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Incidence of *Salmonella* in Imported Day-Old Ducklings. Brazil, 1998-2003

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Salmonella, imported ducklings, isolation.

ABSTRACT

The occurrence of *Salmonella* in a samples of 40 imported day-old duckling flocks was assessed from 1998 to 2003 according to the guidelines of the Brazilian National Poultry Health Program (Programa Nacional de Sanidade Avícola-PNSA). The pathogen was recovered from 26 flocks (65.0%). The most common serovars were *S. Saintpaul* and *S. Kottbus*. Up to four serovars were isolated from a single flock. Transportation box swabs (82.6%) and yolk sac pool (47.1%) showed the highest and the lowest frequency of *Salmonella* isolation, respectively. The high percentage of *Salmonella* isolation from imported day-old ducklings causes concern because of the zoonotic potential of this agent and its economical importance to commercial poultry breeding.

INTRODUCTION

Salmonellae are enterobacteria distributed worldwide and grouped in 2,541 serovars (CDC, 2004). Approximately 2,000 of them are potentially pathogenic to humans (OIE, 2000). It is estimated that 1.4 million people are affected by salmonellosis yearly in the United States, causing around 16,800 hospitalizations (1.2%) and 600 deaths (Mead *et al.*, 1999).

Considering the possible transmission routes of *Salmonella* to humans, it is worthy mentioning the direct contact with animals like reptiles and birds, which is especially important to infections in children (CDC, 2000), and the consumption of contaminated food, mainly poultry-derived foodstuffs (Dansk Zoonosecenter, 1998). In 2002, a total of 16,580 cases caused by foodborne agents were diagnosed in the United States. From these, *Salmonella* was the most common infectious agent (6,028 cases) (CDC, 2003).

Although ducks are very resistant to systemic infection caused by *Salmonella*, they are potential reservoirs of this organism and may shed it in the feces, contaminating the environment (Barrow *et al.*, 1999). Outbreaks of human salmonellosis caused by contact with ducks have been reported in some countries, such as Australia, United States, United Kingdom and Denmark (Merritt & Herlihy, 2003; CDC, 2000; Public Health Laboratory Service, 2000; Dansk Zoonosecenter, 1998). Moreover, the consumption of contaminated duck eggs has been reported as the cause of salmonellosis outbreaks in Italy, Thailand and United States (Nastasi *et al.*, 1998; Saitanu *et al.*, 1994; Baker *et al.*, 1985).

In Brazil, the role of ducks as a source of *Salmonella* infection is unknown and the literature about this issue is scarce. According to a report from the Laboratório de Enterobactérias of the Departamento de Bacteriologia (Instituto Oswaldo Cruz - FIOCRUZ), *Salmonella* has been isolated from 143 samples (6,7%) of the all collected by ducks, from 1962 to 1991 (Hofer *et al.*, 1997).



In spite of the few data about duck production and commercialization, and the consumption of duck meat and by-products in Brazil, more than 70,000 day-old ducklings were imported between 1998 and 2003 (LANAGRO/SP, unpublished results).

Imported animals represent a risk of introducing infectious agents in the country, including different serovars of *Salmonella* (Ribeiro *et al.*, 2003). Therefore, the National Poultry Health Program (Programa Nacional de Sanidade Avícola-PNSA) requires that all imported birds and egg batches should be analyzed for infectious agents, including *Salmonella* (Brasil, 1999).

The aim of the present study was to report the occurrence of *Salmonella* in day-old ducklings imported by Brazilian companies from 1998 to 2003.

MATERIALS AND METHODS

Samplings were performed following current governmental guidelines (Brasil, 1999). All samples were collected in airports by inspectors from MAPA. Twenty day-old ducklings were collected per flock. Dead ducklings, when present, were also sampled. In such cases, a maximum of 20 dead birds per flock were used. Swabs were individually taken from transportation boxes of each imported flock (maximum 30 boxes per flock). After sampling, swabs were placed into 1% buffered peptone water for transportation and taken to the laboratory (Laboratório Nacional Agropecuário-LANAGRO/SP - MAPA). The samples were analyzed according to the National Poultry Health Program (PNSA) guidelines for imported birds (Portaria 126, November 3rd, 1995 - MAPA).

