Salmonella infections in poultry flocks in the vicinity of Tehran

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Abstract

The purpose of this study was to survey infections with Salmonella spp. in poultry flocks in the vicinity of Tehran and to determine the most frequent serogroups and serotypes implicated. Twenty-eight samples of pullet, layer, and broiler flocks were randomly collected (n=1463), including freshly dropped feces from live birds or visceral organs from dead birds. In most flocks, 60 samples were taken and 10 fecal samples of each were pooled. Standard cultural methods were used for *Salmonella* spp. isolation. The slide agglutination or tube agglutination tests were performed using *Salmonella* somatic O poly A-S antisera, and different somatic O monovalent or flagellar H monovalent antisera. Thirty-one *Salmonella* isolates were recovered from 1,463 samples. Nine broiler flocks out of 14 (64.2%) and one layer flock out of 11 (9%) were positive for *Salmonella* spp. but all pullet flocks were negative. One isolate was obtained from the layer flock and the other 30 *Salmonella* isolates were obtained from broiler flocks. The slide agglutination test determined that all isolates belonged to one of the serogroups from A to S. The frequency of serogroups among 30 broiler isolates was found to be 76.6% and 13.3% for groups C and D, respectively. Three (10%) of the broiler isolates and one layer isolate did not belong to any of the A to D serogroups. All group D isolates were found to be *Salmonella enteritidis*. This study showed a high incidence of Salmonella in broilers. Infection of broilers with *Salmonella* spp. poses a high risk to public health.

Introduction

Salmonellosis is one of the most important food-borne diseases worldwide (Hendriksen, 2003; Valkenburgh *et al*., 2007; Gast, 2008). In addition to the risks to public health, *Salmonella* spp. infections impose economical losses to both healthcare systems and the poultry industry (Collard *et al*., 2008). More than 2,600 serovars of *Salmonella* have been identified, some of which are responsible for human illness and diseases in a wide variety of animals (Gast, 2008). Humans most often become infected after the consumption of contaminated eggs, poultry meat, pork, or, less frequently, bovine meat (Velge *et al*., 2005; Soltan Dallal *et al*., 2007; White *et al*., 2007; Collard et al., 2008). In order to manage the risk to public health, it is essential to investigate the prevalence of *Salmonella* infections at the farm level and counteract this problem to reduce the amount of cross contamination which can occur throughout the food chain process. Because animals are most often sub-clinically infected, the disease tends to spread easily within a herd or flock; additionally, because animals can become intermittent or persistent carriers, the prevalence of *Salmonella* spp. can be detected by routine sampling for bacteriological examination (Waltman *et al*., 1998; Gast, 2008). In this study, the prevalence of *Salmonella* infections in different types of flocks around Tehran was investigated and the most prevalent serogroups and serotypes was determined.

Materials and Methods

Sampling

We collected specimens from pullet, layer, and broiler flocks at different ages (Wilks *et al*., 2000). Each house in a farm was considered as a separate flock. In most cases, 60 freshly dropped fecal samples (not less than 1 g each) were randomly collected from each flock, each of which had at least 5,000 birds. Each set of 10 fecal samples were pooled. Anatomical samples (viscera and intestine) were also taken from dead birds in the aforementioned flocks. Totally, 1,463 of these samples were obtained from 28 flocks (Table 1).

Bacteriological culture

Isolation and identification of *Salmonella* spp. was performed according to standard procedures as previously

Table 1: Sampling data and results obtained in bacteriological and serological tests.

