



Salmonella spp. in Meat-type Quails (*Coturnix coturnix coturnix*) in the State of São Paulo, Brazil

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ABSTRACT

In the present study *Salmonella* spp. was surveyed in four flocks of meat-type quails reared in a farm that also had processing plant on site, located in the region of Bastos, state of São Paulo, Brazil. Meconium samples of one-day-old quail chicks were collected from transport cardboard boxes. Cecal content was collected on days 7, 14, 21, 28 and 35 of rearing. At 36 days of age, birds were slaughtered in the farm's processing plant, where two samples of water from the scalding and the chilling tanks and four carcasses per flock were collected. All samples were examined for *Salmonella* spp. using traditional bacteriological methods. *Salmonella* spp. was present in meconium samples of three flocks and in cecal feces of the four flocks. This bacterium was also isolated in the chiller water and in the carcasses of three of the evaluated flocks and in the scalding water of one flock. In this study, *S. enterica* subspecies *enterica* 4, 5, 12; *S. Corvalis*; *S. Give*; *S. Lexington*; *S. Minnesota*; *S. Schwarzengrund*; *S. Rissen* and *S. Typhimurium* were the eight serovars identified.

INTRODUCTION

Quail farming was introduced in Brazil in the early 1960s for egg production purposes. During the last decade, quail meat production has also become an alternative for the Brazilian poultry sector. Due to special features, such as low initial investments, need of small areas, easy management and fast financial returns, quail production has expanded in Brazil.

As well as the industrial production of other domestic poultry, quail meat production has benefitted from genetic improvement, better feed efficiency and the use of modern housing facilities that allow rearing quails at high densities. However, some of these factors have also favored the entrance and dissemination of avian pathogens, such as *Salmonella* spp. (Burkholder *et al.*, 2008; Van Hoorebeke *et al.*, 2011). General biosecurity and hygiene measures adopted in poultry farms and processing plants have reduced, but not prevented the presence of *Salmonella* spp. (Davies *et al.*, 2003; Wegner *et al.*, 2003).

There are three kinds of avian salmonellosis. The chicken-adapted *Salmonella* serovar Gallinarum biovars Pullorum and Gallinarum are responsible for pullorum disease and fowl typhoid, respectively (Barrow & Freitas Neto, 2011). In addition to these two clinically systemic salmonellosis, birds may also be infected by *Salmonella* paratyphoid serovars and develop clinical disease or may become asymptomatic carriers and potential sources of human salmonellosis (Gast, 2003; Gast *et al.*, 2011). The question if avian salmonellosis is a problem in quail production today still needs to be answered, as literature reports on this subject are not conclusive (Edwards, 1936; Pourciau & Springer, 1978; Helm *et al.*, 1999; Kumar *et al.*, 2001; Sander *et al.*, 2001; Erdogru *et*



al., 2002; Takata et al., 2003; Aarestrup et al., 2005; McCrea et al., 2006).

During the last few decades, Brazilian researchers and public health authorities have studied and addressed the problems caused by *Salmonella* spp. in poultry; however, there are very few studies investigating *Salmonella* spp. in Brazilian quail production. It should be mentioned that the Brazilian quail flock is estimated in 7.3 million birds (meat and egg production), which are reared near and or even inside chicken egg producing farms. Therefore, studies to assess the presence of *Salmonella* spp. in quails could generate information to allow the establishment of programs to control *Salmonella* in poultry production and to prevent human foodborne diseases.

MATERIAL AND METHODS

Quail farm and processing plant

Salmonella spp. was surveyed in a quail farm, which also has a quail processing plant, located in the region of Bastos, state of São Paulo, Brazil.

Sample collection

Samples of four different flocks were collected on the farm. Meconium samples of one-day-old quail chicks were collected from transport cardboard boxes. Cecal feces were collected at 7, 14, 21, 28 and 35 days of rearing. Birds were processed at 36 days of age. During processing, two samples of water from the scalding and chilling tanks were collected. Four carcasses per flock were collected.

One day-old-quail chicks were transported to the farm in cardboard boxes containing 250 birds each. After birds were removed from the boxes and housed, the meconium present inside the transport box was collected using sterile swabs moisturized in 1% buffered peptone water (1% BPW) (Oxoid, CM 0509). Each sample corresponded to a pool of five swabs (one swab per box). A total of 23 samples were analyzed.

Twenty samples of cecal feces (five per flock) were collected. Each sample corresponded to a pool of five drag swabs from the same flock taken from the quail litter. Swabs were then placed in sterile glass recipients containing 50 mL of 1% BPW.

During processing, two 500-mL samples of the scalding water and two 500-mL samples of the chilling water were collected from the tanks per flock and placed in sterile flasks. Additionally, at the end of the

processing line, four packaged carcasses per flock were collected.

All flasks and carcasses were placed inside a thermal box with ice and submitted to the laboratory.

