

# Hematological Changes in *Salmonella* Paratyphi A Infected Pullets

A.O. Ogunleye<sup>1</sup> A.T.P. Ajuwape<sup>1\*</sup> A.I. Adetosoye<sup>1</sup>

## Keywords

Chicken – Pullet – *Salmonella* Paratyphi A – Blood – Experimental infection – Nigeria.

## Summary

The hematology of 25 pullets experimentally infected orally with 0.5 mL of  $1 \times 10^7$  cfu/mL of *Salmonella* Paratyphi A was studied. By day 4 post-infection (p.i.), dullness, ruffled or unkempt feathers, somnolence, yellowish-green diarrhea, and decreased water and feed consumption were observed. Hematological parameters of the birds were monitored on days 7, 14 and 21 p.i. The values were analyzed with Duncan's Multiple range test, at  $P < 0.05$ . The packed cell volume (PCV) was significantly higher at day 7 p.i. than during pre-infection and at day 21 p.i. However, it was similar at days 7 and 14 p.i. Likewise, hemoglobin concentrations in the birds were similar during pre-infection, and at days 14 and 21 p.i. In contrast, they were significantly higher at day 7 than during pre-infection and at day 21 p.i. The indicators of anemia showed no significant differences between pre- and post-infection values. The period coincided with that of decreased feed and water consumption in the course of the disease. There were no significant differences in total leukocyte counts for days 7, 14 and 21 p.i., whereas they were significantly ( $P < 0.05$ ) lower during pre-infection than at days 7, 14 and 21 p.i. The course of *Salmonella* Paratyphi A infection depends on the infective dose of the organism. There was no anemia in pullets; leukocytosis was attributable to heterophilic leukocytosis, lymphocytosis, monocytosis and eosinophilia, which are indicators of acute infection and internal stress, to which the pullets adequately responded.

## INTRODUCTION

*Salmonella* is a facultative intracellular pathogen capable of infecting a variety of hosts resulting in several clinical manifestations (5). Domestic poultry constitutes the largest single reservoir of *Salmonella* organisms found in nature (8).

*Salmonella* species are capable of producing changes in the white and red blood cells of the affected animals. For instance, pigeons infected with *Salmonella typhimurium* showed leukocytosis within

the first twenty-one days post-infection (7). A group of cockerels injected with 50 mg/kg body weight of *Salmonella gallinarum* endotoxin intravenously produced severe hypochromic anemia, leukocytosis and increased phagocytic activity of leukocytes (10). In another investigation Asheg et al. (2) experimentally infected a set of day-old chicks orally with  $2 \times 10^2$  cfu/mL of *Salmonella enteritidis* PT4 and recorded a significant ( $P < 0.05$ ) increase in the peripheral lymphocyte count on day 21 post-infection (p.i.), while a significant increase was observed on day 27 p.i. for chicken orally infected with  $2 \times 10^8$  cfu/mL.

*Salmonella enterica* serovars often have a broad host range, and some cause gastrointestinal and or systemic disease. *Salmonella enterica* serotypes Typhi and *Salmonella* Paratyphi A, B, C are responsible for systemic diseases including typhoid fever and paratyphoid enteric fever, respectively (11, 12). In Nigeria, Ogunleye

1. Department of Veterinary Microbiology and Parasitology, University of Ibadan, Ibadan, Nigeria.

\* Corresponding author

Tel.: +234 803 337 1426; Fax: +234 802 810 3641

E-mail: atpajuwape@yahoo.com

et al. (13) reported an outbreak of fulminating disease in a commercial poultry farm attributable to *Salmonella Paratyphi A*. The pathogenicity of the isolate was later tested in pullets which were inoculated orally with 0.5 mL of  $1.3 \times 10^8$  cfu/mL of *Salmonella Paratyphi A* per bird (14). However, the course of the disease was so rapid that the hematological changes in the infected birds could not be monitored and the pullet mortality was 95% by day 16 p.i. The use of hematology as a tool in the diagnosis of diseases of birds is increasing rapidly and can provide essential information, in addition to that obtained from the general clinical examination of animals (7, 15). In Nigeria, there has been no report on the hematological changes associated with *Salmonella Paratyphi A*. This investigation was undertaken to study hematological changes in pullets infected with *Salmonella Paratyphi A*.

