# Salmonella Gallinarum Virulence in Experimentally-Infected Japanese Quails (Coturnix japonica)

■Author(s)

RC Rocha-e-Silva[1]
WM Cardoso*
RSC Teixeira[1]
ÁH Albuquerque[1]
RV Horn[1]
CM Cavalcanti[2]
ES Lopes[1]
VJR Gomes Filho[1]

[1] Students of post grade of Veterinary School of Universidade Estadual do Ceará, Brazil
[2] Student of the School of Veterinary Medicine of Universidade Estadual do Ceará, Brazil

■Mail Adress

Corresponding author e-mail address
William Cardoso Maciel
Av. Rogaciano Leite, 200 Aptº 1303 Bl. Tulipe, Bairro Salinas
CEP 60810-000, Fortaleza, Ceará, Brazil
Phone/fax: (085) 3241 1307 or 31019848 or 9989 4742
E-mail: william.maciel@uol.com.br and robertarochavet@hotmail.com

## ABSTRACT

*Salmonella* Gallinarum is the agent of fowl typhoid in poultry and infects mainly adult galliforms, causing significant economic losses in poultry production. Because quails are susceptible to this disease and quail production is becoming increasingly important in Brazil, this study was carried out to evaluate the virulence of *Salmonella* Gallinarum strain to quails. The inoculum was prepared from *S.* Gallinarum strain resistant to nalidixic acid. Forty eight 16-week-old Japanese quails were randomly distributed in three groups. Before the experiment, cloacal swabs were collected from all birds in order to confirm they were free from *Salmonella* spp. Cloacal swabs and fecal samples were collected on days 03, 06, 09, 12, and 15 post-inoculation. Birds that died during the experiment were submitted to post-mortem examination, and had their organs aseptically collected for bacteriological examination. All eggs produced during the experiment were also examined. The mortality rate recorded during the experiment was 43.75% (21/48). *S.* Gallinarum was recovered from the organs of the birds that naturally died during the experiment, but the agent was not isolated from the organs of sacrificed birds. No egg sample was positive for *Salmonella* Gallinarum. It was concluded that *S.* Gallinarum may be recovered from the organs of experimentally-infected Japanese quails.

## INTRODUCTION

*Salmonella* spp. genetics allows these bacteria to adapt to many environments, fomites and both mammal and non-mammal hosts (Sanchez-Vargas *et al.*, 2011). The consequences of *Salmonella* infections depend on inherent pathogen factors, such as virulence, and on the host's capacity of building an adequate immune response to fight the pathogenic agent (Lahiri *et al.*, 2010).

The nature of pathogenicity of *Salmonella* Gallinarum is multifactorial (Kokosharov, 2003); however, its endotoxin plays an essential role and it is directly linked to the virulence of this microorganism (Kokosharov, 2002). *S.* Gallinarum naturally infects avian species, which body temperature is 5°C higher than that of mammals. It is possible that different environmental stimuli during natural infection influence the host specificity of this bacterium (Pascopella *et al.*, 1995).

Specific *Salmonella* Gallinarum infection is commonly systemic (Evans, 2011), causing sepsis independently of age, but usually affecting birds older than 3 months. Mortally is usually high and may reach 100% in some infected flocks (Uzzau *et al.*, 2000). Common symptoms are depression, weakness, ruffled feathers (Freitas Neto *et al.*, 2007), weight loss, 50-70% drop in egg production (Ezema *et al.*, 2009), prostration, apathy, drooped wings, loss of appetite, dehydration, and greenish-yellow to bloody diarrhea (Garcia *et al.*, 2010). Fowl typhoid affects

primarily chickens and turkeys, but pheasants, quails, and guinea-fowl are also susceptible (Shivaprasad, 2000).

Japanese quail production for meat and eggs has expanded in many countries, including Brazil (Minvielle, 2004). Hence, knowledge on the diseases that may affect Japanese quails, including salmonellosis, is essential (Minvielle, 2004). There are literature reports on the isolation of *S*. Typhimurium, *S*. Virchow, *S*. Meleagridis and Group B and C$_1$ *Salmonella* from the cloaca, liver and intestines of Japanese quails (Al-Nakhli, 2005), *S*. Typhimurium variant Copenhagen, *S*. Typhimurium, and *S.* Hadar from live commercial quails and carcasses, and *S*. Paratyphi from the environment (Sander *et al*., 2001).

