



Revista Brasileira de Ciência Avícola

ISSN: 1516-635X

revista@facta.org.br

Fundação APINCO de Ciência e Tecnologia

Avícolas

Brasil

Ribeiro, SAM; Berchieri Jr, A; Orsi, MA; Mendonça, AO; Ferrati, AR
Experimental infection by *Salmonella enterica* subsp *enterica* serovar *kottbus* in day-old broiler
chickens

Revista Brasileira de Ciência Avícola, vol. 7, núm. 2, abril-junio, 2005, pp. 107-111
Fundação APINCO de Ciência e Tecnologia Avícolas
Campinas, SP, Brasil

Available in: <http://www.redalyc.org/articulo.oa?id=179713987007>

- How to cite
- Complete issue
- More information about this article
- Journal's homepage in redalyc.org



Experimental Infection by *Salmonella enterica* Subsp *Enterica* serovar *Kottbus* in Day-Old Broiler Chickens

■ Author(s)

Ribeiro SAM^{1,3}
Berchieri Jr A¹
Orsi MA²
Mendonça AO²
Ferrati AR³

¹ UNESP (Universidade Estadual Paulista), Faculdade de Ciências Agrárias e Veterinárias – Departamento de Patologia Animal, Campus de Jaboticabal.

² LANAGRO/SP - MAPA (Ministério da Agricultura, Pecuária e Abastecimento)

³ LANAGRO/SP - FACTA (Fundação APINCO de Ciência e Tecnologia Avícolas)

ABSTRACT

The strain used in this work was a *Salmonella enterica* subsp *enterica* serovar *Kottbus* (6,8:e,h:1,5) isolated from imported day-old ducklings in Laboratório Nacional Agropecuário (LANAGRO/SP) of the Ministry of Agriculture of Brazil (MAPA). In view of the lack of information available about this *Salmonella* isolate and also because it was detected in day-old imported birds, this study was carried out to investigate the dissemination of *S. Kottbus* among newly hatched chicks. The birds were placed in three groups: one group of 20 birds received 0.1 mL of *S. Kottbus* culture containing 1.2×10^8 CFU/mL, the second group of 20 birds was inoculated with 1.2×10^5 CFU/mL and the third group of 10 birds was untreated (control group). Results were similar for both infected groups. The bacterium was recovered from cloacal swabs collected from the first day following the experimental infection until the end of the trial (42 days post-inoculation). At 15 and 42 days post-inoculation (dpi), half of the birds of each group were killed for bacteriological examination of cecal contents, liver and spleen. At 15 dpi, viable cell counts of *S. Kottbus* were obtained in all kinds of samples. At 42 dpi, *Salmonella* was present in the liver and spleen of few birds, but in large amounts in the cecal contents of almost all birds.

INTRODUCTION

Large scale production of poultry meat and egg has demanded the control of Pullorum disease and Fowl typhoid in the modern poultry industry. However, commercial poultry are susceptible to infections caused by other *Salmonella* serotypes that may cause clinical disease (Berchieri Jr. *et al.*, 2000). Nevertheless, infections caused by such *Salmonella* serotypes are usually asymptomatic; the pathogens are transmitted vertically and are excreted with the feces. In addition, these serotypes may be associated with human foodborne salmonellosis (OFFICE INTERNATIONAL DES EPIZOOTIES, 2000; Forsythe *et al.*, 1967).

Once infectious pathogens are introduced in a poultry flock, they might spread rapidly. *Salmonella Enteritidis* has been focused by surveillance programs in animal farms since it was responsible for several outbreaks of human foodborne salmonellosis related to food prepared with poultry and eggs (Gast *et al.*, 1998; Gast & Beard, 1992; Lirio *et al.*, 1998; Okamura *et al.*, 2001; Peresi *et al.*, 1998). It is supposed that *Salmonella Enteritidis* reached poultry breeder farms through rodents and was vertically transmitted to the progeny. Eventually, it reached commercial flocks of broilers and laying hens and was spread worldwide. However, there are many other *Salmonella* serotypes that cause currently enteric human salmonellosis, such as *Salmonella Infantis*, *S. Senftenberg*, *S. Anatum*, *S. Agona*, *S. Heidelberg* and the always-important *Salmonella Typhimurium* (Clark & Thatcher, 1973; Okamura

