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Original Article

Seroepidemiological Studies on Poultry Salmonellosis and its Public Health Importance

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ABSTRACT

Non-typhoid *Salmonella* serovars remain a potential threat to human health, and poultry species are possible sources of these organisms. In this study, trials for *Salmonella* isolation from poultry and humans were conducted in the period April 2009 through March 2010 in Beni-Suef Governorate, Egypt. Cloacal swabs were collected from different live poultry species including 150 broilers, 50 breeders, 50 layers, 50 turkeys, and 50 ducks, beside 30 litter samples from various poultry farms. Regarding the humans, stool samples as well as hand swabs were collected from 90 workers and poultry contacts of the examined farms. All poultry and human samples were subjected to bacteriological examination and serological identification for *Salmonella* spp. The recovered *Salmonella* strains were found belonging to *S.* Kentucky, *S.* Typhimurium and *S.* SaintPaul. The obtained results demonstrated that the occurrence of *Salmonella* spp. accounted for 16.66, 10.0, 2.0, 6.0 and 2.0% in broilers, breeders, layers, ducks and turkeys respectively. Investigation of litter samples revealed that the occurrence of *S.* Kentucky was 53.33, 66.66 and 28.57% in broiler's, breeder's and duck's litters respectively. Examination of human samples declared that 8 out of 90 hand swabs were found positive for *S.* Kentucky whereas all stool samples reacted negatively to all *Salmonella* spp. In the present study, conclusively Salmonella serovars (S. Kentucky) isolated from chickens were frequently isolated from hand swabs of the examined poultry contacts, this provided evidence that direct contact with poultry or poultry environment may pose health hazards for humans.

Key words: *Salmonella* spp.; Poultry; Humans; Litter

INTRODUCTION

Salmonellosis is considered to be one of the major bacterial disease problems in the poultry industry world-wide. *Salmonella* spp. cause asymptomatic intestinal infections in birds but acute outbreaks exhibiting clinical disease along with high levels of mortality occur in chicks younger than 2 weeks old (Duchet-Suchaux et al., 1995). In laying hens *Salmonella* can be highly invasive leading to systemic infections that can potentially be deposited in the internal contents of eggs by trans ovarian transmission following colonization of the intestinal tract (Woodward et al., 2005). Due to its endemic nature, high morbidity and association with a wide range of foods, salmonellosis is of high public health concern (Aarestrup et al., 2007 and Kottwitz et al., 2008).

There are more than 2500 serovars of *Salmonella enterica* that have been identified. The majority of human cases of non-typhoidal salmonellosis are caused by a limited number of serovars, which may vary from country to country and over time (Hendriksen et al., 2011). *S.* Enteriditis and *S.* Typhimurium have traditionally been the most frequently isolated serovars from humans worldwide (Fashae et al., 2010 and Hendriksen et al., 2011). However, other serovars have been reported to be more prevalent in specific regions or within countries.

Salmonella spp. in humans, are primarily a cause of self-limiting acute enteritis (diarrhea, abdominal pain, and fever, with a typical duration of 4–7 days). However, invasive *Salmonella* spp. can spread beyond the gastrointestinal mucosa to infect other sites such as the blood stream, the meninges, bone or joint spaces (Crump et al., 2011).

People generally acquire salmonellosis through food-borne exposure, although direct contact with infected animals is another possible route (Mead et al., 1999 and L Plym and Wierup, 2006). A variety of investigations of outbreaks and sporadic cases have indicated that food vehicles identified as the most common source of S*almonella* infections in humans are poultry and poultry products, including raw and uncooked eggs (Hennessy et al., 2004). The objective of the present work was to determine the zoonotic potential of *Salmonella* strains prevalent among various

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different poultry species (chickens, ducks and turkeys) and their human contacts.

MATERIALS AND METHODS

Sample collection

The study was conducted in Beni-Suef Governorate, Egypt (30°13′ N, 31°40′ E), situated 120 kilometers south to Cairo, Egypt with an average altitude of 46 meters. A total of 440 samples, including both poultry (350) and humans (90) were collected during the period from April 2009 to February 2010. The samples were investigated in the department of Hygiene, Management and Zoonoses, Faculty of Veterinary Medicine, Beni-Suef University for demonstrating the occurrence of *Salmonella* in poultry and man.

Poultry samples: Cloacal swabs were collected from different live poultry species including broilers (150), breeders (50), layers (50), turkeys (50) and ducks (50) obtained from various poultry farms. The swabs were directly immersed into sterile tubes containing Buffered Peptone Water (BPW).

Litter samples: A total of 30 litter samples were obtained from the farms of the examined broilers (15), breeders (3), layers (3), turkeys (2) and ducks (7). About 2 g. from each litter was taken and directly immersed into sterile tubes containing BPW.

Human samples: Stool samples as well as hand swabs were collected from 90 workers and poultry contacts of the examined farms. The stool samples were received in sterile plastic containers where a loopful from each sample was inoculated into a sterile tube containing BPW. The hand swabs were also inoculated into sterile tubes containing BPW.