Sample processing and analyses

In the laboratory, carcasses were placed inside sterile plastic bags and washed with 250 mL of 1% BPW, and the liquid was poured into sterile glass flasks. Ten mL of water from the scalding and the chilling tanks were added to sterile glass flasks containing 90 mL of 1% BPW.

Flasks containing the samples in 1% BPW were left at room temperature for one hour, and were then incubated overnight at 37 °C. The next morning, 1.0 mL of the incubated liquid was transferred to tubes containing 10 mL of selenite broth (Oxoid, CM 395) plus novobiocin (Merial, 8041706) and 0.1 mL to tubes containing 10 mL of Rappaport-Vassiliadis broth (Oxoid, CM 669). Tubes were incubated overnight at 37 °C (Davies & Wray, 1994). Subsequently, broths from all samples were plated on the following culture media: Brilliant Green agar (Oxoid, CM 0263) and Mac Conkey agar (Oxoid, CM 0115). Plates were incubated overnight at 37 °C. Out of each plate, five typical colonies were seeded on triple Sugar Iron agar (Oxoid, CM 277) and in Lysine Iron agar (Oxoid, CM 381), which were incubated overnight at 37 °C and submitted to serology using polyvalent sera against O and H *Salmonella* antigens. Isolates were either sent to Adolfo Lutz Institute, São Paulo, Brazil, or to Oswaldo Cruz Foundation (Fio-Cruz), Rio de Janeiro, Brazil for complete identification and serotyping.

RESULTS

Salmonella spp. was surveyed in meconium samples of one-day-old quail chicks collected from four flocks. Three flocks (75%) were infected with *Salmonella* spp. (Table 1).

Table 1 – *Salmonella* serovars isolated from meconium samples of one-day-old quail chicks collected from transport cardboard boxes.

| Flock | Number of | | Result | Serovars |
|-------|-----------|-------|--------|--|
| | Boxes | Birds | | |
| 1 | 33 | 8,250 | + | <i>Salmonella</i> Rissen, <i>S. enterica</i> subspecies <i>enterica</i> 4, 5, 12 |
| 2 | 36 | 9,000 | + | <i>Salmonella</i> Rissen |
| 3 | 17 | 4,250 | + | <i>Salmonella</i> Lexington |
| 4 | 28 | 7,000 | - | - |



Table 2 presents the results of *Salmonella* serovars isolated from cecal feces collected from the litter of the four evaluated flocks. *Salmonella* spp. was detected in all flocks 4. Flock 4, which was negative for *Salmonella* spp. when meconium samples were examined, became positive during rearing.

Table 2 – *Salmonella* serovars isolated from cecal feces collected from the litter at different times in four flocks.

| Age (days) | Flock | | | |
|------------|--------------------------------|------------------------------|-----------------------------------|------------------------------|
| | 1 | 2 | 3 | 4 |
| | Serovars | | | |
| 7 | S. Corvalis S. Lexington | S. Lexington | S. Corvalis S. Lexington | S. Lexington |
| 14 | S. Lexington | S. Lexington | S. Lexington | S. Lexington |
| 21 | S. Lexington S. Corvalis | S. Lexington S. Minnesota | S. Lexington | S. Lexington |
| 28 | S. Lexington | S. Lexington S. Corvalis | S. Lexington S. Schwarzengrund | S. Lexington S. Give |
| 35 | S. Lexington S. Typhimurium | S. Lexington S. Corvalis | S. Typhimurium S. Corvalis | S. Lexington S. Minnesota |

All *Salmonella* serovars isolated from the scalding and chilling tanks and from the processed carcasses are shown in Table 3. *Salmonella* Lexington was recovered from the chilling water and carcasses from flocks 2 and 3. In flock 2, this serovar was also isolated from the scalding tank. In flock 1, *Salmonella* Minnesota was recovered from the scalding water and the carcasses. All samples from flock 4 were negative for *Salmonella* spp.

Table 3 – *Salmonella* serovars isolated during processing from different samples.

| Kind of sample | Flock | | | |
|---------------------|----------------------------|-------------------|-------------------|---|
| | 1 | 2 | 3 | 4 |
| | <i>Salmonella</i> serovars | | | |
| Scalding tank water | - | S. Lexington | - | - |
| Chilling tank water | S. Minnesota | S. Lexington | S. Lexington | - |
| Carcasses | - S. Minnesota | - S. Lexington | - S. Lexington | - |

- Absence of *Salmonella* spp.

DISCUSSION

There is a strong consensus that any program designed to prevent foodborne salmonellosis has to start on the farm where poultry are reared. Quail production has expanded in Brazil; consequently, quail products (meat and eggs) have become increasingly

popular among the Brazilian consumers. Most quail farms are located near or even inside chicken egg production farms and the dynamics of *Salmonella* spp. dissemination between these two avian species is currently unknown. Studies aiming at surveying *Salmonella* spp. in commercial quail farming provide information on the prevalence and epidemiology of this bacterium in this population. The information generated in those studies may aid the design of control programs, and, therefore, help to reduce cases of human foodborne diseases.

