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Research paper

Relationship of *Salmonella* infection and inflammatory intestinal response with hematological and serum biochemical values in laying hens

Mario Alberto Soria, María Agustina Bonnet[1], Dante Javier Bueno*

Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria Concepción del Uruguay, Casilla de Correo N° 6, 3260 Entre Ríos, Argentina

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ABSTRACT

There are few studies about the blood serum of laying hens infected with *Salmonella*. The differential leukocyte count and blood chemistry values are an important aid in the diagnosis of human diseases, but blood parameters in the avian species are not well known. On the other hand, invasive forms of bacterial gastroenteritis, like *Salmonella*, often cause intestinal inflammation so this study was undertaken to find a biomarker of *Salmonella* infection and inflammatory intestinal response in the hematological or serum biochemical parameters in laying hens. Furthermore, we evaluated the association of some farm characteristics with *Salmonella* infection and fecal leukocytes (FL). A fecal sample with at least one fecal leukocyte per field was considered positive for inflammatory intestinal response. False positive serum reactions for *Salmonella* infection, by serum plate agglutination (SPA) test, were reduced by heating the sample to 56 °C for 30 min and then diluting it 5-fold. The range of hematological and biochemical parameter values was very wide, in addition, there was a poor agreement between the SPA and FL results. Comparison of the positive and negative samples in SPA and FL showed that 1.3% and 79.8% of the laying hens were positive and negative in both tests, respectively. Hens with a positive SPA result showed a higher percentage of monocytes than those with a negative SPA result. Hens with a positive FL test had a higher percentage of heterophils, ratio of heterophils to lymphocytes and aspartate aminotransferase values, while the percentage of lymphocytes was significantly lower ($P < 0.05$) than those with a negative FL test. The risk of *Salmonella* infection increased when the age of laying hens and the number of hens per poultry house was greater than or equal to 18 months old and 10,000 laying hens, compared to less than 18 months old and 10,000 laying hens, respectively. On the other hand, the risk of inflammatory intestinal response was higher in laying hens ≥18 months old than in hens <18 months old. Despite the fact that we did not find any specific biomarker of *Salmonella* infection, this is the first report about the change of *Salmonella* infection and inflammatory response in hematological/serum biochemical values for laying hens.

1. Introduction

Infections of poultry with salmonellae can be grouped into two categories. One is infection with the two non motile biovars, *Salmonella* Gallinarun biovar Pullorum (SP) and *Salmonella* Gallinarum biovar Gallinarum (SG), which are avian species-adapted bacteria responsible for

* Corresponding author. Tel.: +54 3442 438073–75; fax: +54 3442 438073–75.
E-mail address: dantejb@yahoo.com.ar (D.J. Bueno).
[1] Present address: Policía Federal Argentina, Gabinete Científico Concepción de Uruguay, San Martín 596, 1er piso, 3260, Concepción del Uruguay, Entre Ríos, Argentina.

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Table 1
Poultry farms sampled in six counties of Entre Rios, Argentina, from August, 2011 to April, 2012.

County of Entre Ríos	City	No. of poultry farms sampled	No. of laying hens sampled
Colon	Colonia Nueva Norte	1	20
	San José	3	50
	Colon	1	30
Diamante	Aldea Protestante	1	30
	Diamante	4	65
Gualeguay	Gualeguay	1	15
Nogoyá	Algarrobitos	1	20
Paraná	Aldea María Luisa	2	60
	Crespo	8	175
	Espinillo	2	25
	Paraná	1	10
	Seguí	1	10
Uruguay	Herrera	1	10
	San Cipriano	3	35
Total	14	30	555

pullorum and fowl typhoid disease, respectively (Waltman and Gast, 2008). The second category is infection with the numerous motile *Salmonella* serotypes (example, S. Enteritidis, S. Typhimurium, and S. Heidelberg), referred to collectively as paratyphoid salmonellae (Gast and Richard, 2013).

A definitive diagnosis of *Salmonella* infection requires the isolation and identification of the *Salmonella* organism (Shivaprasad, 2003; Gast and Richard, 2013). However, the macroscopic tube agglutination (TA) test, whole blood agglutination test, serum plate agglutination (SPA) test, and/or enzyme-linked immunosorbent assay (ELISA) can also be of great value in detecting *Salmonella* infection (Barrow, 1992; Gast, 1997; Jalil and Islam, 2010; Xavier et al., 2011). The TA and SPA tests, with *Salmonella* Pullorum strains, are commonly used for detection and removal of reactor birds in salmonellosis control programs in some countries (Shivaprasad, 2003).

In general, blood examination is performed for several reasons as a screening procedure to assess general health (Simaraks et al., 2004). The differential leukocyte count is important in poultry, but blood parameters in avian species are not nearly as well-known as in humans (Anderson and Stephens, 1970). There are few studies about blood serum in laying hens infected with *Salmonella* (Freitas Neto et al., 2007; Garcia et al., 2010). In addition, hematological values are important to clinico-pathological diagnosis such as bacterial septicemia. Managing abnormality in hens requires an understanding of how disease changes the biochemical functions of the body (Simaraks et al., 2004).

Invasive forms of bacterial gastroenteritis, such as those produced by *Salmonella*, often cause intestinal inflammation whereas most forms with viral, parasitic, and toxin-mediated etiologies do not do so in human beings (Gill et al., 2003). *Salmonella* induces an inflammatory diarrhea response with edema, mucosal bleeding of variable intensity, leukocytic chemoatraction and infiltration. This diarrhea involves the presence of leukocytes in stool (Huicho, 1995). It is known in avian cells that S. Typhimurium and S. Enteritidis invasion produces a strong inflammatory response, which may limit the spread of *Salmonella* largely to the gut, whilst S. Gallinarum does not induce an inflammatory response and may not be limited by the immune system, leading to severe systemic disease fowl typhoid (Kaiser et al., 2000). Rapid stool assays currently available include microscopic examination for leukocytes and erythrocytes and a rapid immunologic assay for fecal lactoferrin in humans (Gill et al., 2003). However, there is a lack of information about these kinds of assays for *Salmonella* diseases in poultry. Therefore, the present study was undertaken to find a biomarker of *Salmonella* infection and inflammatory intestinal response in the hematological or serum biochemical parameters in laying hens. Furthermore, we evaluated the association of some farm characteristics with *Salmonella* infection and fecal leukocytes (FL).

2. Materials and methods

2.1. Hens sampling and study locations

A total of 555 sera samples were collected from commercial laying hens, housed in cages and belonging to 30 poultry farms (40 poultry houses) situated in 6 counties of Entre Ríos, Argentina, from August, 2011 to April, 2012 (Table 1). Ten to twenty blood samples were taken from each laying hen house. There were 18 (250 samples) and 22 (305 samples) poultry houses that had hens of <18 months old and ≥18 months old, respectively. In reference to the number of laying hens per poultry house, 27 poultry houses (325 samples) had less than 10,000 hens with 2 to 6 hens per cage. The others (230 samples) had ≥10,000 hens with 3 to 13 hens per cage; 6 poultry houses (110 samples, 48%) had 8 or more hens per cage. The genetic strains of laying hens were Hy-Line, Bovans, Lohmann and H&N. The age of the hens was between 4 months old and 36 months old. Most of the laying hens were white egg layers and did not receive any vaccine for *Salmonella*.