Isolation, identification and serotyping of *Salmonella*

Salmonella cultures from all samples were performed according to Collee et al. (1996) and Waltman (1999). Briefly, samples that were collected in buffered peptone water (BPW) were taken to the laboratory on the day of collection under refrigeration with minimum of delay and incubated at 37 °C for 24 h. After pre-enrichment, 0.1 ml of the broth culture was transferred into a 10 ml Rappaport–Vasilliadis broth (RV broth, Difco, USA) and was incubated at 42 °C for 24–48 h. The RV broth samples were streaked onto Xylose-Lysine-Desoxycolate agar (XLD, oxoid) plates and incubated overnight at 37 °C. Typical colonies were picked and further tested by standard biochemical methods and serotyped using specific commercial sera according to the Kauffmann–White scheme (Kauffmann, 1974).

RESULTS AND DISCUSSION

The results illustrated in Table 1 demonstrated the occurrence of *Salmonella* spp. among different species of poultry including chickens, ducks and turkeys. In chickens, only *S*. Kentucky could be isolated from broilers, breeders and layers at a rate of 16.66%, 10.0% and 2.0% respectively. The occurrence of *S.* Kentucky in broiler chickens in this study well in line with the reports of Byrd et al. (1997) and Byrd et al. (1999) who declared that *S.* Kentucky and *S.* Heidelberg accounted for the highest percentage of total serovars isolated from commercial broilers production facilities. However these findings are lower than those detected by Caldwell et al. (1995). Furthermore the obtained data are much higher than those reported by Wedderkopp et al. (2001), Hegazy (2002) and Abd El-Hamid et al. (2004). On the contrary in many countries allover the world as e.g. in Ghana, (Sackey et al., 2001), France, (Lahellec et al., 1986), USA, (Payne et al., 2006) and Japan, (Shahada et al., 2006) a wide range of different *Salmonella* serovars were found to contaminate the broiler houses and flocks.

The high level of *Salmonella* isolation in broilers evaluated in this study may be attributed to horizontal and/or vertical transmission of *Salmonella* to the chicks. The horizontal transmission of *Salmonellae* can be mediated by mechanisms including direct bird-to-bird contact, ingestion of contaminated feeds, water or litter, or using contaminated equipments, while vertical transmission to the progeny of infected breeder flocks can result from internal or external contamination of eggs (Gast, 2003). This investigation highlights the importance of isolation of *Salmonella* from broilers as it was reported that slaughtering broiler flocks infected with *Salmonella* can lead to contamination both of the carcasses and the slaughter line (Corry et al., 2002 and Olsen et al., 2003).

On the other hand the percentage isolation of *Salmonella* spp. from breeders (10.0%) is higher than those found by El-Shamy (1999). Even though the breeders may be infected at the farm level by any way of transmission but the birds can also get infected easily from the transporting boxes which are used for different poultry species more than one time without disinfection or from the surrounding environment. Moreover the isolation of *Salmonella* from breeders could explain the theory of vertical transmission which lead to internal or external contamination of eggs followed by infection of newly hatched chicks (Gast, 2003).

Regarding the occurrence of *Salmonella* spp. in layers, the isolation rate (2.0%) is to some extent, in agreement with the data obtained by Ramadan (1997) who demonstrated that *Salmonella* was isolated with a percentage of 2.1% from layer farms. Indeed, *Salmonella* is considered as one of the most important causes of great mortalities and morbidities among laying flocks in addition to its public health importance as it can contaminate table eggs internally or externally (Humphrey, 1998).

The percentage isolation of *Salmonella* spp. from chickens (broilers, breeders or layers) generally in this study is more or less similar to that published by several previous authors as Cardinale et al. (2004), Saad (2007), Badr and Abd El Monaem (2008) and Muhammad et al. (2010) which ranged from 1.7% to 28.6%.

The fore mentioned results in Table 1 clarified that the total occurrence of *Salmonella* spp. in ducks was accounting for 6.0%. The detected *Salmonella* spp. were *S.* Typhimurium (4.0%) and *S.* SaintPaul (2.0%). Such finding is comparable to that achieved by Mohammed (1986). However these data are lower than those recorded by Abd El-Hamid et al. (2004) and Vo et al. (2006). Consequently it was possible in this study to confirm researchers indicating that *S.* Typhimurium was almost certainly the predominant serovar in ducks, as elsewhere, probably because in addition to horizontal transmission, it was the subject of vertical transmission by all routes (Henry, 2000).

The source of duck infection with *Salmonella* could be attributed to litter contamination, residual environmental contamination or true egg transmission occurred when the ducks' egg were infected before oviposition.

