Presence of *Salmonella* spp. in One-day-old Chicks from Hatcheries in the Metropolitan Region of Fortaleza, Brazil

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**ABSTRACT**

**Background:** Salmonellosis is caused by bacteria of the genus *Salmonella* and is a worldwide-considered major zoonosis with a risk for the public health due to the capacity of dissemination between animals and nature. In the poultry industry, day-old chicks may acquire the infection at the hatchery through contact with fomites and eggshells with the presence of contaminated feces, favoring the animal infection and may become a source of infection to other birds in the lot, as well as to several other animals, including man. The aim of this study was to verify the presence of *Salmonella* spp. in one-day-old chicks from hatcheries in the metropolitan region of Fortaleza, Brazil.

**Materials, Methods & Results:** A transversal study with a convenience sampling was performed in 510 day-old chicks acquired from five hatcheries located in the metropolitan region of Fortaleza, Brazil. From each hatchery, 102 day-old chicks were analyzed at the Laboratory of Ornithological Studies (LABEO) of the State University of Ceará. Individual cloacal swab samples were collected immediately after arrival, following euthanasia by cervical dislocation for organ sampling, comprised of a pool of livers and yolk sacs of three birds per sample. Organ and cloacal samples were incubated in pre-enrichment using buffered peptone water 0.1%, following selective enrichment with the broths: Tetrathionate, Rappaport-Vassiliadis and Selenite-Cystine. After incubation, samples were streaked in Brilliant Green agar added Novobiocin (40 µg/mL) and MacConkey agar. For all the microbiological procedures, samples were incubated at 35 to 37°C for 18 to 24 h. Suspect colonies were identified by biochemical tests and confirmed with polyvalent O anti-*Salmonella* serum. *Salmonella* isolates were submitted to disk-diffusion method (Kirby-Bauer) following the National Committee for Clinical Laboratory Standards (NCCLS) guidelines. With the methodology applied, *Salmonella* spp. was not isolated from yolk sac or liver samples. From the five analyzed hatchery, two (40%) were positive for *Salmonella* Enteritidis. From the 510 samples analyzed, three were positive for *Salmonella* Enteritidis, resulting in a prevalence of 0.58% of all the cloacal swabs evaluated and the positive hatcheries presented prevalences of 1.96% and 0.98%. All three isolates were resistant to azithromycin and nalidixic acid in the susceptibility test.

**Discussion:** The resistance of *Salmonella* to nalidixic acid in this study demonstrates the excessive use of this antibiotic as a prophylactic measure in breeder farms, therefore favoring the occurrence of resistant strains, which may be transmitted to other birds, animals and to man. The azithromycin resistant strains isolated in this study are important findings since this antibiotic is widely used in human treatment of infectious diseases that affect the genitourinary tract, respiratory system, oral cavity and skin. The results found in this study suggest further caution with the presence of *Salmonella* Enteritidis in the poultry industry since this pathogen is widely distributed among animals and constant monitoring of these bacteria in broiler breeders and in layer hens is necessary. In addition, strengthening the biosecurity programs of parent stock farms, transportation, eggs manipulation and in hatcheries at the beginning and end of each producing cycle should also be applied. The presence of salmonella in a single day-old chick is a concerning fact considering the dissemination capacity of *Salmonella* in the first days of life to the other birds in the lot during the developing period possibly reaching human contact leading to a public health issue due to foodborne infections.

**Keywords:** *Salmonella* Enteritidis, hatchery, azithromycin, public health.
INTRODUCTION

Salmonellosis is caused by microorganisms of the genus *Salmonella* and is one of the most problematic zoonosis affecting the health of men [17] and animals [4].

In the poultry industry, day-old chicks may get infected at hatchery through contact with fomites and eggshells with the presence of contaminated feces, which may trigger the animal infection through penetration and multiplication of the paratyphoid salmonella inside the egg [2]. Such process may harm the embryonic development and become a source of infection to other chicks [23] as well as several animals, including man [3].

In order to control *Salmonella* spp. in the poultry farming, certain hygiene measures must be taken in the industry and in the field, where the efforts to reduce the level of bacterial colonization of the intestinal tract of birds should be directed [38].

