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ORIGINAL ARTICLE

Identification of Local Eggs Contamination Using Biochemical and Molecular Techniques

Ali Karimi[1*], Ali Ghorbani Ranjbari[2]
[1]Department of Pathobiological Sciences, Faculty of Veterinary Medicine, Kazerun Branch, Islamic Azad University, Kazerun, Iran
[2]Graduated from Faculty of Veterinary Medicine, Kazerun Branch, Islamic Azad University, Kazerun, Iran
[*]Corresponding Author's E-mail: alikarimi224@yahoo.com

ABSTRACT
Salmonella are the group of bacteria which are bacillus, without capsule, facultative aerobic and facultative anaerobic. Its transferring in avian is vertical and horizontal. In human, they can cause food poisoning. The egg is the most important source of salmonella. The native eggs were collected and transferred to the lab for studying the contamination of them. In this research the pattern of drug resistance of bacteria is studied. The shell of eggs was purified with Ethanol 80%, and then the Contents of five eggs were mixed in a dish and incubated with soap in Selenite-f. After 24 hour incubation at 37ºC was analyzed in the terms of suspected colonies to Salmonella. The suspected colonies were inoculated into lysine decarboxylase broth and TSI agar environment. The bacteria which had reactions were related to Salmonella, and they were analyzed by the PCR test, with special primers for Salmonella spp. The results of this study indicated that the eight samples of mixed eggs were contaminated with salmonella of serotype Salmonella enteritidis. Among the separated Salmonella 85.9 % were resist to Ampicillin, 14.5% to Tetracycline, and 42.9% to Kanamycin, but all of them were sensitive to Norfloxacin light in this antibiotic resistance test.
***Keywords**: Identification, PCR, Egg, Drug resistance*

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INTRODUCTION

Salmonella are large group of negative Gram bacillus, all of them have antigen H of the flagellum [1]. Salmonella have a high resistance to chemical agents such as high concentration, bile and also physical factors such as temperature and disinfectant material. It can survive more than 5 months in the soil and maintain its making disease [2]. So far over 2500 serotypes of Salmonella have been identified [3]. In poultry Salmonella can transmit horizontal and vertical and usually it create a stable infection in contaminated flock. Different serotypes of Salmonella cause some disease such as pullorum which its agent is *Salmonella pullorum*. Poultry typhoid caused by *Salmonella gallinarum* which almost transmit horizontally and by bird's faeces.

Salmonella typhimurium and *Salmonella enteritidis* don't habit to a specific host, and are the most important species which are involved in human Salmonella contaminations. Studies show that *Salmonella enteritidis* in most Salmonellosis epidemics are involved with origin eggs. *Salmonella enteritidis* can infect the contents of egg as a result of infection of egg laying hens reproductive tissues. It seems that the major sites of infection, is the upper part of oviduct [4]. Other Salmonella serotype including antibiotic-resistant serotypes, *Salmonella typhimurium* can also be replaced in the egg [5]. Consumption of eggs is the most important sources of Salmonella infections [6, 7]. Salmonella bacteria didn't have a hard growth and after enrichment it is separated from environment, clinical sample and nutrition, and diagnosed by PCR technique as the precise and fast method [8, 9]. Regarding to the important of Salmonella contamination in poultry breeding industry, public health and also increasing antibiotic resistance of Salmonella bacteria [10, 11, 12, 13, 14], this study was conducted to identify the Salmonella contamination of local eggs from

Shiraz County (Fars, Iran), by biochemical and molecular tests, also medical resistance pattern of bacterial isolated to the disk agar diffusion method was performed.

MATERIALS AND METHODS

For isolation of Salmonella bacteria, 150 eggs were collected from areas around Shiraz, each egg was transferred to the laboratory. In the laboratory, the egg shell cleaned and disinfected with the use of 80% ethanol, calcareous shell was broken with sterilized scissors and the content of each 5 eggs were mixed in a sterilized glass dish. The mixture after 24 h incubation at 37 ºC was incubated to environment with swab to the Selenite-F broth. Then the removal samples were cultured surface on solid medium of Salmonella - Shigella agar (containing protein, lactose and iron) and were incubated for 24 hours at 37 ºC. Then the suspected Salmonella colonies were examined. In case of negative result (Salmonella which after this time have no color), the incubation was continued for 24 hours. Pale or yellow colonies with gray and black center were considered negative. The suspicious colonies were removed from Salmonella-Shigella agar medium and were cultured surface and deep in TSI medium.