In the present study, three out of the four quail flocks examined at one day of age were infected with *Salmonella* spp. (Table 1). Commercial broiler and layer (*Gallus gallus*) flocks surveyed immediately after hatching, in the hatchery or in transport cardboard boxes, have shown to be infected with *Salmonella* spp. at rates ranging from 11% to 77% (Zancan *et al.* 2000; Gama *et al.* 2003), which is consistent with our results, where 75% of the quail flocks were already infected with *Salmonella* spp. when they arrived at the farm.

Salmonella spp. can be disseminated either vertically or horizontally in poultry production. Feedstuffs, the presence of rodents, and multiple flocks reared on a single litter batch play an important role in horizontal transmission (Rose *et al.*, 2003; Carrique-Mas *et al.*, 2008; Jones, 2011). Infection in the beginning of life makes the control of *Salmonella* spp. difficult because young birds are more susceptible to this bacterium and excrete microorganisms longer than older birds (Gama *et al.*, 2003). The detection of *Salmonella* spp. during the rearing period (Table 2) is certainly related to their presence in day-old birds, but the re-use of litter in the evaluated farm may have contributed to the persistence of the microorganism until the time of slaughter.

Two out of the six *Salmonella* serovars detected on the farm were also found in the samples collected in the processing plant (Table 3). The scalding tank may spread *Salmonella* if the water is not agitated, feces builds up in the tank, or temperatures are not high enough to kill bacteria (Cox & Pavic, 2010). The absence of *Salmonella* spp. in the scalding water samples collected from flocks 1, 3 and 4 may be explained by the correct temperature of the scalding water (above 60°C) when these three samples were collected. *Salmonella* spp. counts can be reduced up to 5.5 log of colony forming units (CFU) mL when scalding water is at 60 °C (Yang *et al.*, 2001).



The main sources of carcass contamination with *Salmonella* spp. during processing are considered to be head pulling and evisceration, when there may be leakage of crop content and intestinal rupture, respectively (Smith *et al.*, 2007). Once contaminated during these steps, *Salmonella* spp. from one carcass can easily disseminate to other carcasses at chilling step. In order to reduce pathogen carryover in the chiller, sanitizers are traditionally added to chilling water (Hugas & Tsigarida, 2008; Bauermeister *et al.*, 2008). The persistence of *Salmonella* spp. in the chilling water and in the carcasses observed in the present study may be related to the evisceration and chilling steps. Therefore, special care must be taken during these processing steps and perhaps additional measures should be adopted in the processing plant (e.g., the use of sanitizers in the chilling water, better calibration of the evisceration machine) to control or minimize this problem.

Salmonella Lexington has not been isolated in commercial birds in Brazil (Hofer *et al.*, 1997). However, it was the most common serovar (Tables 1, 2 and 3) found in the current study. Its presence was also reported in samples of retail duck meat examined in Vietnam (Pham *et al.*, 2005). *Salmonella* Corvalis was the second isolated serovar from quail cecal feces. Although this serovar is not common in Brazil, it was also the third isolated from samples analyzed in Tunisia between 1994 and 2004 (Benajssaum *et al.*, 2007). In the present study, *S.* Minnesota was isolated from cecal feces samples, chilling water and carcasses; interestingly, it has rarely been reported in Brazil (Taunay *et al.*, 1996; Michael *et al.*, 2002). In the United States, this serovar was also isolated at low rates from human and animal sources between 1996 and 2006 (CDC, 2006).

S. Typhimurium is responsible for most of cases of avian paratyphoid salmonellosis (Gast, 2003). Consistent with the findings of the present study, this serovar was previously described in meat-type quail flocks (Kumar *et al.*, 2001). *S.* Schwarzengrund is not frequent found in commercial poultry; however, it was isolated from cecal feces of quails in this study. It was one of the fifteen most frequently isolated serovars from human cases of food borne diseases in Brazil (Fernandes *et al.*, 2006).

In Finland, *S.* Rissen was one of the most frequent serovars isolated from birds in 2003 (Bangtrakulnonth *et al.*, 2004). In Brazil, there are reports on the isolation of this serovar in samples from infected human beings between 1950 and 1990 (Taunay *et al.*, 1996).

However, the risk of this serovar for the Brazilian population is mainly related to consumption of port, since it has been commonly isolated from pigs (Bessa *et al.*, 2004). *S.* Give was responsible for an outbreak in infants in France in 2008, but in this case it was linked to an infant milk formula (Jourdan *et al.*, 2008). In addition, it was also found in ostrich meat (Higgins *et al.*, 1997) and in commercial broiler feces, feedstuffs and feeds (Hoffer *et al.*, 1998). The results obtained in the present study demonstrated that quails reared under intensive production practices may be infected with *Salmonella* spp., as it is similarly observed in industrial broiler and layer production. Further studies are warranted to elucidate the relationship between *Salmonella* spp. and quails, and particularly on paratyphoid salmonellosis in this species and its possible importance as a human foodborne disease.

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