## ■ MATERIALS AND METHODS

Two hundred and fifty day-old chicks were donated by Amo-Byng (Awe, Oyo State, Nigeria). The birds were raised under strict hygienic conditions for 16 weeks in experimental cages located in the Faculty of Veterinary Medicine University of Ibadan, Oyo State. They were fed *ad libitum* up to week 9 with antibiotic-free chick mash donated by the feed meal unit of Hope Farm (Ibadan, Oyo State), and antibiotic-free growers' mash from week 9 till the end of the investigation. Clean water was provided *ad libitum* in troughs. Vaccines, from the National Veterinary Research Institute (Vom, Plateau State), were routinely administered to the birds against the Newcastle disease on days 1 and 16, and weeks 6 and 10 (intra-ocular and LaSota vaccines, LaSota and Komorov, respectively), and against the infectious bursal disease on days 8 and 18, and week 4 (Gumboro, live attenuated, respectively). The chicks were given an anti-stress vitamin supplement (Vitalyte Laboratories Hipra, Avda, LaSelva SIN Amer., Girona, Spain), at a dosage of 100 g/200 L of drinking water. The birds were dewormed at weeks 9 and 11 using Piperazine (Pfizer Products, Ikeja, Nigeria) at a dosage of 0.7 g/L of drinking water.

At week 16, 25 pullets were randomly selected and the cloacal swab samples were collected and screened for *Salmonella* isolates. Briefly, the swabs were inoculated into sterile broth overnight, subsequently inoculated into 5% sheep blood agar and MacConkey media, incubated at 37°C for 48 h, and identified as described by Barrow and Feltham (3), and Abouzeed et al. (1). The jugular venous blood sample of each bird was collected into bottles with disodium salt of ethylene diamine tetraacetic acid (EDTA) anticoagulant. The blood samples were analyzed by standard methods (7, 9). The packed cell volume (PCV) was determined by the microhematocrit method, hemoglobin (Hb) concentration by the spectrophotometric method, and red blood cells, platelets and white blood cell counts by the hemocytometer method. The mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH) were derived from the red cell values. The differential leukocyte counts were determined by Giemsa stained blood film, and 200 cells were counted, classified and the absolute leukocyte counts were calculated (7, 9).

The *Salmonella Paratyphi A* used in this investigation was isolated from disease outbreaks and pathogenicity studies, earlier documented by Ogunleye et al. (13, 14). Each of the 25 pullets were inoculated orally with 0.5 mL of  $1 \times 10^7$  cfu/mL of overnight broth culture of *Salmonella Paratyphi A* and blood samples were taken for complete hematological analysis at days 7, 14 and 21 p.i.

The data obtained were statistically analyzed with one way analysis ANOVA and the difference among the harmonic samples were

tested for significance at 95% level of confidence with Duncan multiple range test (4).

## ■ RESULTS

No *Salmonella* was found in the pre-infection cloacal swabs examined. However, 0.5 mL of  $1 \times 10^7$  cfu/mL of overnight broth culture of *Salmonella Paratyphi A* orally inoculated produced apparent inactivity by day 3 p.i. By day 6 p.i. the pullets showed dullness, somnolence, decreased feed and water intake, followed by yellowish watery diarrhea. The first death was recorded on day 7 p.i. and when the experiment was terminated on day 21 p.i. the mortality rate was 24%.

The hematological changes observed in pullets experimentally infected with *Salmonella Paratyphi A* during the pre-inoculation day as well as on days 7, 14, and 21 p.i., respectively, are shown in Table I.