Ducklings were killed by decapitation and necropsied, and pools of internal organs (liver, spleen and gall bladder), of yolk sac and caecum were collected from each flock. From ducklings that were dead at the time of sampling, only a pool of internal organs was collected. Pools of internal organs, yolk sac, caecum and swab of transportation box were enriched in both tetrathionate and Rappaport-Vassiliadis enrichment broths for 24 hours at two different temperatures (37°C and 42°C). Afterwards, an aliquot of each broth was transferred to three semi-solid media: MacConkey agar, Hektoen agar and Brilliant-green Phenol-red Lactose Sucrose agar with Novobiocin, which were incubated at 37°C for 24 hours. Isolation of suspected colonies was performed in Rambach agar at 37°C for 24 hours. Suspected colonies were confirmed as *Salmonella* genus by biochemical tests.

Salmonella isolates were serotyped as *S. Enteritidis*, *S. Pullorum*, *S. Typhimurium*, *S. Gallinarum* or *S. sp.* by slide agglutination test. These four serovars were herein considered because they are included in the Brazilian National Poultry Health Program (PNSA). Serotyping was performed or the results were confirmed (in case of the four above mentioned serovars) at a reference laboratory (Laboratório de Enterobactérias - Departamento de Bacteriologia - Instituto Oswaldo Cruz - FIOCRUZ).

RESULTS

From 1998 to 2003, three different companies imported 40 flocks of day-old ducklings from the same country. Among them, 26 (65.0%) flocks were positive for *Salmonella* (Table 1).

Salmonella Saintpaul (29.8%) and *S. Kottbus* (26.3%) were the most frequently isolated serovars. The highest number of different *Salmonella* serovars obtained from a single flock was four. The most common association of serovars was *S. Saintpaul* and *S. Kottbus* (Table 2, Figures 1 and 2). The samples with the highest and the lowest frequencies of *Salmonella* in positive flocks were swabs taken from transportation boxes (82.6%) and yolk sac pools (47.1%), respectively (Table 3 and Figure 3).

Table 1 - Frequency of *Salmonella* isolation in flocks of day-old duckling imported from 1998 to 2003.

Year	Imported flocks (n)	Number of positive flocks (%)
1998	04	04 (100%)
1999	06	01 (16.7%)
2000	02	01 (50.0)
2001	07	04 (57.1)
2002	09	06 (66.7)
2003	12	10 (83.3)
TOTAL	40	26 (65.0)

DISCUSSION

The high frequency of *Salmonella* recovery from imported day-old ducklings causes great concern because of the zoonotic potential of this pathogen and its economical importance to commercial poultry breeding. Macroscopic lesions were not observed in the dead or sacrificed birds, which may be associated with the inability of some serovars to multiply in the duck reticulo-endothelial system (Barrow *et al.*, 1999). However, the isolation of *Salmonella* from different organs suggests that ducks may harbour *Salmonella* in their viscera and shed it in the faeces.



Table 2 - *Salmonella* serovars isolated from positive flocks of day-old duckling imported from 1998 to 2003.

Flock Numbers	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
S. Saintpaul							X		X	X	X	X	X	X	X	X	X	X		X	X	X		X	X	X
S. Kottbus	X	X	X	X						X	X			X	X	X	X		X	X	X	X	X			
S. sp (6,8:ch:-)	X			X	X	X			X	X								X	X							
S. Indiana														X	X			X	X		X	X		X	X	
S. Newport							X							X												
S. sp (4,5:ch:-)												X														X
S. Agona					X																					
S. London					X																					
S. Chester							X																			
S. Kentucky																X										
S. sp (6,8:-1,5)															X											

Table 3 - *Salmonella* isolation from different specimens taken from imported day-old ducklings from 1998 to 2003.

Flock	Liver pool (Live birds)	Yolk sac pool	Caecum pool	Transportation box swab	Liver pool (Dead birds)
1	ND	ND	ND	-	+
2	ND	ND	ND	+	+
3	+	+	WI	WI	ND
4	-	ND	-	+	ND
5	+	ND	-	-	ND
6	+	+	+	+	ND
7	-	ND	+	-	-
8	WI	WI	WI	WI	WI
9	-	-	+	+	ND
10	ND	ND	ND	+	-
11	-	-	-	+	ND
12	WI	WI	WI	WI	WI
13	+	+	+	+	+
14	+	+	+	+	ND
15	+	+	+	+	+
16	+	+	+	+	ND
17	+	-	+	+	ND
18	+	-	+	-	ND
19	+	-	+	+	+
20	+	ND	+	+	+
21	+	+	+	+	ND
22	+	-	+	+	+
23	-	-	-	+	-
24	+	+	+	+	ND
25	+	-	+	+	+
26	+	-	-	+	+

ND = Not done; WI = Without information; (+) = Positive flocks; (-) = Negative flocks.