This study was carried out to evaluate the virulence of *Salmonella* Gallinarum in Japanese quails because there is little information on this subject.

## MATERIALS AND METHODS

### Birds

Forty eight 16-wk-old Japanese quails (24 females and 24 males) were used in the present experiment. Birds were randomly distributed in three groups (G1, G2 and G3) with 16 birds each. Birds were housed per sex in cages, measuring 22x21x16cm and organized in pyramid batteries, located at the inoculation facilities of the Center of Ornithological Studies of the State University of Ceará (UECE). Water and feed were supplied *ad libitum*. Feed did not contain any antibiotics. Birds were not submitted to any vaccination or chemical treatment against parasites. Room temperature (25°C) was controlled, and a 16h light lighting program was applied. The present study was approved by the Committee of Ethics of Animal Use of UECE under protocol number 10244779-9/26.

### Bacteriological monitoring before inoculation

Before the trial started, all birds were checked to ensure they were free from *Salmonella* spp. The test performed according to Wigley *et al*. (2001), with some changes, as follows: individual cloacal swabs were collected, immersed in selenite-cystine broth (M025; HIMEDIA®) containing novobiocin (40µg/mL, SIGMA®) and directly plated (0h) on brilliant green agar (M016; HIMEDIA®) containing nalidixic acid (100 µg). Both cloacal swabs and plates were incubated in a bacteriological oven at 37°C for 24h. After

incubation, cloacal swab samples that were negative for *Salmonella* spp. were again plated in brilliant green agar containing nalidixic acid (100µg) and incubated t 37°C for 24h to confirm the absence of that bacterium in the experimental birds. In addition, six birds were sacrificed for the collection of liver, spleen, cecum, lung, and reproductive tract (testicle or ovarian follicle) samples. Tissue samples were individually ground and placed in test tubes containing peptone water at 0,1% at 37°C for 24h, after which an aliquot was transferred to a tube containing selenite-cystine broth with novobiocin (40µg/mL) and incubated for 24h at 37°C. Samples were then plated in brilliant greed agar with nalidixic acid (100µg) and incubated at 37°C for 24h.

### Inoculum preparation

The inoculum was prepared using a nalidixic acid resistant *Salmonella* Gallinarum strain (SGNalr) isolated from chicken (*Gallus gallus domesticus*) and provide by the Department of Veterinary Pathology of UNESP, Jaboticabal, SP, Brazil. The test performed according to Berchieri *et al*. (2001), with some modifications, as follows: the bacterial culture was prepared in 5mL buffered peptone water at 0.1% and statically incubated in a bacteriological oven at 37°C for 24h, and The culture was then serially diluted in order to determine the number of colony-forming units (CFU; Miles *et al*., 1938), after which three different inocula ($10^4$, $10^5$, and $10^6$CFU de *Salmonella* Gallinarum Nalr/mL) were obtained.

### Inoculation

All birds received via gavage directly in the crop, with the aid of a cannula coupled to a 1mL syringe, 0.1mL of the inoculum. Group 1 (G1) birds received $5x10^5$CFU/mL; group 2 (G2), $1x10^6$CFU/mL; and group 3 (G3), $2x10^7$CFU/mL .

### Post-inoculation monitoring

During the trial, clinical signs and mortality were daily recorded. Dead birds were submitted to gross examination, and their organs collected for microbiological tests for the presence of *Salmonella* Gallinarum.

Fragments of the liver, spleen, cecum, lungs, and reproductive tract were aseptically collected, individually ground and placed in tubes with peptone water at 0.1% (7365A; Acumedia®). Samples were then plated on brilliant green agar with nalidixic acid (100µg) and incubated at 37°C for 24h. Plates were read, and the

samples negative for *Salmonella* Gallinarum were transferred to selenite-cystine broth with novobiocin (40μg/mL), incubated in bacteriological oven at 37°C for 24h, and then plated on brilliant green agar with nalidixic acid (100μg).

