Brazilian Journal of Microbiology (2000) 31:50-52
ISSN 1517-8382

EXPERIMENTAL *SALMONELLA* GALLINARUM INFECTION IN LIGHT LAYING HEN LINES

Angelo Berchieri Júnior[1]*; Gláucia Helaine de Oliveira[1]; Lucas Augusto Soeiro Pinheiro[1]; Paul A. Barrow[2]

[1]Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, SP, Brasil. [2]Division of Environmental Microbiology, Institute for Animal Health, Compton Laboratory, Compton, Newbury, Berkshire, United Kingdom.

Submitted: December 04, 1998; Returned to authors for corrections: May 25, 1999; Approved: March 22, 2000

SHORT COMMUNICATION

ABSTRACT

Although the epidemiology of fowl typhoid in chickens supposedly involves a vertical transmission stage, a previous work run by the authors has suggested that this did not happen in a commercial line of laying hens highly susceptible to systemic disease with *Salmonella* Gallinarum. A new experiment was carried out in two other lines of commercial layers, considerably more resistant than those used in the previous study. Clinical fowl typhoid was not observed, but *Salmonella* Gallinarum was isolated from the spleen and liver four weeks after infection and, sporadically, from the ovary.

Key words: *Salmonella* Gallinarum, White Leghorn, infection, laying hens

Fowl typhoid remains a serious systemic disease of domestic poultry which may cause large scale economic losses through mortality, morbidity and reduction in egg production. The disease is under control in many countries in Europe and North America but remains a major problem in countries where poultry husbandry was recently intensified or where the high ambient temperature causes difficulties to environmental hygiene. Even in countries where the disease is controlled, vigilance remains important. The injudicious introduction of infected poultry has led to local national resurgence of the disease in northern Europe (10). Although the epidemiology of fowl typhoid has been well documented in the literature over the years, the relative contribution of horizontal and vertical transmissions remains unclear. Experimental work has shown that the infection may spread horizontally between pen mates (6, 12) and reports of vertical transmission were made several decades ago (3, 4, 5). There have been numerous reports of isolation of *S*. Gallinarum from the eggs of reactor birds (14, 15, 16) but much less information is available on direct evidence of transmission of the pathogen through eggs to the progeny. Hall *et al.* (13) found that 50% of typhoid reactors, infected either naturally or experimentally, produced infected eggs which resulted in infected chicks. A high percentage of these chickens (33%) died within six months; most died within the first month.

In a recent preliminary investigation on persistent infection of laying hens by *Salmonella* Gallinarum, *S*. Pullorum and *S*. Enteritidis it was found difficult to establish long lasting infections in young or adult commercial laying hens (7). Birds either succumbed to clinical disease or did not become infected. The evidence from the literature indicates that vertical transmission is associated with persistent infection in the absence of clinical disease. The commercial birds used in that preliminary investigation were highly susceptible to infection by this serotype. Therefore, the authors considered that more positive results may follow infection of a more resistant light line of hen. The present report describes the results of infection of

* Corresponding author. Mailing address: Faculdade de Ciências Agrárias e Veterinárias, UNESP, CEP 14870-000, Jaboticabal, SP, Brasil. E-mail: berchier@fcav.unesp.br

commercial layers belonging to a light breed with increased level of resistance to *S.* Gallinarum infection.

Two lines of commercial white laying hens (White Leghorn), which produce light-coloured white eggs, were used in this experiment. Birds from Hy-Line line were 44 weeks old and birds from Babcock line were 46 weeks old. All birds were laying when the experiment started. A commercial layer feed was provided by the farmer.

