Table 1). PCR was carried out in a 25 µl amplification mixture consisting of 200 mM dNTPs, 1 µm of each primer, 40 ng of genomic DNA, 1.5 mM $MgCl_2$ and 1U of *Taq* DNA polymerase (fermentase). Amplification was performed in a thermal cycler (Biosystem). The cycling condition was as follows: initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 60 s, annealing at 65°C for 30 s, elongation at 72°C for 30 s and final extenstion period for 7 min (Zahraei et al., 2005). Amplified products were subjected to electrophresis at 100 V on a 1.2% agarose gel for 1 h and a 100 bp DNA ladder was used as a size reference. After staining with ethidium bromide, the gel was documented and photographed under ultra violet light. *S. typhimurium* with ATCC- 14025 and *S. enteritidis* with RTCC-1624 were used as positive control.

RESULTS

The prevalence of salmonellosis in poultry in the five

Table 2. Prevalence of salmonellosis in the broiler farms of five selected zones of Ardebil province, Iran.

Zone	No. of examined samples	No. positive samples	Prevalence rate (%)
North	60	5	8.33
South	50	4	8
West	85	8	9.4
East	90	8	8.88
Central	115	12	10.43
Total	400	37	9.25

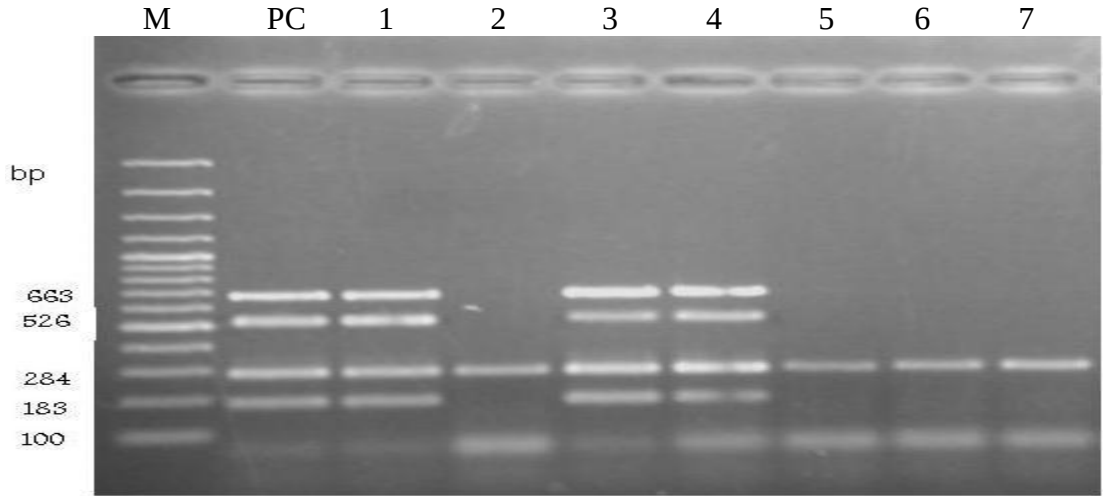

Figure 1. Multiplex PCR with four pairs of primers for *S .typhimurium* isolated from poultry. The 183 bp bands produced by fliC gene (specific for *S .typhimurium*) and the 284 bp bands produced by invA gene (specific for the genus *Salmonella*). The 526 and 663 bp bands produced by fljB and rfbJ genes respectively. M: marker (100 bp). PC: positive control (*S. typhimurium* with ATCC- 14025). Lanes 1, 3 and 4 are positive samples for *S. typhimurium.* Lanes 2, 5, 6 and 7 are positive samples for genus of *Salmonella*.

selected zones of Ardebil province are shown in Table 1. Out of a total 400 samples, 37(9.25%) were positive for *Salmonella* by bacteriological tests. As Table 2 shows the highest, prevalence was recorded in Central zone (10.43%) while the lowest prevalence was in south zone of Ardebil province (8%). MPCR technique which was carried out with specific primers for *S. typhimurium* and *S. enteritidis* (Table1). Among 37 isolated *Salmonella*, 11 serovars of *S. typhimurium* and 21 serovars of *S. enteritidis* were confirmed by MPCR technique. Distribution of *S. typhimurium* and *S. enteritidis* in different geographic area of Ardebil province is shown in Table 2. Also, Figures 1 and 2 shows the MPCR results (Table 3) of *S. typhimurium* and *S. enteritidis* respectively.

DISCUSSION

Salmonella is an important cause of food-borne infections. Most of these infections are caused by *Salmonella* originated from poultry (Doyle and Beuchat, 2007). As Table 1 shows out of 400 samples which was examined by bacteriological tests 37(9.25%) of them had positive results for *Salmonella* strains. This finding is comparable to the reports of previous works from other cities and provinces in Iran. While some previous authors reported higher prevalences of *Salmonella* in poultry, the others reported lower prevalence compared to this study. In a study which was conducted in Fars province, Iran the prevalence of poultry salmonellosis was determined 15.62% (Zahraei et al., 2005). Another survey which was conducted in Mashhad city, Iran; the prevalence of *Salmonella* in poultry carcasses was determined by 8.3% (Jamshidi et al., 2008). In 2010, Akbarmehr et al. (2010) reported the prevalence of poultry salmonellosis in Sarab city, Iran as 7.25%. According to Table 1 although the prevalence of *Salmonella* is widely distributed in all over the five selected geographic area in Ardebil province but the prevalence rates were varied from 8% (South zone) to 10.43% (Central zone). This may be due to confirement system of poultry farms in central zone of Ardebil province which provides the easily distribution of