Samples that did not present colonies with typical morphological profile of *Salmonella* Gallinarum, which are small colonies (2-4mm) and have smooth edges and circular shape (Bergey *et al.*, 1994; Gast, 1997), were submitted to an agglutination test using polyvalent O antiserum poly (Difco®).

### Cloacal swab samples

Individual cloacal swabs were collected of all birds (pool of two birds) on 03, 06, 09, 12, and 15 days post-inoculation (dpi) and processed as described by Zancan *et al*. (2000) with some modifications. Individual cloacal swabs were immersed in selenite-cystine broth (M025; HIMEDIA®) containing novobiocin (40μg/mL, SIGMA®), plated on brilliant green agar containing nalidixic acid (100μg) and then incubated in selenite-cystine broth. Both broth and plates were incubated at 37°C for 24h. Cloacal swab samples negative for *Salmonella* Gallinarum were re-plated on brilliant green agar with nalidixic acid (100μg).

### Fecal samples

Fresh feces from all birds were collected in plastic bags placed under each cage on 03, 06, 09, 12, and 15 dpi. Samples were weighed and placed in tubes containing 5mL peptone water (1g feces: 10mL) and directly plated on brilliant green agar containing nalidixic acid (100μg) and incubated at 37°C for 24h. Samples negative for *Salmonella* Gallinarum were then transferred to selenite-cystine broth with novobiocin (40μg/mL) and incubated at 37°C for 24h, after which samples were plated on brilliant green agar with nalidixic acid (100μg) and incubated at 37°C for 24h.

### Organ sampling

At the end of the experimental period, birds that were still alive were sacrificed by neck dislocation and liver, spleen, cecum, lungs, and reproductive tract samples were aseptically collected and submitted to bacteriological testing for the presence of *Salmonella* Gallinarum. Organs were ground, weighed, and individually placed in tubes containing 5mL peptone water at 0.1% (1g:10mL) and subsequently plated on brilliant green agar containing nalidixic acid (100μg). Samples (tubes and plates) were then incubated at

37°C for 24h. Samples that did not produce colonies with the typical morphological profile of *Salmonella* Gallinarum were transferred to tubes containing selenite-cystine broth with novobiocin (40μg/mL) to try to increase bacterium recovery. Samples were then incubated at 37°C for 24h and plated on brilliant green agar containing nalidixic acid (100μg) and incubated under the same previously described conditions.

### Procedure for the isolation of *Salmonella* Gallinarum in eggs

The eggs produced during the entire experimental period were daily collected, separately placed in quail-egg boxes and stored in a cold room at a temperature of approximately 7°C. At the end of the experiment, all eggs were separated according to production date and experimental group. Eggs were not previously disinfected and were then broken in a sterile beaker (pool of eggs produced during two consecutive days by each group), homogenized (entire egg, including both eggshell and internal content), and placed in a bacteriological oven at 37°C for 24h, as described by Berchieri *et al*. (2001), with some modifications. After this period, samples were homogenized, collected using sterile swabs, and transferred to selenite-cystine broth with novobiocin (40μg/mL) and incubated at 37°C for 24h, after which they were plated on brilliant green agar with nalidixic acid (100μg) and incubated at 37°C for 24h.

## STATISTICAL ANALYSIS

Total mortality was compared using non-parametric tests – Chi-square test with Yates correction or Fisher's exact test, as indicated – at 5% significance level.

## RESULTS

None of the birds evaluated in the pre-inoculation period were positive for *Salmonella* spp., according to the applied microbiological tests.

Mortality started to be observed after 5 dpi and continued for further six days (Figure 1). Total mortality during the experiment was 43.75% (21/48 birds), divided as follows: 8.3% (4/48), 4.16% (2/48), and 31.25% (21/48) in G1, G2 e G3, respectively.

Within groups G1, G2, and G3, mortality rates were 25% (4:16), 12.5% (2:16) e 94.7% (15:16), respectively, with significant difference ($p > 0.05$) between G3 and the two other groups.