The birds were kept in individual wire cages to allow laying to continue. Cloacal swabs and blood samples for bacteriological and serological examination were taken to assess that birds were free of *Salmonella* before starting the experiment. Cloacal swabs were done as shown below. The samples of sera were submitted to slide agglutination test using a commercial pullorum antigen and also a overnight growth of *S.* Gallinarum on Brain Heart Infusion Agar (BHI agar). The birds were allowed to settle for three days before starting the experiment. The birds were numbered from 01 to 21 (Hy-Line) and 22 to 42 (Babcock). A broth culture of a nalidixic acid resistant mutant of *S.* Gallinarum 9 (2,17), prepared in Brain Heart Infusion broth (BHI broth) and incubated overnight at 37ºC in a shaking incubator, was prepared and diluted in BHI broth to contain 1.5 x 10^7 viable cells per ml. From this, 0.5 ml was orally inoculated into the crop of each bird. Three birds from each line were killed for post-mortem examination at 1, 3, 7, 14, 21 and 28 days after inoculation.

During necropsy, samples from liver, spleen, ovary, caecal contents, cloacae and blood were collected. All eggs found inside the oviduct were examined too. The blood samples were used for serological analysis and the other samples were investigated bacteriologically. Twice a week a cloacal swab was taken from all live birds. All eggs laid were inspected to *Salmonella*.

The bacteriological examination followed the methodology adopted previously by Barrow *et al.* (1). After opening the carcass, organs were removed aseptically in this order: ovary, spleen, liver, egg in oviduct and caeca. Samples from liver, spleen and caecal contents were homogenised with a Griffith's tube and decimal dilutions were prepared in PBS, pH 7.4. Viable counts were estimated by plating aliquots of dilutions on Brilliant Green agar containing 25 ug/ml of nalidixic acid and 2 ug/ml of novobiocin (BG Nal/Nov). The plates were incubated at 42ºC overnight. When there was no bacterial growth, an equal volume of double strength selenite broth was added to the sample. After overnight incubation at 42ºC, the broth was streaked on BG Nal/Nov. Cloacal swabs were placed in a tube containing 2 ml of selenite broth. After mixing, this broth was plated on BG Nal/Nov and the swabs in broth were incubated at 42ºC overnight. If no growth of *Salmonella* was obtained by direct plating, the overnight-enriched culture was plated in a similar manner. The ovary was added to 100 ml of selenite broth in a sterile jar, followed by gross maceration with sterile scissors. The jar was incubated at 42ºC overnight and the cultures were plated as mentioned above.

The sera from the blood samples were tested for agglutination on a glass slide with overnight growth of *S.* Gallinarum on BHI agar.

On arrival, bacteriological and serological examination of the birds did not reveal any evidence of *Salmonella* infection.

The results of the microbiological examination of the birds are presented in Table 1. Following oral inoculation of *Salmonella* Gallinarum 9 Nal^r, the bacterium was not isolated one day post infection but was detected in the liver and spleen of 3/6 birds four days post-inoculation, and in the cloaca of one bird after enrichment. Similar results were found in 4/6 birds after one week. The number of bacteria in the tissues had such a decrease that isolations were successful (after enrichment only) in 4/6 birds only after 2 weeks, and 2/6 only after 4 weeks. Isolations from the ovary were obtained after one and two weeks of inoculation. Over a post inoculation period of 34 days, Hy-line birds laid 253 eggs and Babcock birds laid 201 eggs. *Salmonella* was not isolated from these eggs.