After 24 h incubation, colonies suspected to alkaline / acid were red and yellow respectively. All Salmonella species, except *Salmonella paratyphi* A, in the TSI medium, create H_2S gas that the medium knows black. Simultaneously LIA test was performed which salmonella made all the medium purple. Also we provide a spread from the suspicious colonies which were stained with Gram method. If the rods gram-negative bacteria observed, a pure culture was provided and examined [15].

Recognition of *Salmonella enteritidis* and *Salmonella typhimurium* by PCR technique:

For DNA extraction of identified Salmonella in above, colony suspensions in sterile distilled water were prepared. The Suspension centrifuged for 5 min at 18 °C with a speed of 14000 rpm and extract from the precipitated, by phenol - chloroform bacterial genome method. Genome by using specific primers of invasion gene used PCR to detect Salmonella. To determine the serotype, the primers of fimbrial gene sefA-1 (5' GCAGCGGTTACTATTGCAGC 3') and

sefA-2 (5' TGTGACACGGACATTTAG CG 3') for *Salmonella enteritidis* and primers of virulence fimbria pefA-1 (5' TTCCATTATTGCACTGGGTG 3') and pefA-2 (5' GGCATCTTTCGCTGTGGCTT 3') for *Salmonella typhimurium* were used in PCR. PCR products were analyzed by agarose gel electrophoresis, and 100 bases pair DNA ladder were used to determine the molecular weight of DNA fragments. Gel stained with ethidium bromide solution and visualized on a UV transilluminator.

Antimicrobial Resistance: Determination the sensitivity of isolated Salmonella to the antibacterial compounds, a disk agar diffusion method was used. First, colonies on Mueller Hinton liquid environment were provided and simultaneously a disk contain antibiotic was lodge on the culture environment. After 24 h incubation at 37 ºC, Salmonella growth inhibitions were determined. To antibiogram tests, four antibiotics disks containing: ampicillin (10 µg), tetracycline (30 µg), kanamycin (30 µg) and norfloxacin (10 µg) were used. The result of isolated Salmonella sensitivity were estimated on the base of the percentage, in three sensitive, semi-sensitive and-resistant level using standard Kirby and Bauer technique [10, 16].

RESULTS AND DISCUSSION

In this study, 150 eggs were examined for Salmonella contamination. To increase the isolation sensitivity of Salmonella, the enrichment step prior to inoculation to the selected medium was done. From the 30 mixed sample of eggs, 4 mixed samples in biochemical tests and PCR technique were contaminated with *Salmonella enteritidis*.

According to the method of preparing samples for bacterial culture, at least 66.2% of the eggs (one to two eggs in each mixed sample) or up to 33.13% of eggs (all eggs in the mixture) were contaminated with Salmonella. The PCR band pattern differentiate between eggs contaminated with Salmonella and eggs without contamination (figure 1). The PCR results showed no cross reaction to the other common eggs infection especially *E.coli*, Klebsiella, Proteus and Shigella (Figure 2).

The frequency of Salmonella species isolation has been documented variously in different studies. In present study 11.2% of the total samples proved positive for Salmonella; although according to two different studies in England 8% and 25% of total samples were found positive for Salmonella [17, 18]. In similar studies in Canary Island and New Zealand these figures were 16.5% and 16%, respectively [19, 20] and in Korea only 2.2% of samples were found positive for Salmonella [21]. It is evident that percentage of recovery varies from country to country and this applies even to different regions in a particular country, for instance the latter is quite obvious in case of Iran [20]. In a study which was done in US Salmonella contamination of content and shells of 1200 eggs was examined, just in 12 eggs, external contamination with Heidelberg Salmonella was found while all egg's content have no salmonella