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RV Horn, CM Cavalcanti,
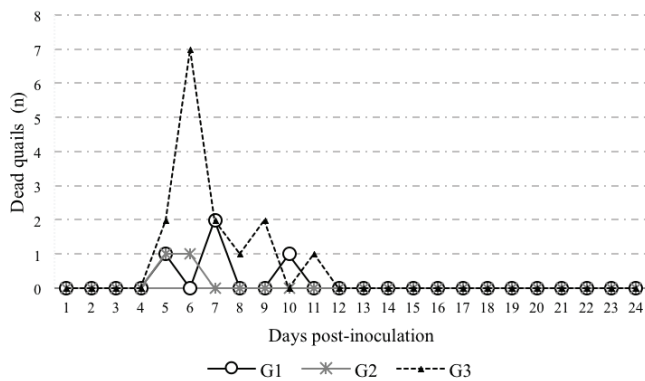ES Lopes, VJR Gomes Filho

Figure 1 – Daily mortality curve of Japanese quails (*Coturnix coturnix japonica*) orally infected with *Salmonella* Gallinarum.

All fecal and cloacal swab samples collected on 03, 06, 09, 12, and 15 dpi were negative for *Salmonella* Gallinarum. Almost all organs collected from birds that naturally died during the experiment were positive for the bacterium, except for four birds that presented negative cecal samples and one bird that had both negative cecal and reproductive tract samples (Tables 1, 2, and 3). All evaluated organs from quails sacrificed at the end of the experimental period (24 dpi) were negative for the agent.

**Table 1 –** Recovery of *Salmonella* Gallinarum from the organs of Japanese quails orally inoculated with 5x105CFU/mL (G1) and that naturally died during the experiment.

| Birds | Spleen | Liver | Lung | Cecum | Reproductive tract |
|---|---|---|---|---|---|
| 02 | + | + | + | + | + |
| 06 | + | + | + | + | + |
| 07 | NR | + | + | + | + |
| 11 | + | + | + | - | - |
| Positive (%) | 100% | 100% | 100% | 75% | 75% |

\* Birds identified as positive in the plating phase

NR: No result available

**Table 2 –** Recovery of *Salmonella* Gallinarum from the organs of Japanese quails orally inoculated with 1x10⁶CFU/mL (G2) and that naturally died during the experiment.

| Birds | Spleen | Liver | Lung | Cecum | Reproductive tract |
|---|---|---|---|---|---|
| 40 | - | - | - | - | - |
| 43 | NR | + | + | + | + |
| Positive (%) | 100% | 50% | 50% | 50% | 50% |

\* Birds identified as positive in the plating phase

NR: No result available

**Table 3 –** Recovery of *Salmonella* Gallinarum from the organs of Japanese quails orally inoculated with 2x10⁷CFU/mL (G3) and that naturally died during the experiment.

| Birds | Spleen | Liver | Lung | Cecum | Reproductive tract |
|---|---|---|---|---|---|
| 17 | + * | + * | + | + | + |
| 18 | + | + | + | + | + |
| 19 | + | + | + | + | + |
| 20 | + | + | + | - | + |
| 21 | + | + * | + | + | + |
| 22 | + | + | + | + * | + |
| 23 | + | + | + | - | + |
| 24 | + | + | + | + | + |
| 25 | + | + | + | + | + |
| 26 | NR | + | + | + | + |
| 27 | + | + | + * | + | + |
| 28 | + | + | + | - | + |
| 29 | NR | + | + | - | + |
| 31 | + | + | + | + | + |
| 32 | + | - | - | - | - |
| Positive (%) | 100% | 93.3% | 93.3% | 62.5% | 93.3% |

\* Birds identified as positive in the plating phase

NR: No result available

The main clinical sign was apathy. Birds remained quiet in the corner of the cage, with their eyes closed, ruffled feathers, and presented greenish-yellow watery feces, which sometimes contained blood. The main findings at gross examination were enlarged and hemorrhagic liver, enlarged gall bladder, enlarged spleen, hemorrhagic ovarian follicles, and petechial hemorrhages in the intestinal tract.

During the experiment, 170 eggs (G1 = 65, G2 = 80; G3 = 25) were collected, and all were negative for *S.* Gallinarum.

