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Occurrence of *Salmonella* sp in Laying Hens

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ABSTRACT

This study was carried out to investigate the presence of *Salmonella* sp in flocks of white laying hens. In different farms, the transport boxes of twelve flocks were inspected at arrival for the presence of *Salmonella*. Four positive (A, B, L and M) and one negative (I) flocks were monitored at each four weeks using bacteriological examination of cecal fresh feces up to 52 weeks. Birds were also evaluated at 52 weeks, when 500 eggs were taken randomly, and at 76 weeks, after forced molt. *Salmonella enterica* serovar Enteritidis and *S. enterica* rough strain were isolated from the transport boxes of the four positive flocks (flocks A, B, L and M). *Salmonella* sp was not isolated from the transport boxes or from the feces after 76 weeks-old in flock I. *Salmonella* sp was isolated in the 1st, 11th, 34th, 42nd and 76th weeks from flock A; in the 1st, 4th, 11th and 76th weeks from flock B; in the first week and in the 17th to 52nd weeks from flock L; and in the 1st and 76th weeks from flock M. *S.* Enteritidis, *S. enterica* rough strain and *Salmonella enterica* serovar Infantis were isolated from the four positive flocks. Besides, *Salmonella enterica* serovar Javiana was isolated from flocks B and L, and *Salmonella enterica* serovar Mbandaka was isolated from flock L. Eggs produced by flock A and by flock L were contaminated with *S.* Enteritidis and *S. enterica* rough strain. According to these results, *Salmonella*-infected flocks may produce contaminated eggs.

INTRODUCTION

Salmonella remains the main food borne bacterial diseases in human (Barrow, 2000), and many of the world outbreaks are related to food containing poultry products (Rodrigue *et al*., 1990; Cox, 1995). Besides, infected birds may develop the illness (Suzuki, 1994). Fowl are the specific host of *Salmonella enterica* serovars Pullorum and Gallinarum, which cause pullorum disease and fowl typhoid, respectively. Other serotypes with no specific host, such as Enteritidis and Typhimurium, may infect chickens and persist in the final poultry product, inducing or not clinical disease during rearing. Thus, the control of *Salmonella* in poultry flocks is crucial for poultry industry success.

Salmonella are introduced in poultry farms by several ways, including day-old infected chicks, domestic animals, human, equipment, water and feed (Barrow, 2000). Once the farm is contaminated, it is very difficult to eliminate *Salmonella* from the environment (Davies & Wray, 1995b). Factors such as the presence of wild birds, rodents, domestic animals and insects, as well as intensive production systems and multiple age flocks keep *Salmonella* on farms, which compromise eradication methods (Berchieri Junior & Barrow, 1995).

Paratyphoid salmonellas are responsible for poor performance of breeders, decreased egg production by layers, infertility and mortality. Furthermore, there is restriction for commercialization and egg consumption also decreases (Barrow, 2000). *Salmonella* infections may cause systemic disease in young and adult chickens, associated with clinical symptoms and lesions in organs like liver, spleen, heart, and mostly the ceca (Suzuki, 1994; Barrow, 2000). In addition, with or without clinical disease, *Salmonella* shedding occurs and the laid egg becomes contaminated (Suzuki, 1994; Barrow, 2000).

Infected breeder flocks are responsible for vertical transmission. The eggs are contaminated either from the ovary tissue or on their passage through the cloaca (Nakamura *et al.*, 1993). Vertical transmission of *Salmonella* was reported by Zancan *et al.* (2000), who found 44.45 % of positive transport boxes, and horizontal transmission of *Salmonella enterica* serovar Enteritidis (SE) was demonstrated by Gast & Holt (1999) and Soncini *et al.* (2000). They reported that birds free of bacteria in contact with inoculated birds became infected and excreted SE in the feces within the following 12 to 24 hours. The duration of fecal excretion is not easily defined. According to Gast & Holt (1998), laying hens experimentally exposed to SE soon after birth may remain infected until maturity, producing contaminated eggs and eliminating the bacteria to the environment. Conversely, Berchieri Júnior *et al.* (2001) reported that chicks orally infected with SE at 7 days of age presented systemic infection, and the bacteria were isolated from liver and ceca for 10 weeks, but not after this period.

Since the 80´s, the number of human food borne salmonellosis has increased worldwide and has been mostly related to the consumption of poultry products (Rodrigue *et al.*, 1990; Taunay *et al.,* 1996). On the other hand, it was demonstrated that the food was prepared with raw egg contaminated by SE (Duguid & North, 1991; Mishu *et al*., 1991; Cox, 1995; Barrow, 2000).