Antibiotics are commonly used as a control measure of bacteria in the poultry industry added in the birds feed aiming a prophylactic effect [30], favoring the occurrence of resistant salmonella strains which is a risk to the public health [29].

In this context, due to the wide distribution of these bacteria in nature, also to the variety of unspecific serotypes and hosts, and the abundance of multidrug resistant strains [5], this study aimed to verify the presence of *Salmonella* spp. in day-old chicks from hatcheries in the metropolitan region of Fortaleza, Brazil.

MATERIALS AND METHODS

Samples

From October 2013 to February 2014, transversal study with a convenience sampling [39] was performed in 510 day-old chicks from five hatcheries located in the metropolitan region of Fortaleza, Ceará State, Brazil.

From each hatchery, 102 day-old chicks were analyzed immediately after arrival at the Laboratory of Ornithological Studies (LABEO) at the State University of Ceará. Individual cloacal swabs were collected from all birds following euthanasia by cervical dislocation.

Laboratory method

From all birds organs were collected and analyzed in a pool of liver and pool of yolk sacs of three birds per sample. The isolation of *Salmonella* spp. from organs and cloacal swabs was performed according to the current law [6]. Samples were incubated in pre-enrichment using buffered peptone water 0.1%, following selective enrichment with the broths: Tetrathionate, Rappaport-Vassiliadis and Selenite-Cystine. After incubation, samples were streaked in Brilliant Green agar added Novobiocin (40 µg/mL) and MacConkey agar.

For all the microbiological procedures, samples were incubated at 35 to 37°C for 18 to 24 h. Suspect colonies were identified by biochemical tests and confirmed with polyvalent O anti-*Salmonella* serum [6].

Isolated *Salmonella* were submitted to disk-diffusion method, Kirby-Bauer, following the National Committee for Clinical Laboratory Standards - NCCLS guidelines [26]. Plates with Müller-Hinton agar were inoculated with the bacterial isolate, antimicrobial disks were placed after incubation, and the inhibition zones were measured and interpreted as susceptible or resistant according to the standards established by NCCLS [26]. The following antimicrobial disks were used with the respective concentrations: ceftiofur (30 µg), ciprofloxacin (5 µg), enrofloxacin (5 µg), streptomycin (10 µg), gentamycin (10 µg), sulfonamide (300 µg), sulfamethoxazole-trimethoprim (25 µg), nalidixic acid (30 µg), ampicillin (10 µg), polymyxin B (300 µg), tetracycline (30 µg) and azithromycin (15 µg).

RESULTS

In this study, there was no isolation of *Salmonella* spp. from yolk sacs or liver samples.

Table 1 demonstrates the relative and absolute frequencies of day-old chicks positive for *Salmonella* sp. from hatcheries in the Metropolitan region of Fortaleza, Brazil, analyzed by cloacal swabbing.

From the five hatcheries analyzed, two (40%) presented positive results for *Salmonella* Enteritidis. Among the 510 samples, three were positive, which resulted in a 0.58% prevalence of all cloacal swabs evaluated, and from the two positive hatcheries the prevalences were 1.96% and 0.98%. All *Salmonella* isolates were identified as serotype Enteritidis (Table 1).

The antimicrobial susceptibility test performed in the three *Salmonella* isolates demonstrated the same pattern, which was resistance only to azithromycin and nalidixic acid (Table 2).
Table 1. Relation between chicks positive for *Salmonella* sp. (cloacal swab) and total analyzed birds from hatcheries in the Metropolitan region of Fortaleza, Brazil.

<table>
<thead>
<tr>
<th>Hatchery</th>
<th><em>Salmonella</em> sp.</th>
<th>Positive</th>
<th>Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Isolated</td>
<td>2/102</td>
<td>1.96</td>
</tr>
<tr>
<td>2</td>
<td>Not isolated</td>
<td>0/102</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Not isolated</td>
<td>0/102</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Isolated</td>
<td>1/102</td>
<td>0.98</td>
</tr>
<tr>
<td>5</td>
<td>Not isolated</td>
<td>0/102</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>-</td>
<td>3/510</td>
<td>0.58</td>
</tr>
</tbody>
</table>

Table 2. Antimicrobial susceptibility of *Salmonella* sp. isolated from day-old chicks from hatcheries in the Metropolitan Region of Fortaleza, Brazil.