contamination [22]. In the other study, more than 5700 eggs of 15 flocks which naturally contaminated with *Salmonella enteritidis*, the mass in the shell and content of egg were tested. According to the findings, contents of 32 eggs (6%) were shown the infection [4]. Organisms inside the egg through direct transfer or egg shell contamination penetrate to the egg [23]. The existence and flexible transmission of integrons was proven suitable for the spread of drug resistant genes and the acceleration of multidrug resistance [24]. The investigation on drug resistance of 51 strains of human *Salmonella enterica* serovar Typhimurium conducted by Biendo [25] showed that multidrug resistance of the strains was 98%, with more than 90% of isolates resistant to sulfonamides, ampicillin, streptomycin and tetracycline, and sensitive to amikacin and cephalosporins. Analysis of the drug resistance of animal and human *Salmonella enterica* serovar Typhimurium isolated by Graziani [26] showed that 64% of strains were resistant to more than four drugs and most strains were resistant to sulfamethoxazole. In studding drug resistance pattern of isolated salmonella, resistance rate to the antibiotic of ampicillin, kanamycin and Tetracycline of the sample was 11%, 21% and 36% respectively, while there was not observed resistant sample to Norfloxacin. Regarding to antibacterial used, the most sensitivity is to Norfloxacin (table 1) which may result from not using these antibiotics in the region and findings no resistance to it. Having no complete sensitivity to Tetracycline and Kanamycin can result from excessive use of antibiotics in the treatment and resistance to it, is not unexpected. Observed Ampicillin resistance may be due to the more effect of these antibiotics on Gram-possitive bacteria than Gram- negative bacteria [27].

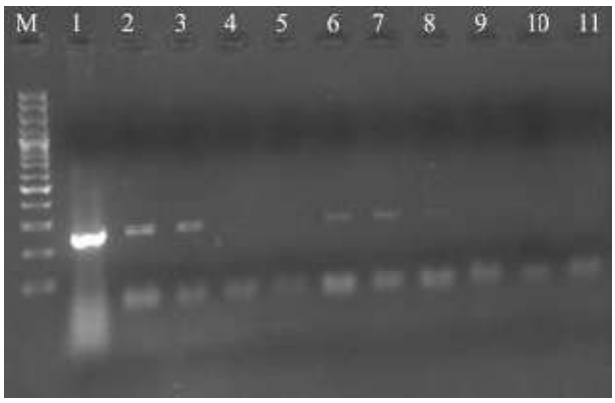

Figure 1: PCR identification of *Salmonella enteritidis*. Lane M, 100 bp DNA size marker; Lane 1standard isolate of *S.enteritidis*; Lane 2, 3, 6, 7 and 8 represent eggs contaminated with *S.enteritidis*; Lane 4, 5, 9 and 10 represent eggs without *S.enteritidis* contamination; Lane 11, negative control

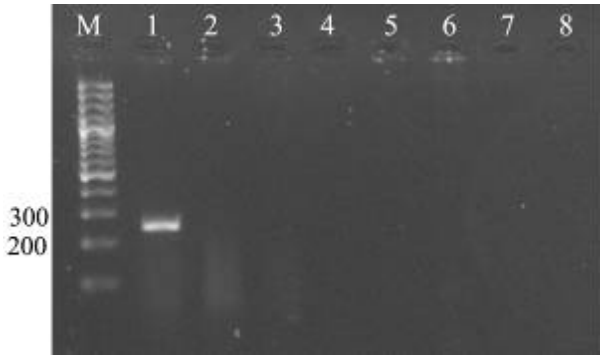

Figure 2: PCR specificity. Lane M, 100 bp DNA size marker; Lane 2 to 7 represent S.typhi, S.paratyphi A, Shigella, E.coli, Klebsiella and Proteus respectively; Lane 8, negative control

Table 1: Pattern of drug resistance of Salmonella isolated from the native eggs

Result	Ampicillin	Kanamycin	Tetracycline	Norfloxacin
Sensitive (%)	0	14	17	100
Semi sensitive (%)	7	25	19	0
Resistance (%)	11	21	36	0

CONCLUSION

Native chickens are usually kept with domestic animals and their laying egg also takes place on the bed, so the contamination probability to bacteria with intestinal origin, such as Salmonella, will increase. In addition to the control programs and Salmonella eradications don't perform, therefore to reduce the risk of salmonella, we can propose these actions:

1 - Full cooking eggs (70 ºC to 10 minutes) that can remove Salmonella.