## ■ DISCUSSION

In an earlier study, Ogunleye et al. (14) administered orally 0.5 mL of  $1.3 \times 10^8$  cfu/mL of *Salmonella Paratyphi A* to each bird. The mortality rate was 95% by day 16 p.i.; the progression of the disease was so rapid that the hematological changes in the infected birds could not be monitored. In the present study, the course of the disease was not as rapid as in the earlier experiment, and the hematological changes in the bird were monitored at days 7, 14 and 21 p.i. When the experiment was terminated on day 21 p.i., the mortality rate was 24%. These findings suggest that the course of *Salmonella Paratyphi A* in birds is dose dependent.

The hematological pre-inoculation parameters of the pullets were within the range earlier recorded by Jain (6). In the current investigation, PCV on day 7 p.i. was significantly higher than pre-infection PCV, as well as PCV on day 21 p.i., but PCVs on days 7 and 14 were similar. Hb concentration values during pre-infection, and on days 14 and 21 p.i. were similar, while on day 7 it was significantly higher than during pre-infection as well as on day 21 p.i. MCV and MCH showed no significant differences in the pre- and post-infection values indicating that no anemia was present. MCHCs on days 7, 14 and 21 p.i. were similar, although they were significantly ( $P < 0.05$ ) lower on days 7 and 14 than during pre-infection, and during pre-infection and on day 21 p.i. they were similar. Hence, the above values were due to relative increases in PCV and Hb values. Birds generally have lower MCHCs than mammals because of the space occupied by the nucleus (6). The period, however, coincided with the period of decreased feed and water consumption in the course of salmonellosis in the birds. The same phenomenon might have been responsible for the relative thrombocytosis recorded on day 7 p.i. In an earlier investigation by Kokosharov, intravenous injection of *Salmonella typhimurium* endotoxin into cockerels (10) produced hypochromic anemia, leukocytosis and increased phagocytic activities of the leukocytes. The relative increases in PCV and Hb observed in this investigation were attributable to *Salmonella Paratyphi A*, contrarily to Sharma et al. (16) who observed a decrease in PCV and in the total erythrocyte count in goats infected with *Salmonella enterica* serovar *typhimurium*. The observed differences in response to the *Salmonella* infection may be due to the physiology of chicken erythrocytes, which are immature-nucleated, while in goats they are non-nucleated.

There was no significant difference in the total leukocyte counts on days 7, 14 and 21 p.i., whereas the pre-infection total leukocyte count was significantly ( $P < 0.05$ ) lower than those on days 7,

Table 1

Hematological changes in pullets experimentally infected with *Salmonella Paratyphi A*

Hematological value	Pre-infection	Day 7 post-infection	Day 14 post-infection	Day 21 post-infection
PCV (%)	35.7 <sup>b</sup>	40.8 <sup>a</sup>	39.0 <sup>ab</sup>	36.4 <sup>b</sup>
Hb (g/dL)	11.7 <sup>b</sup>	13.0 <sup>a</sup>	12.5 <sup>ab</sup>	11.7 <sup>b</sup>
RBC x 10 <sup>6</sup> /μL	2.9 <sup>b</sup>	3.3 <sup>a</sup>	3.7 <sup>a</sup>	3.0 <sup>b</sup>
Platelet	210,457.2 <sup>b</sup>	325,000.0 <sup>a</sup>	151,800.0 <sup>b</sup>	199,600.0 <sup>b</sup>
MCV (fl)	124.6 <sup>a</sup>	123.3 <sup>a</sup>	105.5 <sup>a</sup>	125.5 <sup>a</sup>
MCH (pg)	40.8 <sup>a</sup>	39.4 <sup>a</sup>	33.9 <sup>a</sup>	40.6 <sup>a</sup>
MCHC (%)	32.8 <sup>a</sup>	31.9 <sup>b</sup>	32.2 <sup>b</sup>	32.3 <sup>ab</sup>
WBC/μL	12,928.6 <sup>b</sup>	16,870.0 <sup>a</sup>	18,730.0 <sup>a</sup>	18,210.0 <sup>a</sup>
Heterophils/μL	2,706.4 <sup>b</sup>	6,906.0 <sup>a</sup>	5,317.6 <sup>a</sup>	4,987.5 <sup>a</sup>
Lymphocytes/μL	9,652.1 <sup>b</sup>	8,979.4 <sup>c</sup>	12,343.8 <sup>a</sup>	11,591.1 <sup>b</sup>
Monocytes/μL	148.3 <sup>b</sup>	186.3 <sup>b</sup>	263.1 <sup>b</sup>	479.6 <sup>a</sup>
Eosinophils/μL	351.4 <sup>c</sup>	798.3 <sup>b</sup>	728.3 <sup>b</sup>	1,158.8 <sup>a</sup>
Basophils/μL	0	0	0	0