<table>
<thead>
<tr>
<th>Strains (Hatchery)</th>
<th>azi</th>
<th>est</th>
<th>sul</th>
<th>cip</th>
<th>sut</th>
<th>tet</th>
<th>eno</th>
<th>cft</th>
<th>gen</th>
<th>amp</th>
<th>nal</th>
<th>pol</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Enteritidis (1)</td>
<td>r</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>r</td>
<td>s</td>
<td>s</td>
</tr>
<tr>
<td>S. Enteritidis (1)</td>
<td>r</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>r</td>
<td>s</td>
<td>s</td>
</tr>
<tr>
<td>S. Enteritidis (4)</td>
<td>r</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>r</td>
<td>s</td>
<td>s</td>
</tr>
</tbody>
</table>

azi: azithromycin (15 µg); est: streptomycin (10 µg); sul: sulfonamide (300 µg); cip: ciprofloxacin (5 µg); sut: sulfamethoxazole-trimethoprim (25 µg); tet: tetracycline (30 µg); eno: enrofloxacin (5 µg); cft: ceftiofur (30 µg); gen: gentamycin (10 µg); amp: ampicillin (10 µg); nal: nalidixic acid (30 µg); pol: polymyxin B (300 µg).

**DISCUSSION**

The results from the microbiological analysis of organs showed similarity with the studied performed by Perdoncini *et al.* [28], which also collected liver and yolk sacs to isolate *Salmonella* spp. from 129 day-old chicks also as a pool of three organs per sample. Only one sample of a liver pool was positive (2.32%) and *Salmonella* spp. was not isolated from yolk sac samples. According to the authors, the absence of this pathogen in the organs studied may occur due to the reduced volume of these viscera at this stage of life. However, although the pool method may not identify the real number of infected animals, the occurrence of carrier animals becomes important since the presence of such may serve as a source of infection for other animals or to man.

Cloacal swab analysis revealed an important result, since two out of five analyzed hatcheries were positive for *Salmonella*, however, considering the overall prevalence, a low isolation rate was observed. Other studies have demonstrated that *Salmonella* rates in day-old chicks may be considerably more elevated when compared to the results of the present study, such as demonstrated by Dougherty [10], which isolated this bacterium from 37.5% of day-old chicks arriving at the farm. Similar results may be compared to the study of Tessari et al. [38] which found two distinct serotypes in 130 day-old lots and, from these, 32 (24.26%) were positive for *Salmonella*, of which S. Enteritidis were present in 24 lots (18.46%). Rocha et al. [34] verified that in a total of 18 chick lots, 11.1% were positive for *Salmonella* and 92.3% of these were identified as S. Enteritidis.

With the methodology applied in this study, the source infection of the analyzed chicks cannot be detected, however some hypothesis may be suggested. The infection in birds from hatcheries can occur from the broiler breeders, either by transovarian transmission or feces. Hens artificially infected with *Salmonella* Enteritidis are important in the egg contamination process through transovarian transmission [14]. However, naturally infected birds with this bacterium may also produce contaminated eggs [13]. Besides transovarian contamination, vertical transmission may also occur from parent stocks infected with paratyphoid...
salmonella to day-old chicks through the egg shells contaminated with feces [24]. When the intestinal tract of the bird is colonized by Salmonella, the egg may be contaminated through the passage by the cloaca and, in few min, when layed in the environment at a temperature of 37ºC the Salmonella may penetrate the eggshell and cracks may favor the penetration [25]. This penetration may result in direct transmission of the bacteria to the chick embryo, or may even expose other chicks to the infection of Salmonella when the eggshell is broken at birth [15].