■ Mail Address

Simone Alves Mendes Ribeiro
R. Raul Ferrari, s/n
Caixa Postal 5538
13.091-907. Campinas, SP, Brazil
Phone: +55 +19 3252 0155 extension 193

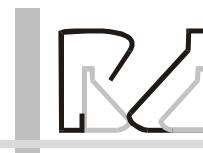
E-mail: simone_ribeiro2002@yahoo.com.br

■ Keywords

Duck, poultry, *Salmonella* Kottbus, salmonellosis

■ Acknowledgement

MAPA/LANAGRO/SP, FACTA and CNPQ for financial support.



et al., 2001; Parimal et al., 2001; Taunay et al., 1996; Tavechio et al., 1996; Tavechio et al., 2002). Based on the episode caused by *Salmonella* Enteritidis, the Brazilian Government has established that imported birds must be free of *Salmonella* serotypes Gallinarum, Pullorum, Enteritidis and Typhimurium (BRASIL, 1999). However, the surveillance plan does not consider other serotypes of *Salmonella*, which allowed them to be introduced in the country and flocks containing day-old infected birds were contaminated during rearing (Gama et al., 2003).

Recently, *Salmonella* Kottbus was isolated in Brazil from imported day-old ducklings (Galletti et al., 1999). This serotype had been previously isolated from other imported flocks (Ribeiro et al., 2004) and no prevention measures were taken. *Salmonella* Kottbus has been implicated in several cases of salmonellosis. Over a period of 11 years, 5.7% of 245 hospitalized horses were infected by *S.* Kottbus, and 21.4% of these died due to the bacterium. Sick animals excreted fluid feces with large amounts of bacteria and contaminated the environment, other animals and human beings (Carter et al., 1986).

It has been reported that 4.29% of carcasses and organs of healthy pigs were contaminated by *Salmonella*, and *S.* Kottbus was one of the isolated serotypes. In regard to environmental contamination, *Salmonella* was isolated from adult muscoid flies collected from commercial poultry farms, including *Salmonella* Kottbus (Mian et al., 2002). According to Hoszowski & Wasyl, 2002, the majority of *Salmonella* strains isolated in veterinary laboratories in Poland in 2001 were recovered from poultry; and *S.* Kottbus was isolated from ducks. *Salmonella* Kottbus was isolated from lambs in a group of 200 animals, from which 16 died over a period of 3 days (VETERINARY LABORATORIES AGENCY, 2003).

According to the Information System of Public Health Laboratory in USA (1968-1998), the mean number of cases of human salmonellosis was 43 per year and in 2001 *Salmonella* Kottbus infection was observed in 23 people who had eaten alfalfa sprouts (CENTER OF DISEASE CONTROL AND PREVENTION, 2002). In Libya, a multi-drug resistant strain of *S.* Kottbus was among 21 *Salmonella* strains isolated from 16 children with diarrhea (El_Ghodban et al., 2002). According to Nelius et al. (1969), most *Salmonella* infections remain as enteric diseases, however, *S.* Kottbus may cause septicemia that is often related to immunodeficiency. Human salmonellosis is one of the most important foodborne diseases, and foods containing poultry meat

and eggs are usually involved in outbreaks (Jakabi et al., 1999).

Since *Salmonella* serotypes may be introduced by vertical transmission through imported birds and some of these serotypes are not compulsorily investigated according to the surveillance plan adopted by the poultry breeding industry, this work was carried out to study the pathogenicity of a *Salmonella enterica* subsp *enterica* serovar Kottbus strain in commercial broiler chicks reared from one to 42 days of age.

MATERIAL AND METHODS

Salmonella strain

It was used a *Salmonella enterica* subsp *enterica* serovar Kottbus (6,8:e,h:1,5) strain that had been isolated from imported day-old ducklings and was resistant to nalidixic acid and novobiocin (Galletti et al., 1999). The culture of *S.* Kottbus was prepared in 5 mL of nutrient broth (Oxoid, CM225), and incubated at 37°C/24h in a shaking water bath (100 rpm).