Flocks	Type	Age[1]	Sample	No. of samples (pooled)	Positive samples	Serogroup (serotype)
F1, H3	Layer	70 W	Feces	60 (6)	0	-
F1, H4	Layer	70 W	Feces	60 (6)	0	-
F1, H5	Layer	70 W	Feces	60 (6)	1	Not A to D
F1, H6	Layer	70 W	Feces	60 (6)	0	-
F2	Layer	44 W	Feces	20 (2)	0	-
F3, H6	Layer	66 W	Feces	60 (6)	0	-
F3, H7	Layer	66 W	Feces	60 (6)	0	-
F3, H10	Layer	151 W	Feces	60 (6)	0	-
F3, H11	Layer	152 W	Feces	60 (6)	0	-
F3, H12	Layer	128 W	Feces	60 (6)	0	-
F3, H13	Layer	94 W	Feces	60 (6)	0	-
F4, H1	Pullet	50 D	Feces	60 (6)	0	-
F4, H2	Pullet	50 D	Feces	60 (6)	0	-
F4, H3	Pullet	80 D	Feces	60 (6)	0	-
F5, H1	Broiler	8 D	Feces	60 (6)	0	-
F5, H2	Broiler	8 D	Feces	60 (6)	4	D (SE)
F5, H3	Broiler	11 D	Feces	120 (12)	9	C
F5, H4	Broiler	12 D	Liver - ceca	6 (2)	1	Not A to D
F5, H5	Broiler	12 D	Liver - ceca	6 (2)	1	Not A to D
F5, H6	Broiler	15 D	Feces	60 (6)	5	C
F5, H7	Broiler	15 D	Liver - ceca	6 (2)	2	C
F6	Broiler	45 D	Feces	120 (12)	0	-
F7, H1	Broiler	15 D	Feces	60 (6)	2	1- C 2- Not A to D
F7, H2	Broiler	15 D	Feces	60 (6)	0	-
F8	Broiler	1 D	Viscera - yolk sac	25 (5)	0	-
F9, H1	Broiler	43 D	Liver - ceca	10 (4)	2	C
F9, H2	Broiler	43 D	Feces	60 (6)	4	C
F10, H1	Broiler	1 D	Litter papers	10	0	-

1W = week, D = day

described (Waltman *et al*., 1998). Briefly, selective enrichment of samples in selenite F at 41°C for 24 hr was followed by sub-cultivation on *Salmonella-Shigella* and MacConkey agar at 40-41°C (37°C for tissue samples) for 24 hr. During the next step, the suspect colonies were identified and further characterized by biochemical identification. Positive samples were kept at -70°C and liquid nitrogen for future use.

Determination of serogroups and serotypes

The slide agglutination test was carried out using *Salmonella* somatic O poly A-S antisera (ProLab, England), as previously described (Waltman *et al*., 1998). Each suspect Salmonella culture was mixed with a drop of polyvalent antisera and incubated for up to 2 min at room temperature. Positive reactors in the slide agglutination test with polyvalent antisera were then tested separately with different somatic O monovalent (O2, O4, O5, O7, O8, O9, O12) and flagellar H monovalent (H2, H6, HL, Hgm) antisera (ProLab, England) available in our laboratory in order to determine the serogroups and serotypes of the isolates. Controls were run simultaneously in parallel in all tests. All negative results were re-tested by the tube agglutination test (Waltman *et al*., 1998).

Results

Thirty-one *Salmonella* isolates were recovered out of 1,463 samples from 28 flocks. In total, 10 infected flocks were found. Nine broiler flocks out of 14 (64.2%) and one layer flock out of 11 (9%) were positive for *Salmonella*, but all pullet flocks were negative. One isolate was obtained from the layer flock and the other 30 *Salmonella* isolates were obtained from broiler flocks. The slide agglutination test determined that all isolates belonged to one of A to S serogroups, as tested with somatic O antisera poly A-S. The frequency of serogroups among 30 broiler isolates was found to be 76.6% and 13.3% for groups C and D, respectively. Three (10%) of the broiler isolates and one layer isolate did not belong to any of the A to D serogroups. In five out of 10 *Salmonella* positive flocks, only group C were identified. One flock only showed the presence of *Salmonella* group D. Because individual antisera other than A to D were not available in our laboratory, further characterization was not performed. All group D isolates were found to be *Salmonella enteritidis*. The results are shown in Table 1.

Discussion

Salmonellosis (the illness caused by the *Salmonella* bacterium) is one of the most important zoonotic diseases of birds due to its economic impact and public health concerns. It is estimated that in the United States, the annual economic cost of salmonellosis is more than 2.6 billion USD in humans (Anonymous, 2009). Recent studies in the USA have found 4.3% rate of *Salmonella* positive samples from meat, poultry, and egg products (White et al., 2007). Recent surveys of *Salmonella* infection in the UK also detected a prevalence of 11.7% (54 out of 454) and 10.7% (41 out of 382) in commercial layer and broiler flock holdings, respectively (Snow *et al*., 2007; Snow *et al*., 2008).