## DISCUSSION

The high mortality recorded in the present experiment indicates that Japanese quails are sensitive to *Salmonella* Gallinarum (SG). This result was expected, because scientific literature mentions that SG produces a severe disease, with significant mortality rates in chickens (Compra *et al*., 2008). Mortality caused by SG may be higher than 80% in broilers (Paiva *et al*. 2009), and may reach almost 100% in inoculated young brown layers (Oliveira *et al*., 2005). Accordingly, 94.7% mortality was recorded in G3 in the present experiment.

Although all birds were shown to be sensitive to the pathogen, the administration of inocula with different concentrations caused different mortality rates, in agreement with studies with semi-heavy layers receiving inocula with different CFU levels (Berchieri Jr. *et al* .,

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2001; Garcia *et al.*, 2009). Despite receiving a more concentrated inoculum than G1, mortality in G2 was lower, albeit not statistically significant, demonstrating that increasing SG concentration from $5 \times 10^5$ CFU to $1 \times 10^6$ CFU did not influence mortality rate. On the other hand, the administration of the inoculum with $2 \times 10^7$ CFU resulted in significantly higher mortality in G3 birds compared with G1 and G3. This was expected due to the high pathogen concentration in that inoculum. Buchholz & Fairbrother (1992) recorded mortality on 6 and 7 dpi in quails orally inoculated with $10^5$, $10^6$ and $10^7$ CFU/mL of *S*. Pullorum, which concentrations are similar to those used in the present study. Those authors also found that the intravenous administration of inocula at $10^6$ and $10^7$ CFU resulted in mortality on 3 dpi. Therefore, the period birds start dying does not depend only on the concentration of the inoculum, but also on the inoculation route.

There are few reports on the evaluation of SG virulence in quails, but studies with chickens are consistent with the results obtained in the present study, particularly relative to the time mortality was recorded, which was between 5 and 11 dpi. Shivaprasad (2000) suggested that birds infected with SG may die within four days after exposure, but they usually start dying five days later and mortality commonly persists for 10 days. Chadfield *et al.* (2003) orally inoculated young broilers with SG and found that mortality started five days after inoculation. Assoku *et al.* (1970) also recorded the same time for the beginning of mortality, with a persistence of six further days, as observed in the present study.

Although the results of the present study are consistent with other reports, it must be noted that mortality rates caused by fowl typhoid may considerably vary, and many factors may influence the severity of this disease, such as host's susceptibility (Sato *et al.*, 1997). Freitas Neto *et al.* (2007) found that semi-heavy commercial layers orally inoculated with approximately $3.3 \times 10^5$ CFU/mL and that were sensitive to SG started to die on 4 dpi, whereas the SG-resistant birds started dying on 6 dpi, demonstrating the bird sensitivity to the microorganism may determine the beginning of mortality. The high mortality recorded in this experiment indicates that quails may be more susceptible to SG than chickens, as the mortality rate related with the concentration of the inoculum was relatively higher than those presented in studies with chickens. Oliveira *et al.* (2005), working with layers, recorded 94% mortality, close to that recorded in the present experiment in G3 birds, when using

a SG inoculum at $8.5 \times 10^8$ CFU/mL. Other studies administered higher concentrations than those used in this trial. Barrow *et al.* (1987) reported 50% mortality in 2-wk-old broilers orally inoculated with $1.8 \times 10^9$ CFU/mL and Rychlik *et al.* (1998) observed 60% mortality in 3-wk-old layers inoculated with $1.3 \times 10^9$ CFU/mL.

Relative to the presence of SG in fecal and cloacal swab samples, the behavior of this microorganism in inoculated quails was different than that of chickens inoculated with different *Salmonella* strains. Research studies demonstrated that, when orally inoculated with SG, layers presented positive cloacal swab samples for more than 40 dpi (Oliveira *et al.*, 2005), and those inoculated with *Salmonella* Entetitidis were positive both in fecal (Nakamura *et al.*, 2004; Ishola *et al.*, 2009), and cloacal swab (Maddadi *et al.*, 2010) samples. Other serotypes, such as *S*. Pullorum (Berchieri *et al.*, 2001) and *S*. Typhimurium (Beal *et al.*, 2004), have also been recovered from cloacal swabs of laying chickens submitted to oral inoculation. *S*. Typhimurium was isolated in cloacal swab samples up to seven weeks post-infection (Beal *et al.*, 2004).