All control programs of infectious diseases are based on the knowledge of the routes of introduction and pathogen dissemination. In view of the lack of information about the epidemiology of avian salmonellosis in commercial poultry in Brazil, this work was carried out to investigate the presence of *Salmonella* in naturally infected flocks of commercial laying hens from newly hatched chicks up to 76 weeks of age. The presence of *Salmonella* was also investigated in eggs laid by hens at 56 weeks of age.

MATERIAL AND METHODS

Twelve flocks of white laying hens were inspected prior the beginning of the experiment (Table 1). *Salmonella* was searched inside all transport boxes when one-day-old chicks arrived at different farms (100 chicks/box). Four Salmonella-positive flocks and one negative flock were used in the experiment. Samples of feces were collected for bacteriological examination starting on the seventh day and at every four weeks thereafter, up to 52 weeks. Additional evaluations were performed in 500 eggs laid by hens with 52 weeks of age, and after molting in fecal samples from 76-week-old birds.

Experimental procedure was done according Wray and Davies (1994) recommendations.

Sampling of transport boxes

Samples were taken from the internal wall and bottom of the transport boxes using one large gauze swab per box, moistened in PBS pH 7.4. Five swabs were placed in a glass jar containing 100mL selenite broth (CM395) plus novobiocin (40mg/L) and incubated overnight at 42ºC. The broth was plated on brilliant green agar (Oxoid CM263) and MacConkey agar (Oxoid CM115) and plates were incubated overnight at 42ºC. Suspected colonies were inoculated

Table 1 – Isolation of *Salmonella* sp from transport boxes of one-day-old birds.

Flock	Number of birds	Serovar
A*	4,800	Enteritidis
B*	2,000	Enteritidis and R strain***
C	8,000	Negative
D	3,500	Negative
E	7,000	Negative
F	7,000	Negative
G	4,000	Negative
H	10,000	Negative
I**	2,000	Negative
J	7,000	Negative
L*	2,100	Enteritidis
M*	4,000	Enteritidis

* Positive flocks used to carry on the research.
**Negative flock used to carry on the research.
***R: rough strain.

in TSI agar (Oxoid CM277) and lysine iron agar (CM281), incubated overnight at 37ºC and confirmed using polyvalent sera against O and H *Salmonella* antigens (Probac). Complete identification and serotyping of the isolates were performed at the Adolfo Lutz Institute in São Paulo, Brazil.

Feces

Fresh samples of cecal feces were collected under the cages of the birds. Each sample corresponded to 200 birds up to 17 weeks and 100 birds afterwards. The samples were processed according to the procedure described above.

Eggs

Eggs were collected and placed in sterile trays, The trays were put in plastic bags and taken to the laboratory under refrigeration. Each egg was placed in a sterile glass jar and broken by agitation. The contents were mixed and the jars were incubated at 37ºC for 24 h. Bacteriological procedure was the same described above, except that Hektoen agar (Oxoid CM409) was used instead MacConkey agar.

RESULTS

The presence of *Salmonella* was investigated in 614 boxes corresponding to 12 flocks of one-day-old birds. *Salmonella* Enteritidis was detected in four flocks (129 boxes). In one of these flocks, *S. enterica* rough strain (R strain) was also isolated (Table 1). Samples were collected from four positive flocks (A, B, L and M) and one negative flock (I) until the end of the experiment (Table 2). The positive flocks showed the same result at the second sampling, but isolation varied afterwards. In flock A, *Salmonella* was isolated from fecal samples at 11, 34, 42 and 76 weeks of age. *Salmonella* was isolated from fecal samples collected from flock B at 4, 11 and 76 weeks of age. Flock L presented positive results for *Salmonella* isolation in all samplings from 17 to 52 weeks of age. Flock L was not evaluated at 76 weeks of age because it was depopulated. *Salmonella* was not isolated from flock M between 4 and 52 weeks of age, but it was detected at 76 weeks of age. Flock I presented negative results throughout the experimental period, even after molting. Several serovars of *Salmonella* were isolated (Table 3). *S.* Enteritidis, rough strain and *S. enterica* serovar Infantis were isolated from fecal samples from the four positive flocks, *S. enterica* serovar Javiana was isolated from fecal samples from flocks B and L, and *S. enterica* serovar Mbandaka was isolated only from flock L.