In the present study, we sampled 28 flocks of different types and obtained 1,463 tissue samples, of which 31 (2.11%) were found to be positive for *Salmonella*. Broiler flocks were considerably more implicated in *Salmonella* infection than layer flocks. The reason for the lower prevalence of *Salmonella* spp. in layer flocks could be the declining rate of colonization and fecal shedding two weeks post-infection in laying

chickens. Consequently, it would be difficult to isolate Salmonella from pullet and layer flocks. Interestingly, it has been shown that *Salmonella* persists in the intestinal tracts or visceral organs of these birds for several months. Moreover, in some farms, the birds may be infected with *Salmonella* without showing signs of the illness, which means the presence of a sub-clinical infection in the flock. Feces from these flocks may contain Salmonella in low numbers (Hendriksen, 2003).

There are some risk factors for the prevalence and persistence of *Salmonella* in broiler chicken flocks (Angen *et al*., 1996; Namata *et al*., 2009). If a broiler flock is infected by *Salmonella*, the bacteria can persist within the flock if the prerequisites and procedures for cleaning and disinfection of the house are not adequate (Brown *et al*., 1992). Important factors in this respect could be if the house standards do not allow for satisfactory cleaning or if bacteria survive in or on beetles that live in the insulation of the building. The ability of *Salmonella* to resist desiccation allows it to survive for long periods in the environment. It has been shown that *Salmonella* remains in dust of ventilation filters for several months (Kim *et al*., 2007). Using well-trained workers for proper cleaning and disinfecting procedures will dramatically reduce such risk factors (Huneau-Salaun *et al*., 2007).

The exposure of flocks to external contamination is another important risk factor. In one study, there was a significantly increased risk of *Salmonella* contamination of the broiler flocks if there were more than three houses on a farm (Brown *et al*., 1992), which might increase the possibilities of transmission of *Salmonella* between houses. This might be due to the shorter time available for cleaning and disinfection before new stock is introduced to the farm, making it more difficult to follow the 'all in-all out' principle. Animal density might influence the infection pressure within the flock (Martin *et al*., 1987). Introducing only *Salmonella*-free chicks, such as by vaccinating the parental flocks against *Salmonella*, is an effective way to control vertical transmission but will not prevent the infection of birds with *Salmonella* from environmental sources if no additional hygienic measures are taken simultaneously. Measures to reduce horizontal transmission include ensuring Salmonella-free feed and water, effective cleaning and disinfection of the farm, the use of feed additives, applying 'all in-all out' procedures, appropriate biosecurity measures against animated or unanimated vectors, and other activities (Wales *et al*., 2007).

One study in Iran showed a high prevalence of group C *Salmonella* (S. Thompson) in poultry product samples (Soltan Dallal *et al*., 2007) that corresponds with our findings. Many studies have shown *Salmonella* group C serotypes such as S. hadar as the most common serogroup and serotype in chicken broiler flocks and chicken carcasses (Caldwell *et al*., 1995; Uyttendaele *et al*., 1998; Antunes *et al*., 2003). Unfortunately, due to lack of some serotype-specific antisera in our laboratory, we did not proceed with the serotyping to determine the predominant serotypes within the group C isolates.

Salmonella enteritidis (SE) has been one of the most common causes of food-borne infections in the last three decades (Velge *et al*., 2005). This serotype ranked among the top two most frequently isolated serotypes from human sources, as reported to the Center for Disease Control in 2006 (Anonymous, 2006). Poultry and poultry products are considered as major sources of SE infections for humans (Velge *et al*., 2005). Recent studies in the USA have shown that 1.3% of Salmonella isolates among samples from meat, poultry, and egg products were SE (White *et al*., 2007). In another US study, it was reported that 4.4% of Salmonella isolates from 51,327 broiler rinses were SE (Altekruse *et al*., 2006). Studies from the UK and The Netherlands' poultry flocks have also demonstrated that SE was the predominant serotype (van de Giessen et al., 2006; Snow et al., 2007; Snow et al., 2008). In our study, all group D isolates were identified as SE, which included 13.3% of broiler isolates.

In the present study, we have shown a high incidence of *Salmonella* spp. in broilers, which might be a potential vehicle for the transmission of drug-resistant *Salmonella* spp. to humans. These findings are important for the Iranian poultry industry and public health authorities.

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