Oliveira *et al.* (2005) reported that the pathogen may be isolated from feces from dying chickens, and that those with no clinical symptoms that survive the infection may excrete *Salmonella* for 35 days. Freitas Neto *et al.* (2007) observed that the agent may be isolated from fecal samples on 7 dpi in semi-heavy layers. In the present experiment, only the organs of the quails that died were positive for SG; according to Beer (1998), the microorganisms present in the animal may significantly replicate after death, which may have allowed SG isolation in the present experiment. On the other hand, SG was not recovered from the organs of birds that survived until the end of the experiment (21 dpi). It is possible that these birds were able to overcome the infection because, in addition of presenting negative microbiological results, they were apparently healthy, consuming feed and water and producing eggs. Consistent findings were obtained with inoculated and sacrificed chicks: at the end of the experiments, it was not possible to recover SG from their liver, spleen, and ceca (Barrow *et al.*, 1987; Jones *et al.*, 2001), and the birds that survived were apparently healthy (Bumstead & Barrow, 1993).

The birds that died during the experiment presented classical fowl typhoid symptoms. Birds remained quiet in the corner of the cage, with their eyes closed, ruffled feathers, and presented greenish-yellow watery feces, which often contained blood. The presence of blood in the feces indicates sepsis, which causes gastrointestinal

hemorrhages and ulceration of the intestinal wall (Chappell *et al*., 2009).

In the present study, all analyzed egg samples were negative for SG, in agreement with previous studies using experimental infections (Berchieri Jr. *et al*, 2000; Berchieri Jr. *et al*, 2001; Oliveira *et al*, 2005). The vertical transmission of SG occurs easier in poultry strains that are genetically resistant to fowl typhoid.

Although *Salmonella* is able to penetrate the egg, there are other important extrinsic factors that may interfere in transmission, such as bacterial strain, temperature, humidity, number of microorganisms, and storage conditions (Messens *et al*., 2005). Some authors assert that eggs produced by SG-infected layers may not present any contamination (Oliveira *et al*., 2005), differently from layers that carry *Salmonella* Pullorum (SP) and may vertically transmit this agent through infected eggs due to the quantitative increase of SP in the reproductive tract during sexual maturation. It was found that gonadal hormones may aid the transport of infected macrophages to the reproductive system; however, the mode of SP transmission to the eggs has not been elucidated yet (Wigley *et al*., 2001).

## CONCLUSIONS

*Salmonella* Gallinarum is virulent for Japanese quails and the mortality it causes is possibly influenced by the concentration of inoculated bacteria. SG may be recovered from the liver, spleen, cecum, lungs, and reproductive tract of experimentally-infected quails; however, inoculated birds may not show any clinical symptoms or shed the inoculated SG strain.

## ACKNOWLEDGEMENTS

## REFERENCES

Al-Nakhli HM. Occurrence of Paratyphoid Infection Among japanese quails (*Coturnix coturnix japonica*) in Saudi Arabia. Journal of Bio-Science, 2005; 12(1):59 -66.

Assoku KG, Penhale WJ, Buxton A. Haematological changes in acute experimental *Salmonella* Gallinarum infection in chickens. Journal of Comparative Pathology 1970; 80(3):473-485.

Barrow PA, Simpson JM, Lovell MA, Binns MM. Contribution of *Salmonella* Gallinarum large plasmid toward virulence in fowl typhoid. Infection and Immunity 1987; 55(2):388-392.

Beal RK, Wigley P, Powers C, Hulme SD, Barrow PA, Smith AL. Age at primary infection with *Salmonella enterica* serovar Typhimurium in the chicken influences persistence of infection and subsequent immunity to re-challenge. Veterinary Immunology and Immunopathology 2004; 100(3-4):151-164.

Beer J. Doença infecciosa em animais domésticos. 1 ed, São Paulo, SP; 1998.

Berchieri Jr. A, Murphy CK, Marston K, Barrow PA. Observations on the persistence and vertical transmission of *Salmonella enterica* serovars Pullorum and Gallinarum in chickens: effect of bacterial and host genetic background. Avian Pathology 2001; 30(1):221-231.