Sample size: 25

Harmonic mean sample used: 5.385

<sup>a, b, c</sup> Values with different superscripts are significantly different at  $P < 0.05$ 

PCV: packed cell volume; Hb: hemoglobin; RBC: red blood cell count; MCV: mean corpuscular volume; MCHC: mean corpuscular hemoglobin concentration; WBC: white blood cell count

14 and 21 p.i., respectively. This finding agrees with that of Jansotovic et al. who observed within the first 21 days p.i. leukocytosis in pigeons infected with *Salmonella typhimurium* (6). Similarly, Kokosharov (10) reported a leukocytosis in cockerels injected with *Salmonella gallinarum* endotoxin, and Asheg et al. (2) recorded the same in a set of day-old chicks infected orally with  $2 \times 10^2$  cfu/mL of *Salmonella enteritidis* PT4.

The pre-infection absolute lymphocyte count was not significantly different from the value observed on day 21 p.i., whereas the lymphocyte count on day 7 p.i. was significantly ( $P < 0.05$ ) lower than the values recorded during pre-infection, and on days 14 and 21 p.i. However, the lymphocyte count on day 14 p.i. was significantly ( $P < 0.05$ ) higher than the values obtained during pre-infection and in day 21 p.i. Initial lymphopenia followed by lymphocytosis before the lymphocyte count normalized in this investigation indicated that the birds were adequately responding to the infection. Asheg et al. (2) recorded a significant ( $P < 0.05$ ) increase on day 21 p.i. in the peripheral lymphocyte count of day-old chicks orally infected with  $2 \times 10^2$  cfu/mL of *Salmonella enteritidis* PT4, whereas a significant increase was observed on day 27 p.i. in chicken infected orally with  $2 \times 10^8$  cfu/mL.

On days 7, 14 and 21 p.i. absolute heterophil counts were not significantly different, whereas they were significantly ( $P < 0.05$ ) higher than the value obtained during pre-infection. The heterophilic leukocytosis (neutrophilia) observed in the current investigation matches reports of neutrophilia observed by Sharma et al. (16) in goats infected with *Salmonella enterica* serovar *typhimurium*.

The absolute monocyte counts obtained during pre-infection and on days 7 and 14 p.i. did not show significant differences, whereas all three were significantly ( $P < 0.05$ ) lower than that obtained on day 21 p.i. This result suggests monocytosis in the experimentally

infected pullets. The absolute eosinophil counts on days 7 and 14 p.i. were not significantly different but they were significantly ( $P < 0.05$ ) higher than the pre-infection value, respectively. However, on day 21 it was significantly ( $P < 0.05$ ) higher than during pre-infection as well as on days 7 and 14 p.i.

## CONCLUSION

This experimental infection of pullets with *Salmonella Paratyphi A* showed that the disease course was infective dose-dependent and that the organism did not cause anemia. There was a leukocytosis attributable to heterophilic leukocytosis (neutrophilia), lymphocytosis, monocytosis and eosinophilia, which are indicators of acute infection and internal stress, to which the pullets adequately responded.