At necropsy, typical fowl typhoid-like alterations were seen in the liver and spleen of several birds. After infection, the following was observed: three days post-inoculation (dpi), birds 16 and 37 presented enlarged and congested liver and hemorrhagic ovary follicles; 7 dpi: birds 14, 15, 35 and 36 presented fowl typhoid-like alterations; 14 dpi: bird 32 presented ovary atrophy and 33 presented fowl typhoid like alterations; 21 dpi : birds 7, 8, 29 and 30 with fowl typhoid-like lesions. The main fowl typhoid-like lesions were enlarged liver and spleen with necrotic and hemorrhagic points and green-yellowish liver colour. Typical changes in the structure of the ovaries were also seen. The diarrhoea was stronger for Hy-line than for Babcock birds. Both lines had a drop in egg production for 12 days from the fifth day after infection. However, in contrast to two susceptible line of birds also infected with *S.* Gallinarum in a similar study, there was no progress in the illness and no mortality was noticed. The assay using birds from susceptible lines demonstrated that the mortality is high and initiated 5-7 dpi. In those few remaining birds which did not develop the disease, *S.* Gallinarum was not isolated in samples taken from liver, spleen, ovary, heart and caecal contents (7). Thus, the result does suggest that lines of chicken genetically resistant to systemic salmonellosis may harbour the pathogen for several weeks. During this experiment the organism was not isolated from the laid eggs but it is possible that insufficient eggs were examined, although a figure of 6% of hatched eggs was mentioned by Hall *et al.* (13). The resistance seen by Bumstead and Barrow (8, 9), manifested by a difference in the rate at which the pathogen multiplied in the reticulo-endothelial system cells and the rate of decay in the tissues, was not involved since this could not, anyway, be measured. Thus, it is possible that whereas transmission of infection between birds may be primarily horizontal in susceptible lines due to ingestion of faeces and mucus containing *S.* Gallinarum and to cannibalism, vertical transmission is much more likely to occur in lines which are

Table 1. Positivity for *Salmonella* Gallinarum infection in two white eggs laying hens (White Leghorn).

	4 days*						1 week*						2 weeks*						4 weeks*					
Bird number**	16	17	18	37	38	39	13	14	15	36	35	34	10	11	12	33	32	31	04	05	06	27	26	25
Collected sample																								
Liver	3.5***	-	-	3.2***	3.7***	-	-	3.0***	2.5***	2.8***	3.3***	-	-	-	-	+****	+****	-	-	-	+****	-	-	-
Spleen	2.8***	-	-	3.0***	3.4***	-	-	2.5***	2.3***	2.9***	3.2***	-	-	-	-	+****	-	-	-	-	+****	-	-	+****
Ovary	-	-	-	-	+****	-	-	+****	-	-	+****	-	+****	+****	-	+****	-	-	-	-	-	-	-	-
Egg inside oviduct	-	na	-	-	-	-	-	na	-	na	na	-	-	na	-	na		-	-	-	-	-	-	-
Cloacal swab	-	-	-	+****	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Caecal contents	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* Killing time after inoculation with *S.* Gallinarum
** Birds 01 to 21 are Hy-line and 22 to 42 are Babcock
*** log10
**** Positive result after enrichment in selenite broth; na = not available.

genetically resistant to clinical fowl typhoid. This has a number of implications for management and breeding of lines which are more resistant to clinical disease. The rate of destruction of *Salmonella* in the tissues of resistant birds may be increased by vaccination. There is some indication that killed vaccines, although not very effective in *Salmonella*-susceptible mouse lines, could be more effective in genetically resistant lines (11).

ACKNOWLEDGEMENTS

The authors wish to acknowledge Mr. A.J. dos Santos and Mrs. A. R. Batista for assistance in various ways and to thank CNPq and FAPESP for financial support.

RESUMO

Infecção experimental por *Salmonella* Gallinarum de aves leves de postura comercial

Estudo anterior, realizado pelos mesmos autores com aves consideradas susceptíveis ao agente do tifo aviário, sugeriu que a relação entre a bactéria e a aves restringe-se ao período da enfermidade. Neste trabalho avaliou-se a relação hospedeiro-parasita entre *Salmonella* Gallinarum e aves leves de postura comercial, consideradas mais resistentes ao tifo aviário. As aves não desenvolveram a doença clínica, mas a bactéria foi isolada do baço e do fígado quatro semanas após a infecção e, em algumas ocasiões, também do ovário.

Palavras-chave: *Salmonella* Gallinarum, White Leghorn, infecção, aves de postura

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