

Monitoring housefly (*Musca domestica*) as carrier of *Salmonella* in poultry farm in Iran

Aynaz Yamrali¹, Mahmood khormali¹, Parastoo Saniee², Fatemeh Ezzatifar³, Babak Asghari³ and Taghi Zahraei Salehi¹

¹Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran, Tehran-Iran; ²Department of microbiology, Faculty of Sciences, University of Tehran, Tehran-Iran; ³Antimicrobial-Resistance Research Centre and Department of Microbiology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran-Iran

Abstract

Musca domestica is a major medical and veterinary insect that causes irritation, spoils food and acts as a vector for many pathogenic organisms. *Musca domestica* have been demonstrated to be carriers of several species of *Salmonella*. This study was carried out to determine the presence of *Salmonella* on houseflies caught in poultry farm. Bacteria were isolated from houseflies samples with microbiological, serological and PCR for *invA* gene. According to serological test, 2 different serogroups were found among 3 *Salmonella* isolates. Two isolates belonged to group D and one isolate belongs to group C. According to this study, housefly could be considered as an important carrier of *Salmonella* among different poultry farms. This explains that house fly poses a possible risk to communities and environmental cleanliness.

Keywords: *Musca domestica*; *Salmonella*; poultry

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Introduction

Salmonella is a threat of food borne bacterial pathogens for humans throughout the world. *Salmonella* spp. is omnipresent in nature, can continue to exist several weeks in a dry environment and numerous months in water. Aquatic vertebrates, birds and reptiles are included as the main carriers of *Salmonella* (Holt et al., 2007). Human infections have occurred from consumption of insufficiently cooked or contaminated food; other potential sources include contaminated environment such as water and in contact with file (Patrick et al., 2004; Cogan and Humphrey, 2003).

Houseflies (*Musca domestica*) have been demonstrated to be carriers of several species of *Salmonella* due to their close relation with decaying organic matters, faeces and garbage (Nazni et al., 2005). The hairy proboscis and feet with glandular hairs and pads produce a sticky liquid material able the

houseflies to pick up the pathogens onto their bodies (Choo et al., 2011). Additionally, the regurgitation of vomitus and deposit of fecal droplets during feeding process are considered to be the causes of the houseflies' opportunity to spread the pathogens (Rosef and Kapperud, 1983; Nazni et al., 2005). Several researchers have isolated pathogens, such as *Escherichia coli* O157:H7 (Sasaki et al., 2007), *Vibrio cholera* (Fotedar, 2001) and *Salmonella* (Olsen and Hammack, 2000) from houseflies and reported them as a potential source for transmission and spread of these pathogens. This study with carried out to determine the presence of *Salmonella* on houseflies caught in poultry farm.

Materials and Methods

Flies and sampling

Samples of houseflies were collected from poultry farm. The flies were caught using four adhesive

Corresponding author: Taghi Zahraei Salehi, Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran, Tehran-Iran, Tel: +98 21 61117052; Fax: +98 21 66427517

absorbent flies from 10 poultry farm that each has two rooms and the hall. Fly samples were also collected by the adhesive from two laying poultry each had three hall from the East of Golestan province (Total of 12 poultry farm= 26halls).The dead flies were retrieved, using sterile forceps, one forceps per fly, and deposited into individual zip-sealable plastic bags and transferred to microbiology laboratory.

Bacterial isolation

In microbiology laboratory, flies were thoroughly crushed and placed in Selenite F broth (Merck) for non-selective pre enrichment. After 24 hours incubation at 37°C, one hundred microliters of the sample was inoculated onto 2 Mac Conkey agar plates and incubated at 37°C for 24 hours. Yellowish non lactose fermenting colonies were considered as *salmonella* suspect colonies. These colonies were subcultured on Xylose lysine desoxycholate (XLD) agar selective media .After incubation, typical *Salmonella* colonies with slightly transparent zone of reddish color and a black center were picked up and cultured on Choromagar (CHROM agar, France) for further confirmation. Purple colonies were considered as *Salmonella* and subjected to gram staining and biochemical tests such as Oxidase, Triple Sugar Iron (TSI) agar, Urea broth, Indole , MR-VP, Simon citrate, Motility and Lysine Iron Agar (LIA).

Serological tests

The colonies that showed typical characteristics of *Salmonella* were then subjected to *Salmonella* agglutinating antiserum Poly A-Z. Colonies that gave positive reaction to slide agglutination test were cultured on nutrient agar for further confirmation of serotyping by commercial *Salmonella* specific O antigen antisera (Difco, USA).