Other embryonated egg contamination forms may occur, however in an indirect manner. Hatchable eggs may be contaminated by Salmonella from feces excreted by broiler breeders at the nests [34]. Embryos may also be contaminated at the setters and with greater frequency at hatchers, during the pipping and hatching [9]. The contamination at the hatchery facilities may result in exposure of the recently hatched birds to salmonella, at the moment in which they are most susceptible to intestinal tract colonization [1,7] characterizing a horizontal transmission [34].

The presence of Salmonella Enteritidis resistant to nalidixic acid and azithromycin may be explained by several factors which contribute to the increase of microorganisms with the ability to surpass the mechanism of action of drugs, favoring the occurrence of resistance. Among these, the use of antibiotics by medics without proper control, the indiscriminate use by the general people and the use of these drugs in intensive animal production [16], but also the use of antibiotics without previous susceptibility test to identify the best option and the increase in broad spectrum antimicrobial drugs use [31].

Several studies report the occurrence of resistance among Salmonella Enteritidis poultry isolates to the nalidixic acid [11,19,33]. Some of them [12,27] found more than 90% of Salmonella Enteritidis strains susceptible to this antimicrobial. The extensive use of quinolones in birds is due to the flexible administration in water or feed, the low cost and the efficacy against Salmonella [35].

Nalidixic acid is used in poultry industry therapeutically, however when used in prophylaxis selects resistant strains, which may be transmitted to other birds, animals and to man [39]. Layers and broilers alike represent the main reservoir of resistant Salmonella [33]. Such a fact generates a concern in veterinary and human medicine as well, since it may impair the clinical treatments due to the occurrence of resistant strains [36], leading to a public health threat.

Another relevant fact found in this study is the resistance to azithromycin identified in all three strains of Salmonella Enteritidis isolated. This result differs from the research performed by Kaushik et al. [20] which isolated Salmonella from 23.7% (54/228) of chicken carcasses and 7.7% (11/142) from milk samples and detected resistance to several antibiotics, such as: ampicillin, penicillin, erythromycin, vancomycin, amikacin and clindamycin; however presented susceptible to azithromycin and ceftriaxone and also moderately susceptible to ofloxacin, ciprofloxacin and tetracycline.

The isolation of strains resistant to azithromycin in this study is an important result, since this antibiotic is widely used in the treatment of infections in humans and available in the veterinary medicine, often used as an alternative in the treatment of genitourinary, respiratory, oral and skin infections [32]. Also being used inadequately in feed as a prophylactic measure to the treatment of infections in animals, as well as a growth promoter, which contribute to the perpetuation of resistant and pathogenic strains [30].

A study performed by Molloy et al. [22] reports the first case of a Salmonella Paratyphi A strain resistant to azithromycin isolated from a 48 years old patient in the United Kingdom and the authors affirmed that the difficulty in the treatment was due to a strain resistance to ciprofloxacin and azithromycin. The emergence of Salmonella Paratyphi A strains resistant to azithromycin is an important fact, since the infection by this pathogen is becoming an important finding in the Indian subcontinent with high levels of resistance to fluoroquinolones.

The results found in this study suggest further caution with the presence of Salmonella Enteritidis in the poultry industry. The presence of salmonella in a day-old chick is a serious threat, since in the first days of life a single bird infected by Salmonella is enough to transmit it to the other birds in a lot during the growth period [18]. This pathogen is considered the most common serotype in human infections [8,12,19,21], becoming a serious public health issue since the 1990 decade in Brazil, due to several reports as the most identified serotype in foodborne infections in humans [37].
CONCLUSION

Due to the important risk *Salmonella* spp. presents to the public health, as well as the wide distribution among animals, constant monitoring of these bacteria in broiler breeders and in layer hens, as well as a strengthening in biosecurity in parent stock farms, transportation, egg manipulation and in hatcheries at the beginning and end of each producing cycle are necessary. In order to monitor and attenuate the antimicrobial resistance occurrence, the constant training of professionals concerning inadequate prescription of drugs should be applied, as well as more complex elucidations of the microbiological functionality of *Salmonella*.

REFERENCES


SOURCES AND MANUFACTURERS

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3 Probac do Brasil®, São Paulo, SP, Brazil.

4 Laborclin®, Pinhais, PR, Brazil.

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