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An Investigation into *Salmonella* Infection Status in Backyard Chickens in Iran

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Abstract: Salmonellosis is one of the most prevalent diseases in birds, causing high losses in poultry industry and food poisoning in human. For this reason, 422 apparently healthy chickens from 35 backyard flocks in different geographical regions of the rural area of Ahvaz (a city in the southwestern Iran) were sampled from January 2004 to June 2005 by cloacal swab as per OIE standards. The swabs, from each flock, were pooled in groups of up to five, then cultured and confirmed by routine biochemical and serological tests. Out of pooled 85 samples, five (5.8%) were positive for *Salmonella*. Of these, four isolates were identified as *S.* Serovar Typhimurium and one isolate as *S.* Serovar Enteritidis. The isolates belonged to 3 flocks. *S.* Serovar Enteritidis was sensitive *in vitro* to tetracycline, chloramphenicol, furazolidone, neomycine, nalidixic acid and sulfamethoxazole and trimethoprim, but the other four isolates were resistant to tetracycline and sulfamethoxazole and trimethoprim. This is the first report of the isolated serotypes in backyard chickens in Iran.

Key words: *Salmonella*, backyard, chicken, Iran

Introduction

Salmonellosis is one of the most prevalent diseases in birds caused by a vast range of *Salmonella* serotypes. The non-motile serotypes (*S.* Serovars Pullorum and Gallinarum) are generally host-specific for chickens and turkeys, being less prevalent in the world (Shivaprasad, 2003), whereas the motile serotypes often referred to as paratyphoid (PT) *Salmonellae* occur throughout the world and can infect a very wide variety of hosts, including wild and domestic animals and humans. PT infections have long been known to cause great losses in poultry. In addition, they play a very important role in food-borne salmonellosis in humans (Gast, 1997). For example, more than one-third of food-borne salmonellosis outbreaks in humans in USA between 1983 and 1987 were associated with poultry meat or eggs (Tauxe, 1991).

Backyard chickens can also be infected through contact with wild animals, domestic mammals and commercial poultry that are carriers of *Salmonellae* and consequently may play a role in the transmission of the organism to other animals and humans. In Iran, the rearing of backyard chickens is very common, providing some part of nutritional requirements for villagers and even citizens. However, there have been not any reports about the prevalent *Salmonellae* in the backyard chickens in Iran. For this reason, the current study was undertaken to characterize the *Salmonella* infection status in the native chickens.

Materials and Methods

From January 2004 to June 2005, 422 apparently healthy chickens from 35 backyard flocks in different geographical regions of the rural area of Ahvaz (a city in the southwestern Iran) were sampled by cloacal swab as per OIE standards (1992) (Table 1). The cloacal swabs, from each flock, were pooled in groups of up to five, so that a total of 85 pooled samples were collected. Each sample was placed in 10 mL of tetrathionate broth (Merck, Germany) and kept at 42°C for 24 hr. Then, one loop was streaked on both BG and XLD agars (Hi-Media, India) and incubated at 37°C for 24 hr. To inhibit the growth of *Proteus* species, novobiocin (Hi-Media, India) was added to the plating media and TT broth at a rate of 20 μg mL^{-1} and 40 μg mL^{-1} respectively. After purified on blood agar, the colonies characteristic of salmonella were confirmed by routine biochemical and serological tests as recommended by Douglas Waltman *et al.* (1998). The isolated *Salmonellae* were tested for *in vitro* susceptibility to tetracycline, chloramphenicol, furazolidone, neomycine, nalidixic acid and sulfamethoxazole and trimethoprim by disk diffusion method as described by Treagan and Pulliam (1982).

Table 1: The number of birds in a flock sampled by cloacal swab

No. of birds in a flock	No. of birds sampled	No. of birds in a flock	No. of birds sampled
25-29	20	60-89	40
30-39	25	90-199	50
40-49	30	200-499	55
50-59	35	>500	60

Table 2: Regional distribution of *Salmonellae* isolated from backyard chickens

Region	Flocks sampled	Positives	Percent positives	Isolated serotypes
North	9	0	0	-
West	9	2	22	*S.* Serovar Typhimurium
South	9	1	11	S. Serovar Enteritidis
East	8	0	0	-
Total	35	3	8.5	*S.* Serovars Typhimurium and Enteritidis