Experimental infections

Fifty newly hatched chicks from a broiler breeder flock were hatched at Laboratório Nacional Agropecuário (LANAGRO/SP) to be used in the experiment. Ten of these were sacrificed by cervical dislocation for bacteriological examination of the liver, spleen, egg yolk and cecal contents. The remaining birds were separated in three groups: one group of 20 birds received 0.1 mL of *S.* Kottbus culture containing 1.2×10^8 CFU/mL, the second group of 20 birds was inoculated with 1.2×10^5 CFU/mL and the third group of 10 birds was untreated (control group). Infected birds were housed in isolation units in a Biosafety Level 2 research facility and given water and antibiotic-free diet ad libitum. All birds were evaluated daily for clinical signs of disease and mortality. Dead chickens were removed from the isolation units, necropsied and examined for the presence of *Salmonella*.

Fifteen and 42 days post-infection, half of the birds were sacrificed by cervical dislocation and necropsied. Liver, spleen and cecal contents were collected to determine the number of *Salmonella* using Brilliant Green Agar plates containing 25 µg/mL nalidixic acid and 40 µg/mL novobiocin (Merck 7232) (VBNalNov), as described by Smith et al. (1980) (Table 1).

Cloacal swabs were taken at 24hs, 3 days, 7 days and then weekly after the infection, until the birds were 42 days old. Swabs were streaked directly onto VBNalNov plates and then kept in a tube containing 2

Table 1 - Recovery of *Salmonella* Kottbus in liver, spleen and cecal contents of chickens after infection. (\log_{10}/g).

Bird #	<i>Salmonella Kottbus</i> inoculum								
	1.2×10^8 (CFU/mL)			42 dpi			1.2×10^5 (CFU/mL)		
	L	S	C	L	S	C	L	S	C
01	3.78	0	7.00	0	0	6.90	0	0	8.30
02	4.30	3.78	7.70	0	0	7.00	0	<1	7.30
03	3.30	3.30	<1	0	0	6.30	5.30	0	6.30
04	<1	<1	7.70	0	0	6.60	<1	0	7.60
05	<1	<1	7.90	0	0	6.00	<1	0	7.30
06	3.30	<1	6.60	0	0	7.30	0	0	8.30
07	3.60	3.30	7.70	0	0	6.30	<1	<1	8.00
08	4.60	4.90	7.60	0	2.48	6.30	<1	0	8.60
09	3.78	3.60	7.60	0	0	<1	<1	<1	7.95
10	4.90	4.30	7.78	ND	ND	ND	ND	ND	ND

L= liver; S= spleen; C=cecal contents; ND= not done (mortality in the first week).

mL Selenite Broth (Merck 107717). Both plates and tubes were incubated at 37°C/24h. In case there was no growth, the swab was plated again onto VBNalNov and incubated at 37°C/24h (Barrow *et al.*, 1988).

RESULTS

No *Salmonella* was recovered from the 10 newly-hatched birds that were evaluated.

Although four birds died in the first week after oral inoculation with *S. Kottbus*, there were no clinical signs of disease throughout the experiment.

Table 1 presents the viable counts of *Salmonella* in birds sacrificed 15 and 42 days post inoculation (dpi). Higher numbers of *S. Kottbus* were recovered in the groups inoculated with culture of *S. Kottbus* containing 1.2×10^8 CFU/mL. In both groups, isolation from liver and spleen decreased with time, but remained high in cecal contents.

Isolation of *Salmonella* Kottbus from cloacal swabs using either direct plating (D) or plating after enrichment (T) is shown in Table 2. Organisms were detected in the feces from 24 h post-inoculation until 42 dpi.

DISCUSSION

Salmonellosis is still nowadays the main foodborne disease in humans. Modern practices in the poultry industry are even now very favorable to the maintenance and dissemination of *Salmonella* serotypes. The pathogenicity of many serotypes in chickens, including *S. Kottbus*, remains unknown. The host-parasite relationship between *Salmonella* and chicken can be assessed following oral inoculation of day-old birds (Smith & Trucker, 1980; Gast & Beard, 1992; Gast *et al.*, 1998). In the present study, day-old broiler chicks were orally infected with *Salmonella*

Kottbus to assess fecal shedding until 42 days of age and to examine the liver, spleen and cecal contents at 15 and 42 days of age.