Berchieri Jr. A, Oliveira GH, Pinheiro LAS, Barrow PA. Experimental *Salmonella* Gallinarum infection in light laying hen lines. Brazilian Journal of Microbiology 2000; 31(1):50-52.

Bergey DH, Holt JG, Krieg NR. Bergey's: manual of determinative bacteriology. 9 ed. Baltimore: W. & Williams; 1994.

Buchholz PS, Fairbrother A. Pathogenicity of *Salmonella* Pullorum in northern bobwhite quail and mallard ducks. Avian Diseases 1992; 36(2):304-312

Bumstead N, Barrow P. Resistance to *Salmonella* Gallinarum, *S*. Pullorum and *S*. Enteritidis in Inbred Lines of Chickens. Avian Diseases 1993; 37(1):89-93.

Chadfield MS, Brown DJ, Aabo S, Christensen JP, Olsen JE. Comparison of intestinal invasion and macrophage response of *Salmonella* Gallinarum and other host-adapted *Salmonella enterica* serovars in the avian host. Veterinary Microbioogy 2003; 92(1-2):49-64.

Chappell L, Kaiser P, Barrow P, Jones MA, Johnston C, Wigley P. The immunobiology of avian systemic salmonellosis. Veterinary Immunology and Immunopathology 2009; 128(1-3):53-59.

Evans EE. Zoonotic diseases of common pet birds: psittacine, passerine, and columbiform species. Veterinary Clinics of North America: Exotic Animal Practice 2011; 14(3):457-476.

Ezema WS, Onuoha E, Chah KF. Observations on an outbreak of fowl typhoid in commercial laying birds in Udi, South Eastern Nigeria. Comparative Clinical Pathology 2009; 18(4):395-398.

Freitas Neto OC, Arroyave W, Alessi AC, Fagliari JJ, Berchieri Jr. A. Infection of Commercial Laying Hens with *Salmonella* Gallinarum: Clinical, Anatomopathological and Haematological Studies. Brazilian Journal of Poultry Science 2007; 9(2):133-141.

Garcia KO, Santana AM, Freitas Neto OC, Simplício KMMG, Alessi AC, Berchieri Jr. A, Fagliari JJ. Experimental infection of commercial layers using a *Salmonella enterica* sorovar Gallinarum strain: blood serum components and histopathological changes. Brazilian Journal of Veterinary Pathology 2010; 3(2):111-117.

Garcia KO, Berchieri Jr. A, Santana AM, Freitas-Neto OC, Fagliari JJ. Experimental infection of commercial layers using *Salmonella enterica* serovar Gallinarum strain: Leukogram and serum acute-phase protein concentrations. Brazilian Journal of Poultry Science 2009; 11(4):263-270.

Gast RK, Porter Jr. RE, Holt PS. Applying tests specific yolk antibodies to predict contamination by *Salmonella* Enteritidis in eggs from experimentally infected laying hens. Avian Diseases 1997; 41(1):195-202.

Ishola OO. Effects of challenge dose on faecal shedding of *Salmonella* Enteritidis in experimental infected chickens. African Journal of Biotechnology 2009; 8(7):1343-1346.

Jones MA, Wigley P, Page KL, Hulme SD, Barrow PA. *Salmonella enterica* serovar Gallinarum Requires the *Salmonella* Pathogenicity Island 2 Type III Secretion System but Not the *Salmonella* Pathogenicity Island 1 Type III Secretion System for Virulence in Chickens. Infection and Immunity 2001; 69(9):5471-5476.

Kokosharov T. Acid tolerance, bile salts and serum resistance of *Salmonella* Gallinarum from hens. Veterinarski arhiv 2003; 73(5):277-283.

Kokosharov T. Clinical and hematological effects of *Salmonella* Gallinarum endotoxin in cockerels. Veterinarski arhiv 2002; 72(5):269-276.

Lahiri A, Lahiri A, Iyer N, Das P, Chakravortty D. Visiting the cell biology of *Salmonella* infection. Microbes and Infection 2010; 12(11):809-818.