Salmonella was studied in 500 eggs from each flock (Table 4). SE and R strain were found in one egg from flock A (0.2%) and R strain was also isolated from 10 eggs from flock L (2.0%).

DISCUSSION

Salmonella was detected in four transport boxes (33.3%) from a total of 614 boxes with one-day-old laying hens. Similarly, *Salmonella* was found in 20% of the samples of meconium and fluff (Bhatia & Mcnabb, 1980), 17% of the samples taken from commercial hatchery residues (Bailey *et al*., 1994), 26% of the samples of transport boxes and egg shell (Cox *et al*., 1997) and 44.45% of transport boxes (Zancan *et al*., 2000).

Table 2 – Isolation of *Salmonella* sp from transport boxes and feces of laying hens.

FLOCK	AGE (weeks)															
	0(1day)	1	4	8	11	14	17	20	25	30	34	38	42	48	52	76(molt)
A	+	+	_	_	+	_	_	_	_	_	+	_	+	_	_	+
B	+	+	+	_	+	_	_	_	_	_	_	_	_	_	_	+
L	+	+	_	_	_	_	+	+	_	+	+	+	+	+	+	NP
M	+	+	_	_	_	_	_	_	_	_	_	_	_	_	_	+
I	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_

(+): Isolation of *Salmonella*.
(-) : No isolation of *Salmonella*.
NP: not performed.

Table 3 – *Salmonella* sp serovares isolated from transport boxes and feces of light white laying hen flocks.

AGE (weeks)	FLOCKS			
	A	B	L	M
0 (1 day)	Enteritidis	Enteritidis, R strain*	Enteritidis	Enteritidis
1	Enteritidis, R strain	Enteritidis, Infantis, R strain	Enteritidis, R. strain	Enteritidis, R. strain
4	- **	Enteritidis	-	-
8	-	-	-	-
11	Enteritidis	Enteritidis	-	-
14	-	-	-	-
17	-	-	Infantis	-
20	-	-	Enteritidis, Javiana, Mbandaka, Infantis	-
25	-	-	-	
30	-	-	R strain	-
34	Infantis	-	R strain	-
38	-	-	Enteritidis, R strain	-
42	Enteritidis, R strain	-	Enteritidis, R strain	-
48	-	-	R strain	-
52	-	-	Enteritidis, R strain	-
76	Infantis, R strain	Javiana	-	Infantis

*R: rough strain.
**No isolation of *Salmonella.*

Table 4 – Serovar of *Salmonella* sp isolated from eggs produced by 52-week-old layers.

Flocks	Number of analyzed eggs	Number of positive eggs	Positive eggs (%)	Serovars
A	500	1	0.2	Enteritidis and *R strain
B	500	0	-	-
L	500	10	2.0	R strain
M	500	0	-	-
I	500	0	-	-

*R strain: rough strain.

The presence of *Salmonella* sp in transport boxes of day-old birds strongly suggests the occurrence of vertical transmission (Lister, 1988; Mc Ilroy & Mac Craken, 1990). Bacteria are present in chicks for a long period of time when they are exposed to *Salmonella* at the end of the hatchery period or during the first hours of life, and may be disseminated to other susceptible chicks in the same flock or other flocks (Gast & Holt, 1998). Therefore, the first step to prevent *Salmonella* introduction in farms is to obtain *Salmonella*-free chicks, avoiding lateral transmission. Since there are many sources of *Salmonella* contamination in commercial poultry production, the negative results found in flock I cannot be attributed only to the introduction of non-infected chicks. However, the results suggest that obtaining *Salmonella* free-birds is the first step for preventing *Salmonella* on the farm.

Salmonella serotypes other than those isolated from the transport boxes were detected during rearing and production in the four positive flocks. Besides vertical transmission, *Salmonella* may be introduced in poultry farms by several ways, mainly by feed containing meal of animal origin (Berchieri Júnior *et al*., 1993). Rodents have great importance on the epidemiology of avian salmonellosis, because they maintain paratyphoid salmonellas such as serotypes Enteritidis and Typhimurium in the poultry houses (Davies & Wray, 1995a). According to these authors, even after cleaning and disinfection, poultry houses are re-contaminated by infected resident rodents. Furthermore, the deficiency on the disinfecting process may contribute to the maintenance of *Salmonella* at farms (Davies & Wray, 1995b). It should be also considered that light lines of birds are supposed to be resistant to *Salmonella*. In fact, the hens did not show signs of illness although they were infected and bacteria were shed for some time. Therefore, although natural and individual resistance of birds might be considered (Protais *et al*., 1996; Duchet-Suchaux *et al*., 1997; Bumstead, 2000), it is necessary to remember that while commercial lines of chickens are homogeneous for production, there is not enough knowledge about their susceptibility to infection. Moreover, genes responsible for the resistance to systemic infection are not related to resistance to the colonization of the intestinal tract by *Salmonella* (Bumstead, 2000).