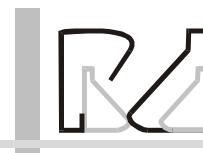
Table 2 - *Salmonella* Kottbus isolated from the cloaca after infection.

Time (pi)	<i>Salmonella Kottbus</i>								
	1.2×10^8 CFU/mL			1.2×10^5 CFU/mL			D	E	T
	D	E	T	D	E	T			
24 h	03	04	07/20	02	03	05/20			
03d	06	03	09/20	05	04	09/18*			
07d	16	01	17/19*	06	02	08/17*			
14d	10	07	17/19	13	03	16/17			
21d	05	04	09/09**	06	02	08/08**			
28d	09	00	09/09	04	02	06/08			
35d	08	01	09/09	06	01	07/08			
42d	04	01	05/09	03	04	07/08			

D = *S. Kottbus* isolated by direct plating on VBNalNov agar; E = *S. Kottbus* isolated after enrichment in selenite broth; T = D + E / total number of examined birds; * = total number reduced due to mortality; ** = total number reduced due to necropsy. pi = post-inoculation; d = days post-inoculation; h = hours post-inoculation

The results of oral inoculation in day-old commercial broiler chicks demonstrated that *S. Kottbus* colonized the liver, spleen and ceca at diverse intensity. It was recovered from these organs and persisted in the intestinal tract resulting in cecal colonization. Pathogenesis studies associated with virulent strains suggested that organisms multiply in the liver and spleen after invasion and then disseminate to other organs, producing a systemic infection (Barrow *et al.*, 1987). Gast & Holt (1998) recovered *S. Enteritidis* from the liver and spleen at seven dpi and in the ceca 24 weeks post-inoculation. Similar situations have been reported in chicks experimentally inoculated with other *Salmonella* serotypes (Smith & Turker, 1980; Barrow *et al.*, 1987; Gast & Beard, 1992; Parimal *et al.*, 2001).

In the present study, birds showed no signs of disease caused by *S. Kottbus*, but there was

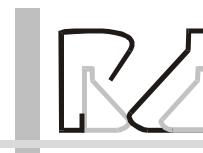


colonization of the cecum and shedding of the pathogen in the feces. *S. Kottbus* was detected in the feces since 24 h post-infection until 42 dpi, similarly to other serotypes of public health concern, including *Salmonella Typhimurium* and *Salmonella Enteritidis* (Barrow *et al.*, 1988; Gast & Holt, 1998; Smith & Tucker, 1980). In the alimentary tract, the main site of colonization is the cecum in both young and old chickens, but persistence is greater in younger birds. This has been previously reported (Smith & Turcker, 1980) and may be due to the fact that the gut flora in newly hatched chicks is simpler than in older birds (Barrow *et al.*, 1988). Since this serotype persists for a long time in birds, they may be contaminated until slaughter age. The stress to which infected birds are subjected during transportation to the abattoir enhances *Salmonella* shedding in the feces (Smith *et al.*, 1980). Consequently, bacteria numbers in the slaughterhouse are also increased, which makes it harder to obtain *Salmonella*-free carcasses (Boes *et al.*, 2001).

According to Jakabi *et al.* (1999), food prepared using poultry meat, eggs and egg products have been the most common source of *Salmonella* infections to humans. Synnot *et al.* (1998) reported human foodborne salmonellosis due to *Salmonella Agona* present in turkey meat. The authors also said that the disease was controlled after the etiologic agent had been investigated and the government had established a plan, including a monitoring program and general measures of hygiene and disinfection. This report should be considered as an alert to the programs adopted in the breeding poultry industry, i.e., control directed to only a few serotypes of *Salmonella* may not protect commercial poultry farms from the presence of *Salmonella* in their flocks. In Brazil, the National Plan of Surveillance of Avian Diseases (PNSA, Brasil, 1991) is currently focused on serotypes Gallinarum, Pullorum, Enteritidis and Typhimurium. Based on the present experiment with *Salmonella* Kottbus, any unexpected serotype present in imported birds might be intensively shed in the feces in the first days of life. It should be remembered that any food of animal origin should be free of *Salmonella*. In conclusion, not only *Salmonella* Kottbus but other serotypes might be introduced in Brazilian poultry farms through imported genetic material.