Table 1. Recovery of *Salmonella* spp. from cloacal swabs of the examined poultry species

Poultry species		Examined number	Number of samples positive for:			
			***S.* Kentucky (%)**	***S.* Typhimurium (%)**	***S.* SaintPaul (%)**	**Total (%)**
Chickens	Broilers	150	25 (16.66)	0 (0)	0 (0)	25 (16.66)
	Breeders	50	5 (10.0)	0 (0)	0 (0)	5 (10.0)
	Layers	50	1 (2.0)	0 (0)	0 (0)	1 (2.0)
Ducks		50	0 (0)	2 (4. 0)	1 (2.0)	3 (6.0)
Turkeys		50	0 (0)	1 (2.0)	0 (0)	1 (2.0)
Total		350	31 (8.86)	3 (0.86)	1 (0.29)	35 (10.0)

Table 2. Occurrence of *Salmonella* spp. in the examined poultry litters

Type of litter		Examined number	Number of samples positive for:		
			***S.* Kentucky (%)**	***S.* Typhimurium (%)**	**Total (%)**
Chickens	Broilers	15	8 (53.33)	0 (0)	8 (53.33)
	Breeders	3	2 (66.66)	0 (0)	2 (66.66)
	Layers	3	0 (0)	0 (0)	0 (0)
Ducks		7	0 (0)	2 (28.57)	2 (28.57)
Turkeys		2	0 (0)	0 (0)	0 (0)
Total		30	10 (33.33)	2 (6.66)	12 (40.0)

Table 3. Occurrence of *Salmonella* spp. in different specimens from the examined humans

Type of sample	No. of samples	Recovered *Salmonella* strains	
		***S.* Kentucky**	
		No.	**%**
Fecal samples	90	0	0
Hand swabs	90	8	8.88

Also in the view of the data illustrated in Table 1 it was clear that *S.* Typhimurium was the only serovar that could be recovered from turkeys at a rate of 2.0% . The current results support the report of Ferris et al. (1999) who revealed that the most commonly identified paratyphoid serovars in turkeys were *S.* Senftenberg, *S.* Heidelberg, *S.* hadar, *S.* Muenster, and *S.* Typhimurium. Furthermore high levels of *Salmonella* spp. were recorded by Aury et al. (2010).

In the contrary the current data are much higher than those reported by Abd-Allah (2003) who did not recover any *Salmonella* organism from the detected turkeys. Presumably infection of turkeys with *Salmonella* was transmitted laterally by direct contact between infected birds and uninfected turkeys or by indirect contact with contaminated environments through ingestion of *Salmonella* organisms. Among valuable turkey's breeder flocks, infection with *Salmonella* was generally accompanied by severe economic losses, because of their chronic nature and the difficulty of eradication. In many cases, the infection seriously impairs fertility, hatchability and egg production. The most important aspect, however, is the continuing effect of *Salmonella* contaminated

turkey meat and meat products on public health (Hafez and Jodas, 2000).

On discussing the hazards of poultry litter in the epidemiology of salmonellosis, Table 2 showed that the detected *Salmonella* spp. were *S.* Kentucky (53.33% in broiler's litter and 66.66% in breeder's litter) and *S.* Typhimurium (6.66% in duck's litter) while no *Salmonella* organisms could be recovered from layer and turkey litters. The obtained results are in harmony with that achieved by Orji et al. (2005). However the current data are higher than other findings in previous studies, as those obtained by Samaha et al. (2004) and Bayomi et al. (2006). Anyhow in the present study, *S.* Kentucky was the most prevalent serovar identified in litter samples. The current results are, to some extent, in agreement with the data obtained by Payne et al. (2006) who recorded that *S.* Kentucky was the most commonly isolated serovar from both litter and fecal dropping samples, and Li et al. (2007) who also demonstrated that *S.* Kentucky was the most commonly isolated serovar from processed fecal samples.

In this work, conclusively *Salmonella* serovars isolated from broiler's litter were frequently isolated from cloacal swabs of live birds, this provided evidence that litter play an important role as a source of *Salmonella* infection in poultry farms as the infection may arise from the contaminated litter or the infected birds may contaminate litter via their droppings.

Regarding the occurrence of *Salmonella* spp. in different specimens from the examined humans as shown in Tables 3 it was clearly obvious that all stool samples reacted negatively to all *Salmonella* spp. whereas 8 out of 90 (8.88%) hand swabs from poultry workers were *S.* Kentucky. The occurrence of *Salmonella* spp. in hand swabs are much higher than those obtained by Abd-Allah (2003) who isolated *Salmonella* spp. at the rate of 3.1% from hand swabs. It is worth mentioning that all the examined humans were apparently healthy (absence of fever and diarrhea). The detected isolation rate of *Salmonella* spp. in hand swabs of the examined humans in this study should be considered as a serious threat to public health, because *Salmonella* bacteria are usually transmitted to humans by the fecal-oral route (ingestion of foods contaminated with feces or contaminated by the unwashed hands of an infected food handler).

In the present study, conclusively *Salmonella* serovars (*S.* Kentucky) isolated from broilers, breeders and layers were frequently isolated from hand swabs of the examined individuals (poultry workers), this provided evidence that direct contact with poultry or poultry environment may pose health hazards for humans especially whom their occupation necessitate their contact with poultry.

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