Ducks are probably asymptomatic reservoirs of *Salmonella* and, consequently, may act as infection sources to other species, including humans. The isolation of *Salmonella* Indiana in frozen duck meat for human consumption (Food Microbiology Department, LANAGRO/SP-MAPA, unpublished results) corroborates this hypothesis.

The low frequency of *Salmonella* in yolk sacs occurred because this is a vestigial organ in ducks and it is quickly absorbed in the first days after birth. In most cases, the sampling of this organ was not possible (see Table 3). Nevertheless, the high percentage of isolation from liver, caecum and swabs from transportation boxes (75.0% to 82.6%), suggest that these are the most appropriate specimens for *Salmonella* recovery.

Brazilian official guidelines recommend that breeder farms should be free of *S. Gallinarum*, *S. Pullorum*, *S. Enteritidis* and *S. Typhimurium*, but other serovars are not considered (Brasil, 2003). However, it is known that some serovars like *S. Indiana*, *S. Newport* and *S. Saintpaul* have caused outbreaks of salmonellosis in human (Public Health Laboratory Service, 1996; CDC, 2002; Hata *et al.*, 2003). It is suggested that the current guidelines should be reviewed in order to include other serovars, especially those of public health importance.

In the present study, more than one *Salmonella* serovar were recovered from most of the positive flocks and four serovars were cultured from a single flock (Figure 2). These results indicate that ducklings may eliminate different serovars concomitantly,

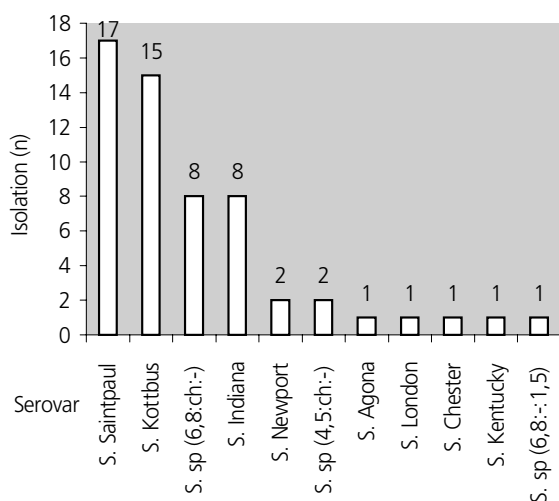


Figure 1 - *Salmonella* serovars isolated from imported day-old ducklings from 1998 to 2003.

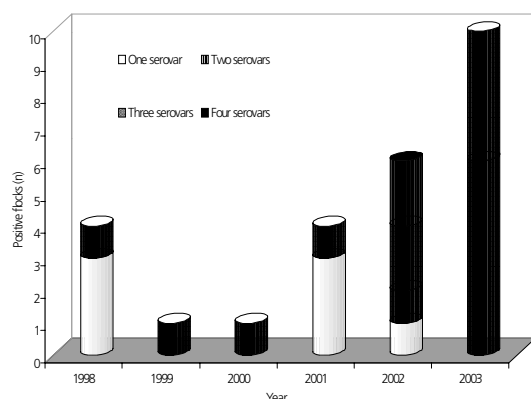


Figure 2 - Number of different *Salmonella* serovars isolated from a single flock of imported day-old ducklings.

showing the importance of this species for *Salmonella* spreading.

Further studies on salmonellosis pathogeny in ducks and epidemiological surveillance data from commercial duck breeding are fundamental to clarify the importance of this species on the epidemiology of *Salmonella* and to establish more severe procedures to control this important zoonosis.

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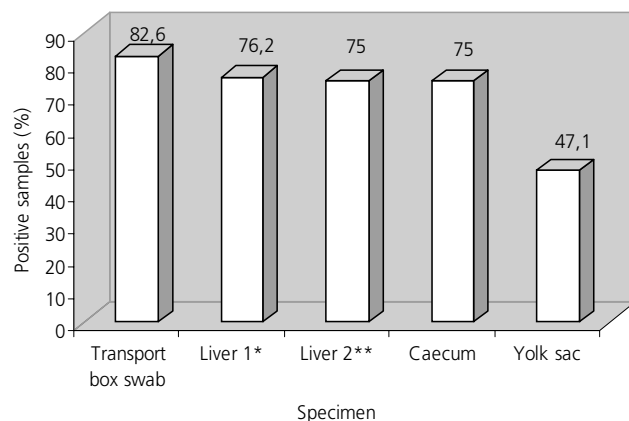


Figure 3 - Percentage of positive samples for *Salmonella* isolation, according to the material analyzed, among imported day-old ducklings flocks positives to *Salmonella* isolation.

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