REFERENCES

- Barrow PA, Huggins MB, Lovell MA, Simpson JM. Observations on the pathogenesis of experimental *Salmonella typhimurium* infection in chickens. Veterinary Science 1987; 42:194-199.
- Barrow PA, Simpson JM, Lovell MA. Intestinal colonization in the chicken by food-poisoning *Salmonella* serotypes; microbial characteristics associated with faecal excretion. Avian Pathology 1998; 17:571-588. Ausente no texto
- Bäumler AJ, Hargis BM, Tsolis RM. Tracing the origins of *Salmonella* outbreaks. Science 2000; 287:50-52.
- Berchieri Jr A. Salmoneloses Avíárias. In: BERCHIERI JR A, MACARI M. Doenças das aves: FACTA 2000; 185-195.
- Boes J, Dahl J, Nielsen B, Krog HH. Effect of separate transport, lairage and slaughter on occurrence of *Salmonella typhimurium* on slaughter carcasses. Berl Munch Tierarztl Wochenschr 2001; 114(9-10):363-5.
- Brasil. Programa nacional de sanidade avícola. Atos Legais. Instrução Normativa 3. Diário Oficial da República Federativa do Brasil, Poder Executivo, Brasília – DF 1991. Seção 1.
- Carter JD, Hird DW, Farver TB, Hjerpe SA. Salmonellosis in hospitalized horses: seasonality and case fatality rates. Journal of the American Veterinary Medical Association 1986; 188(2):163-167.
- Center of Disease Control and Prevention. Outbreak of *Salmonella* serotype Kottbus Infections Associated with Eating Alfalfa Sprouts. Morbidity and Mortality Weekly Report 2002; 51(1):7-9. Available at <www.cdc.gov/mmwr/preview/mmwrhtml>. Accessed on May 15th, 2003.
- Clark DS, Thatcher FS. Análisis microbiológico de los alimentos. In: Alimentos – Microbiología Manuais de laboratório. Zaragoza: ACRIBIA 1973; 271.
- El_Ghodban A, Ghengesh KS, Marialigeti K, Abeid S. Serotypes, virulence factors, antibiotic sensitivity, beta-lactamase activity and plasmid analysis of *Salmonella* from children with diarrhea in Tripoli (Libya). Acta Microbiologica Hungarica 2002; 49(4).
- Forsythe RH, Ross WJ, Ayres JC. Salmonellae Recovery Following Gastro-Intestinal and Ovarian Inoculation in the Domestic Fowl. Poultry Science 1967; 46(4):849-855.
- Galletti MCM, Ribeiro SAM, Reis EMF, Doretto JR L, Orsi MA. Isolamento de *Salmonella enterica* subsp *enterica* serovar Kottbus em aves importadas. In: Conferência APINCO de Ciência e Tecnologia Avícolas. Anais... Campinas:FACTA; 1999:36.
- Gama NMSQ, Berchieri Jr A, Fernandes SA. Occurrence of *Salmonella* sp in Laying Hens. Brazilian Journal of Poultry Science 2003; 5(1):15-21.
- Gast RK, Holt P. Persistence of *Salmonella enteritidis* from one day of age until maturity in experimentally infected layer chickens. Poultry Science 1998; 77:1759-1762.
- Gast RK, Beard CW. Evaluation of a chick mortality model for predicting the consequences of *Salmonella enteritidis* infections in laying hens. Poultry Science 1992; 71:281-287.



Hoszowski A, Wasyl D. *Salmonella* serovars found in animals and feeding stuffs in 2001 and their antimicrobial resistance. *Bulletin of the Veterinary Institute in Pulawy* 2002; 46:165-178.