Molecular identification

For molecular confirmation, bacteria were harvested in phosphate-buffered saline (PBS 1X). DNA extraction was performed by boiling for 10 min and centrifuging at 6,000 rpm for 5min. Amplification of the *Salmonella*-specific *invA* gene was performed using 5'-GTGAAATTATCGCCACGTTCGGGCAA - 3' and 5'- TCA TCGCACCGTCAAAGGAACC-3' as forward and reverse primer, respectively. PCR was performed in a total volume of 25 µL containing 2.5 µl 10X PCR buffer, 1.25 µl dNTPs (10mM), 1.6 µl MgCl₂, 0.5 µl of each primer, 0.5µl of Taq DNA polymerase (Fermentas, Lithuania) and 1.5 µl of each extracted DNA. Amplification was conducted in Master-gradient Thermocycler (Eppendorf, Germany). The cycle conditions were as follow: An initial incubation at 94°C for 60 sec, followed by 35 cycles of denaturation at 94°C for 60 sec, annealing at 64°C for 30 sec and

elongation at 72°C for 30 sec, followed by 7 min final extension period at 72°C. PCR products were electrophoresed on 1% agarose gel and visualized by UV transilluminator.

Results

A total of 90 fly samples were isolated from poultry farms. By culturing on Mac Conkey agar and XLD agar three *Salmonella* suspect colonies were isolated. Interaction with chromagar and biochemical test confirmed the isolates as *Salmonella* species. According to serological test, two different serogroups were found among three *Salmonella* isolates. Two isolates belonged to group D and one isolate belongs to group C.

Electrophoresis of the amplified products of *invA* gene from all 3 isolates showed the band size of 284bp. The size of amplicons was homologous to that of the control *Salmonella* (Fig. 1).

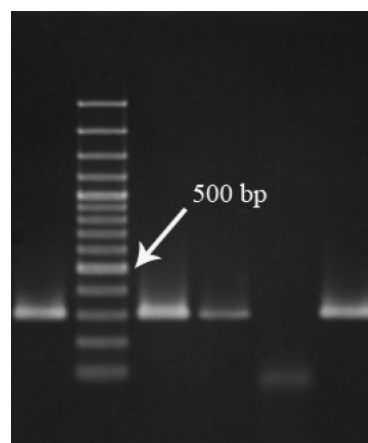


Fig. 1: Electrophoresis of PCR products of *salmonella*-specific *invA* gene. 284 bp fragment was amplified from all 3 isolated *Salmonella* species examined. Lanes 1, 3 and 4 PCR products from 3 isolates, Lane 2: 100-bp ladder, lane 5: No template, lane 6: Control *Salmonella*.

Discussion

Salmonella species is considered the source of infection is usually contaminate animal products, especially poultry-derived food and cause human disease. In this study, three *Salmonella* species were isolated from 90 fly samples (3.3%). Flies were thoroughly crushed so whether these organisms were carried externally or internally was not investigated in this study. Bailey et al. (2001) isolated *Salmonella* from 19% of flies captures in broiler farms. Olsen and

Hammack (2000) found a 22% carrier rate for *Salmonella* serovar *Enteritidis*, in flies captured in facilities housing laying hens (Olsen and Hammack, 2000). In one similar study, various bacteria were isolated from house flies (Sulaiman et al., 2000). To the author's knowledge, no studies have followed the rate of *Salmonella* species carried by flies, collected from poultry farms in Iran. Flies are known as vehicles of pathogenic bacteria. The findings of this study indicate that houseflies can transmit *Salmonella* species and subsequently deposit pathogenic bacteria on food and water that result in food born infection.

According to serological test, one of the 3 isolated *Salmonella* belonged to serogroup C. Different studies show that as the prevalence of *S. enteritis* and *S. typhimurium* decreased because of eradication efforts in the last decade, the proportion of *Salmonella* sero group C in human salmonellosis cases, have been gradually increased (van Duijkeren et al., 2002).

Some antibiotics are currently used for preventive therapy and growth promotion of animals used as food source (Chopra and Roberts, 2001). Many reports indicate that long term use of antibacterial drugs produce resistance and multiple resistance genes could be exchange between bacterial pathogens of human and animals (Kruse and Sørum, 1994). Accordingly houseflies could be considered as an important carrier of multiple resistance bacteria among different poultry farms and humans. This explains that house fly poses a possible risk to communities. For clean environment mechanical, chemical and biological treatment is necessary to discourage flies around poultry farms.

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