Maddadi MS, Hassanzadeh M, Bozogmehrifard M, Shojaei H, Yazdani A, Karimi V, Barin A. Study on two inoculation routs of *Salmonella* Enteritidis in abilities to colonize in internal organs and to contaminate of eggs in broiler breeder hens. International Journal of Poultry Science 2010; 9(3):254-258.

Messens W, Grijspeerdt K, Herman L. Eggshell penetration by *Salmonella*: a review. World's Poultry Science Journal 2005; 61(1):71-86.

Miles, A.A.; Misra, S.S.; Irwin, J.O. (1938). The estimation of the bactericidal power of the blood. J. Hyg. (Lond), 38 (6), p.732–749.

Minvielle F. The future of japanese quail for research and production. World's Poultry Science Journal 2004; 60(4):500-507.

Nakamura M, Nagata T, Okamura S, Takehara K, Holt PS. The Effect of Killed *Salmonella* Enteritidis vaccine prior to induced molting on the shedding of *S*. Enteritidis in laying hens. Avian Desease 2004; 48(1):183-188.

Oliveira GH, Berchieri Jr. A, Fernandes AC. Experimental infection of laying hens with *Salmonella enterica* sorovar Gallinarum. Brazilian Journal of Microbiology 2005; 36(1):51-56.

Paiva JB, Penha Filho RAC, Argüello YMS, Silva MD, Gardin Y, Resende F, Berchieri Jr. A, Sesti L. Efficacy of several *Salmonella* vaccination programs against experimental challenge with *Salmonella* Gallinarum in commercial brown layer and broiler breeder hens. Brazilian Journal of Poultry Science 2009; 11(1):65-72.

Pascopella L, Raupach B, Ghori N, Monack D, Falkow S, Small PL. Host restriction phenotypes of *Salmonella* Typhi and *Salmonella* Gallinarum. Infection and Immunity 1995; 63(11):4329-4335.

Purchase C, Picard J, McDonald R, Bisschop SPR. A comparison of the oral application and injection routes using the onderstepoort biological products fowl typhoid vaccine, its safety, efficacy and duration of protection in commercial laying hens. Journal of the South African Veterinary Association 2008; 79(1): 39-43.

Rychlik I, Lovell MA, Barrow PA. The presence of genes homologous to the K88 genes faeH and faeI on the virulence plasmid of *Salmonella* Gallinarum. FEMS Microbiology Letters 1998; 159(2):255-260.

Sanchez-Vargas M, Abu-El-Haija MA, Gómez-Duarte OG. *Salmonella* infections: An update on epidemiology, management, and prevention. Travel Medicine and Infectious Diseases 2011; 9(6):1-15.

Sander J, Hudson CR, Dufour-Zavala L, Waltman WD, Lobsinger C, Thayer SG, Otalora R, Maure JJ. Dynamics of *Salmonella* Contamination in a Commercial Quail Operation. Avian Diseases 2001; 45(4):1044-1049.

Sato, Y., G. Sato, L. Tuchili, G. S. Pandey, A. Nakajima, H. Chimana, and H. Sinsungwe. 1997. Status of *Salmonella* Gallinarum- Pullorum infections in poultry in Zambia. Avian Diseases 1997;41(2):490-495.

Shivaprasad HI. Fowl typhoid and pullorum disease. Office International des Epizooties, Scientific and Technical Review 2000; 19(2):405-424.

Uzzau S, Brown DJ, Wallis T, Rubino S, Leori G, Bernard S, Casadesús J, Platt DJ, Olsen JE. Host adapted serotypes of *Salmonella enterica*. Epidemiology & Infection 2000; 125(2):229-555.

Wigley P, Berchieri Jr. A, Page KL, Smith AL, Barrow PA. *Salmonella enterica* serovar Pullorum persists in splenic macrophages and in the reproductive tract during persistent,disease-free carriage in chickens. Infection and Immunity 2001; 69(12):7873-7879.

Zancan FT, Berchieri Jr. A, Fernandes AS, Gama NMSQ. *Salmonella* investigation in transport boxes of day-old birds. Brazilian Journal of Microbiology 2000; 31(3):230-232.