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## Résumé

**Ogunleye A.O., Ajuwape A.T.P., Adetosoye A.I.** Modifications hématologiques chez des poulettes infectées par *Salmonella Paratyphi A*

Une analyse hématologique a été effectuée sur 25 poulettes infectées expérimentalement par voie orale avec 0,5 mL de  $1 \times 10^7$  CFU/mL de *Salmonella Paratyphi A*. Quatre jours après l'infection (p.i.), les animaux ont présenté de l'apathie, un plumage ébouriffé, de la somnolence, des diarrhées vert jaunâtre, une baisse de la prise d'eau et d'aliments. Les paramètres hématologiques des volailles ont été enregistrés aux jours 7, 14 et 21 p.i. Les valeurs ont été analysées avec le test de comparaisons multiples de Duncan, avec  $P < 0,05$ . L'hématocrite a été significativement plus élevé au jour 7 que lors de la pré-infection et qu'au jour 21 p.i. Il a cependant été semblable aux jours 7 et 14 p.i. De même, les concentrations d'hémoglobine chez les volailles ont été semblables lors de la pré-infection et aux jours 14 et 21 p.i. Elles ont été significativement plus élevées au jour 7 que lors de la pré-infection et qu'au jour 21 p.i. Les indicateurs d'anémie n'ont pas révélé de différences significatives entre les valeurs pré-infection et post-infection. La période a coïncidé avec celle de la baisse de prise d'eau et d'aliments lors de l'évolution de la pathologie. Il n'y a pas eu de différences significatives dans le nombre de leucocytes total aux jours 7, 14 et 21 p.i., mais ce nombre a été significativement ( $P < 0,05$ ) plus bas lors de la pré-infection qu'aux jours 7, 14 et 21 p.i. L'évolution de l'infection à *Salmonella Paratyphi A* dépend de la dose infectante du micro-organisme. Les poulettes n'ont pas manifesté d'anémie ; la leucocytose pouvait être attribuée à la leucocytose hétérophile, à la lymphocytose, à la monocytose et à l'éosinophilie, qui sont des indicateurs d'infection aiguë et de stress interne auxquels les poulettes ont répondu de façon satisfaisante.

**Mots-clés :** Poulet – Poulette – *Salmonella Paratyphi A* – Sang – Infection expérimentale – Nigeria.

## Resumen

**Ogunleye A.O., Ajuwape A.T.P., Adetosoye A.I.** Cambios hematológicos en pollos infectados con *Salmonella Paratyphi A*

Se estudió la hematología de 25 pollos infectados oralmente en forma experimental con 0,5 mL de  $1 \times 10^7$  cfu/mL *Salmonella Paratyphi A*. Al día 4 post infección (p.i.), se observó apatía, plumas hirsutas y sin aseo, somnolencia, diarrea verde amarilla, disminución en el consumo de agua y alimento. Los parámetros hematológicos de las aves se monitorearon en los días 7, 14 y 21 p.i. Los valores se analizaron con el test de rango múltiple de Duncan, a  $P < 0.05$ . El hematocrito (PCV) fue significativamente más elevado al día 7 p.i. que durante la pre infección y al día 21 p.i. Sin embargo, fue similar en los días 7 y 14. De la misma manera, la concentración de hemoglobina en las aves fue similar durante la pre infección y a los días 14 y 21 p.i., mientras que fue significativamente más elevada al día 7 que durante la pre infección y al día 21 p.i. Los indicadores de anemia no mostraron diferencias significativas entre los valores pre infección y la post infección. El periodo coincidió con el periodo de disminución en el consumo de agua y alimento durante el curso de la enfermedad. No se encontraron diferencias significativas en el conteo total de leucocitos para los días 7, 14 y 21 p.i., mientras que fueron significativamente ( $P < 0,05$ ) más bajos durante la pre infección que en los días 7, 14 y 21 p.i. El curso de la infección por *Salmonella Paratyphi A* depende de la dosis infecciosa del organismo. No hubo anemia en los pollos; en cambio, la leucocitosis se atribuyó a leucocitosis heterofílica, linfocitosis, monocitosis y eosinofilia, los cuáles son indicios de una infección aguda y estrés interno, al cual los pollos respondieron adecuadamente.

**Palabras clave:** Pollo – Gallina – *Salmonella Paratyphi A* – Sangre – Infección experimental – Nigeria.