Several factors might be related to the non-isolation of *Salmonella* sp from fecal samples in flocks B and M during the first production cycle and the absence of infection in Flock I. Birds acquire natural resistance against enteric pathogens with the gradual development of the intestinal flora and the immune system (Suzuki, 1994; Gast, 1997). In addition, an appropriate cleaning and disinfection program associated with a good quality of living birds might decrease the risk of environmental contamination (Barrow, 1999).

Molting is a debilitating process used to extend the productive life of the birds. During molting, birds become more susceptible to infection by pathogens. In fact, flocks A, B and M were evaluated after molting (76 weeks-old) and were excreting *Salmonella*, similar to what was reported by Nakamura *et al.* (1994), Holt (1995), and Macri *et al*.(1997).

The percentage of contaminated eggs was 0.2 and 2.0% in Flocks A and L, values in accordance to the values of 0.1 to 10.0% reported by Humphrey (1994). Although *Salmonella* was detected in few eggs, inadequate storage conditions and food prepared with raw eggs can be a serious threat to human health (Humphrey *et al*., 1989). Eggs may become contaminated by *Salmonella* in the ovary and oviduct (Thiagarajan *et al*., 1994; Keller *et al*., 1995; Miyamoto *et al*., 1997). Most of the times, however, contamination occurs in the cloaca during egg passage. It is well known that the number of *Salmonella*-contaminated eggs decreases, together with a decrease in fecal shedding.

Most food-borne infections caused by *Salmonella* in humans were associated to food like mayonnaise, ice cream and frozen desserts, which are consumed without being cooked after raw egg is added. Provided that few *Salmonella* organisms are present on egg shells or egg contents, they multiply in a few minutes during storage at room temperature (Duguid & North, 1991).

Similar to the results described by Zancan *et al*. (2000), SE was also predominant among the serotypes isolated from transport boxes of one-day-old chicks. This serotype was also frequently isolated from chicken feces after one week of age and was detected in eggs laid by flock A. SE has been associated to food-borne disease since the last century (Barrow, 1993). However, in the last decades, there was an increase in human cases, and most cases were associated to poultry products (Barrow, 2000). In the present work, SE was detected in chicks from flocks A from one-day old until the end of the trial, resulting in the production of contaminated eggs. Besides, SE intoxication also increased in Brazil in the last years (Taunay *et al*., 1996; Hofer *et al*, 1997) and thus, it is evident that more efficient control measures are needed.

Salmonella Infantis, also isolated from fecal samples from four flocks, was implicated in food

borne human salmonellosis in Finland related to consumption of poultry meat (Nurmi & Rantala, 1973). *S.* Infantis and *S.* Mbandaka were isolated from eggs, from flies and other insects present in the chicken houses, and from swab samples taken from the environment (Jones *et al*., 1995; Kinde *et al*., 1996; Olsen & Hammack, 2000). These two serotypes were also isolated from humans presenting symptoms of salmonellosis (Scheil *et al*., 1998; Dera-Tomaszewska & Glosnicka, 1999). According to Hoszowski & Wasyl (2001), domestic poultry is the main source of *S.* Mbandaka infection for men.

Salmonella Javiana isolated from feces in Flocks B and L had already been detected in domestic chickens by Adesiyun *et al*. (1998). It also causes gastroenteritis in human and was considered the sixth most incident serotype in 1996 by CDC, in USA. In Brazil, according to Tavechio *et al*. (1996) and Villa (2000), S.Infantis, S. Mbandaka and S. Javiana are among the serotypes frequently isolated in samples from human, poultry and other animal sources, from the environment and from feed and feedstuffs.

In many occasions rough strains of *Salmonella* were isolated. The biochemical behavior was compatible with the profile of paratyphoid strain. Some evidence suggested they might be of the serovar Enteritidis, nevertheless, the impossibility of identifying them hinders any epidemiological analysis.

In the present experiment the presence and the persistence of *Salmonella* were shown in laying hen flocks since their arrival until the end of the production period. These results demonstrated that improvements are needed in *Salmonella* control program because those currently adopted have not been able to prevent the introduction and dissemination of salmonellas on poultry farms, as well as egg contamination.

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