Ionova I, Monov G, Kunev ZH. Carrier state and body distribution of *Salmonella* bacteria in healthy piglets and calves. *Veterinarno Meditsinski Nauki* 1981; 18(7):98-104.

Jakabi M, Buzzo AA, Ristori CA, Tavechio AT, Sakuma H, Paula AMR, Gelli D. Observações laboratoriais sobre surtos alimentares de *Salmonella* sp, ocorridos na grande São Paulo, no período de 1994 a 1997. *Revista do Instituto Adolfo Lutz*, 1999; 58(1):47-51.

Lirio VS, Silva EA, Stefoni DC, Recco EAP, Maluf YT, Miyazawa TT, Neves DVDA, Oliveira VMR. Freqüência de 17 sorotipos de *Salmonella* isolados em alimentos. *Higiene Alimentar* 1998; 12(55):36-41.

Mian LS, Maag H, Tacal JV. Isolation of *Salmonella* from muscoid flies at commercial animal establishments in San Bernardino Count, California. *Journal of Vector Ecology* 2002; 27(1):82-85.

Nelius D, Neumann P, Hermann H, Kohler F. Septicopyemia caused by *Salmonella* Kottbus (6,8:eh:1,5). *Deutsche Gesundheitswesen* 1969; 24(51):2408-2411.

Office International des Epizooties. Manual of Standards Diagnostic Tests and Vaccines, part 3, section X, chapter X.4, 2000. Available at <www.oie.int/eng/normes/mmanual/A_summary.htm>. Accessed on October 20th, 2003.

Okamura M, Kamijima Y, Miroyuki T, Kazumi S, Baba E. Differences among six *Salmonella* serovars in abilities to colonize reproductive organs and to contaminate eggs in laying hens. *Avian Diseases* 2001; 45:61-69.

Parimal R, Dhillon AS, Shivaprasad HL, Schaberg DM, Bandli D, Johnson S. Pathogenicity of different serogroups of avian salmonellae in specific-pathogen-free chickens. *Avian Diseases* 2001; 45:922-937.

Peresi JTM, Almeida IAZC, Lima SI, Marques DF, Rodrigues ECA, Fernandes AS, Gelli, Irino K. Food borne disease outbreaks caused by *Salmonella* Enteritidis. *Saúde Pública* 1998; 32(5).

Ribeiro SAM, Orsi MA, Ferrati AR, Mendonça AO, Doretto JR L. Incidence of *Salmonella* in imported day-old ducklings. Brazil, 1998-2003. *Brazilian Journal of Poultry Science* 2004.

Smith HW, Tucker JF. The virulence of *Salmonella* strains for chickens: their excretion by infected chickens. *Journal of Hygiene* 1980; 84(3):479-488.

Smith HW, Tucker JF. The virulence of *Salmonella* strains for chickens: their excretion by infected chickens. *Journal of Hygiene* 1980; 84(3):479-488.

Synnot MB, Brindley M, Gray J, Dawson JK. An outbreak of *Salmonella* agona infection associated with precooked turkey meat. *Communicable Disease and Public Health* 1998; 1(3):176-179.

Taunay AE, Fernandes AS, Tavechio AT, Neves BC, Dias AMG, Irino K. The role of public health laboratory in the problem of salmonellosis in São Paulo, Brazil. *Revista do Instituto de Medicina Tropical* 1996; 38(2):119-127.

Tavechio AT, Fernandes AS, Neves BC, Dias AMG, Irino K. Changing patterns of *Salmonella* serovars: increase of *Salmonella* Enteritidis in São Paulo, Brasil. *Revista do Instituto de Medicina Tropical* 1996; 38(5):315-332.

Tavechio AT, Ghilardi AC, Peresi JT, Fuzihara TO, Yonamine EK, Jakabi M, Fernandes SA. *Salmonella* serotypes isolated from nonhuman sources in São Paulo, Brazil, from 1996 through 2000. *Journal of Food Protection* 2002; 65(6):1041-1044.

Veterinary Laboratories Agency, Surveillance Report Small Ruminants, Quarterly Report 2003; 6(4):7-9. Available at <www.defra.gov.uk/corporate/vla.htm>. Accessed on May 15th, 2003.