2- Teaching the danger of salmonellosis to people and preventing method of salmonella transmit to the food in the kitchen which this is the most practical method to prevent Salmonellosis caused by eggs.

3- An Antibiogram should be performed before of antibiotics therapy in poultry to control antibiotic resistances.

REFERENCES

1. Crum-Cianflone NF. (2008). Salmonellosis and the gastrointestinal tract: more than just peanut butter. Curr Gastroenterol Rep.;10:424-431.
2. Jensen AN, Dalsgaard A, Stockmarr A, Nielsen EM, Baggesen DL. (2006). Appl Environ Microbiol; 72(3): 1833–1842.
3. Chu C, Chiu CH. (2006). Microbes and infection; 8:1931-1936.
4. Humphrey TJ, Whitehead A, Gawler AH, Henley A, Rowe B. (1991). Epidemiol infect; 106(3): 489- 496
5. Saif YM, Barnes HJ, Glisson JR, Fadly AM, McDougald LR, Swayne DE. (2003).Diseases of poultry.11 ed, Iowa State Press: 583-599.
6. De Boer E, Hahne M. (1990).J food protection; 53: 1067-1068.
7. Nunes IA, Helmuth R, Schroeter A, Mead GC, Santos MA, Solari CA.(2003). J Food Protection. 66:324-327.
8. Woodward, M. J. and Kirwan, S. E. S. (1996). Detection of Salmonella enteritidis in eggs by the polymerase chain reaction. Veterinary Record, 138 (17). pp. 411-413. ISSN 0042-4900
9. Soumet, C., Ermel, G., Rose, N., Rose, V., Drouin, P., Salvat, G., Colin, P. (1998). Evaluation of a multiplex PCR assay for simultaneous identification of Salmonella. Let Appl Microbiol ; 28: 113-117.
10. Adesiym A, Offah N, Seepersadsingh N, Rodrigo S, Lashley V, Musai L. (2007).Food Cont; 18: 306-311.
11. Cailhol J,Lailler R, Bouvet P, La Vieille s, Gauchard F, et al. (2006).Epidemiol Infect; 134:171-178.
12. Graziani C, Busani L, Dionisi AM, Lucarell S, Owczarek S, et al. (2008).Vet Microbiol; 128: 414-418.
13. Pan Z, Wang X., Zhang X, Geng S, Chel X, Pan W, et al. (2009). Vet Microbiol; 136: 387-392.
14. Van Duijkeren E, Wannet WJ, Houwers DJ, Van Pelt W. (2003). J Clin Microbiol; 41: 3574-3578.
15. Quinn PJ, Markey BK, Carter ME, Donnelly WG, Leonard FC. (2002). Vet Microbiol and Microbial dis. First published. Blackawell Science: 113-118.
16. Quinn PJ, Carter ME, Markey B, Carter GR. (1994). Clin Vet Microbiol.Wolf publishing, London. 95-102.
17. Jorgensen F, Bailey R, Williams S, Henderson P, Wareing DRA et al. (2002).Int J Food Microbiol. 76: 151-164.
18. Meldrum RJ, Tucker D, Edwards C. (2004).J Food Prot. 67: 1226-1228.
19. Hernandez TA, Sierra A, Rodriguez-Alvarez C, Torres A, Arvalo MP, Calvo M. Arias A.(2005). J Food Prot ; 68: 2702-2706.
20. Javadi A, Rasavilar V, Talebi A. (2005).Food Technol Nut; 2: 52-59.
21. Chung YH, Kim SY, Chang YH. (2003). J Food Prot; 6: 1154-1157.
22. Schutze GE, Fawcett HA, Lewno MJ, Flick EL, Kirby RS. (1996). South Med J; 89(9): 889-891.
23. Nazer AHK, Safari GH. Indian J Animal Sci (1994); 64(10): 1006-1009.
24. Chen L, Wu CM, Shen JZ. (2008). Chin J Vet Med; 44: 6–9.
25. Biendo M, Laurans G, Thomas D. Int J Antimicrob Agents (2005); 26: 219–229.
26. Graziani G, Busani L, Dionisi AM. (2008). Vet Microbiol; 128: 414–418.
27. Adams, R.H. (1995). Veterinary. Pharmacology and therapeutics. 7th ed.Iowa State University Press, Iowa, p: 1112-1114