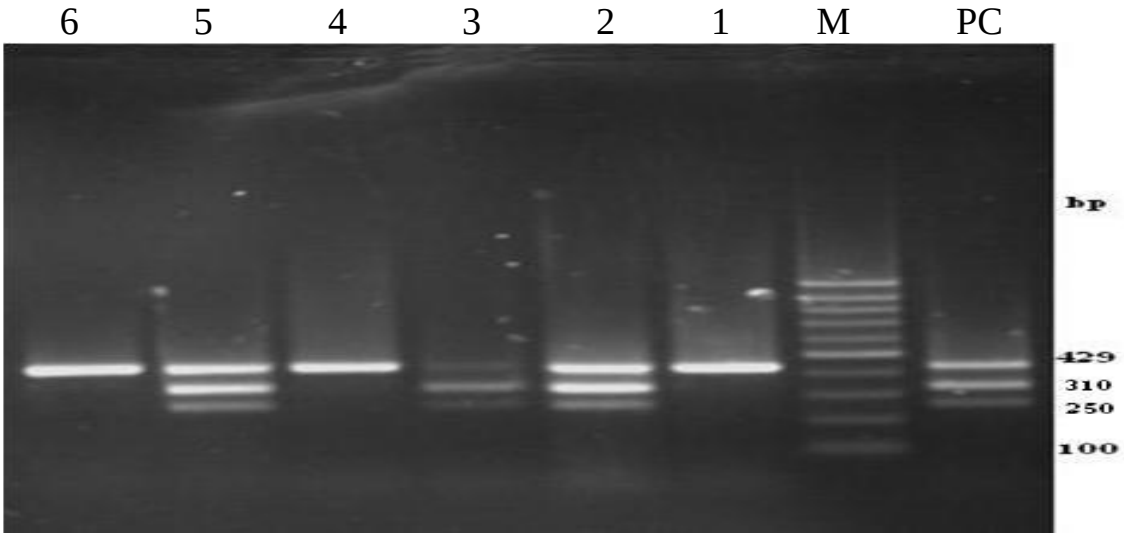

Figure 2. m-PCR with three pairs of primers for *S. enteritidis* isolated from poultry: the 250 bp bands produced by *Salmonella* plasmid virulent gene. The 310 bp bands produced by S.*enteritidis* fimbrial antigen gene and the 429 bp bands produced by randomly cloned sequence which is specific for the genus *Salmonella*. M: marker (100 bp). PC: positive control (*S. enteritidis* with RTCC-1624). Lanes 2, 3 and 5 are positive samples for *S. enteritidis*. Lanes 1, 4 and 6 are positive samples for genus of *Salmonella*.

Table 3. Distribution of *S. typhimurium* and *S. enteritidis* in different geographic area of Ardebil province based on MPCR results.

Zone	No. of positive samples	*S. typhimurium*		*S. enteritidis*	
		No	%	No	%
North	5	1	20	3	60
South	4	1	25	3	75
West	8	2	25	5	62.5
East	8	3	37.5	4	50
Central	12	4	33.33	6	50
Total	37	11	29.72	21	56.75

Salmonella between poultry farms. Although there were no significant differences between different prevalence rates (P>0.05). According to Figure 1, *S. typhimurium* serovars confirmed with four pairs of primers by m-PCR method. As Figure 1 shows the 284 bp bands produced by invA genes which were found in all of the *S. typhimurium* serovars. Primers targeting the rfbJ, fliC and fljB genes were used for specific identification of *S. typhimurium* which produced 663, 183 and 526 bp bands respectively. Olivera reported that the m-PCR technique using invA gene for detection of *Salmonella* and fliC gene for identification of *S. typhimurium* from poultry- related samples was 100% specific (Oliveira et al., 2002). Also, *S. enteritidis* serovars comfirmed with three pairs of primers in this study (Figure 2).

The 429 bp bands were found in all of the *Salmonella* serovars produced by randomly cloned sequenced which were specific for the genus *Salmonella*. *Salmonella* plasmid virulent gene (Spv) and *S. enteritidis* fimbrial antigen gene (sefA) (which produced 250 and 310 bp bands respectively) were used for specific identification of *S. enteritidis* serovars (Madadgar et al., 2008). Based on m-PCR results, *S. enteritidis* with 56.75% frequency and *S. typhimurium* with 29.72% frequency were the most common serovars in five different zones of poultry farms in Ardebil province. Out of 37 *Salmonella* isolates, 5 (13.51%) were other seovars which were not identified in this study. It should be considered that the predominant *Salmonella* serovars differ in different countries. But in Iran many authors showed that *S. enteritidis* and *S. typhimurium* are the most prevalent serotypes of *Salmonella* (Zahraei et al., 2005; Akbarmehr et al., 2010; Madadgar et al., 2008). Because of the ability of *Salmonella* in poultry meat and egg that are not thoroughly cooked, poultry originated products are the main vehicle of transmission (Gianella, 1973). Finally the present research is the first precise study about epidemiology of salmonellosis in poultry farms of Ardebil

province, Iran using conventional culture and m-PCR Assay and our results revealed an important public health and veterinary problem which must be considered by agriculture and public health organizations in Iran.

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