Results and Discussion

In the absence of other microflora, *Salmonellae* are apparently able to adhere, multiply and colonize at any point along the GI tract of chicks (Soerjadi *et al.*, 1982) and may be shed in the feces, forming a source of contamination for other animals, humans and the environment (Poppe, 2000). For this reason, cloacal swabs have been used to provide evidence of persistent intestinal colonization by *Salmonellae* in broiler chicks (Yagoub and Mohammad, 1987), layers (Blaszczak and Binek, 1999), pigeons (Cena *et al.*, 1989) and parrots (Orosz *et al.*, 1992). In the present study, five of 85 pooled samples (5.8%) were positive for *Salmonella*. Of these, four isolates were identified as *S.* Serovar Typhimurium and one isolate as *S.* Serovar Enteritidis. Out of 35 flocks, three (8.5%) were identified to be infected (Table 2). *S.* Serovar Typhimurium was isolated from two flocks (5.7%) and *S.* Serovar Enteritidis from one flock (2.8%). The literature shows that there have been made fewer studies on the *Salmonella* infections in backyard chickens than in commercial poultry flocks. Bouzoubaa *et al.* (1992) assessed 500 cloacal swabs, as 100 pooled samples, taken from village chickens in 50 different farms in Morocco and stated that two cultures were positive for *S.* Serovar Pullorum and one for *S.* Serovar Gallinarum. *S.* Serovars Gallinarum and Pullorum were also recovered from commercial and backyard flocks in Switzerland (Hoop and Albicker-Rippinger, 1997). Istiana (1992) could isolate *S.* Serovars Hadar, Weltevreden, Paratyphi B var. Java and Ouakam from village chickens in south Kalimantan. Moreover, some seroprevalent studies suggested the presence of antibodies against *S.* Serovars Gallinarum/Pullorum in Tanzania (Permin *et al.*, 1997), Benin (Chrysostome *et al.*, 1995), Nepal (Jha *et al.*, 1994), Calfornia (McBride *et al.*, 1991) and Mauritania (Bell *et al.*, 1990). But the isolated serotypes have not yet been reported in backyard chickens in Iran. As showed in Table 2, *Salmonellae* were isolated only from flocks occurred in south and west regions. This might be due to the fact that the most of commercial broiler farms have concentrated in these regions. So this vicinity can increase the risk of spread of *Salmonellae* from backyard flocks to commercial flocks as well.

The drug resistance test showed that *S.* Serovar Enteritidis was sensitive to all antibacterial agents used, but the other four isolates were resistant to tetracycline and sulfamethoxazole and trimethoprim. Factors such as serovar and geographical region can influence the resistance of *Salmonellae* to antibacterial agents. It is assumed that the resistance of isolated *Salmonellae* to the two drugs might be due to their frequent application in local commercial poultry flocks. Istiana (1992) reported that *S.* Serovars Paratyphi and Weltevreden isolated from village chicks in Kalimantan were sensitive to tetracycline, chloramphenicol, trimethoprim, neomycin, polymyxin B and streptomycin, whereas *S.* Serovars Oubakam and Hadar were both resistant to tetracycline and streptomycin. Jha *et al.* (1994) also reported that nalidixic was one of five antibiotics highly effective in controlling *S.* Serovar Pullorum isolated from village chickens in Nepal. All 37 *S.* serovar Gallinarum-Pullorum strains isolated from commercial flocks (37) and backyard flocks (4) have been showed to be sensitive to the same antibiotics used in our study (Hoop and Albicker-Rippinger, 1997).

Although *S.* Serovar Typhimurium may still be reported as the most prevalent serotype in some countries, *S.* Serovar Enteritidis has replaced *S.* Serovar Typhimurium during the last 10-15 years as the commonest serotype in poultry in many countries (Poppe, 2000). In our study, four of 5 isolates were *S.* Serovar Typhimurium, indicating that it is the predominant serotype in the native chickens. This is in agreement with the authors' earlier study, resulting in the isolation of *S.* Serovar Typhimurium (4 isolates) and *S.* Serovar Enteritidis (one isolate) from 300 backyard hens' eggs sampled (unpublished data). Invasiveness and systemic dissemination have been documented very exclusively for *S.* Serovar Enteritidis. This potential, resulting in a more effective egg contamination, along with the increasing human's demand for poultry products rendered it to become the dominant isolated serotype in recent years from cases of human food poisonings in many countries (StLouis *et al.*, 1988). So, the isolated serotypes, particularly *S.* Serovar Enteritidis, can pose a risk for local industrial poultry and public health. Considering that the cloacal swabs were not assessed individually and that the shedding of *Salmonellae* in the feces of infected birds is often intermittent (Fanelli *et al.*, 1971), it is concluded that the actual infection rate in the chickens is likely to be more than our results (at least 5 of 422 chicks). Therefore, any prophylactic program aimed at controlling *Salmonella* infections must take into account the backyard chickens.

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