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HAEMATOLOGICAL CHANGES IN LAYERS EXPERIMENTALLY INFECTED WITH SALMONELLA GALLINARUM

Chiroma Mohammed Adam[1], Adamu Sani[2], Gadzama Joseph John[1], Esievo King Akpofure Nelson[2], Abdulsalam Hassan[1], Sani Nuhu Abdulazeez[3], Joshua Luka[4], Muhammad Ya'u[5]

1. Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Maiduguri, Borno State, Nigeria.
2. Department of Veterinary Pathology, Ahmadu Bello University, Zaria, Zaria, Nigeria.
3. Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Abuja, Abuja, Nigeria
4. Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Maiduguri, Maiduguri, Nigeria
5. Department of Animal Health and Production, Binyaminu Usman Polytechnic Hadejia, Jigawa State

Corresponding Author: Adam CM
Email: drmohammedchiroma78@gmail.com

Abstract

Aim: The present study was conducted to investigate the haematological changes in layers experimentally infected with Salmonella gallinarum.

Methods: A total of 20 eighteen-week- old ISA Brown layers were used for the experiment. The birds were randomly divided into two groups, infected and control, of 10 birds each. To establish the infection, each bird in the infected group was orally administered 0.5 ml of the inoculum containing $9x10^8$CFU/ml. Similarly, birds in the control group were each administered 0.5 ml normal saline only. Following the inoculation, all experimental birds were closely monitored for clinical signs of fowl typhoid. Blood samples were collected from each group at day zero (Day 0), 2, 4, 7, 14, 21, 28, 35 and 42, post-infection (pi) and used for determination of haematological parameters. By day seven post infection, all birds in the infected group showed clinical signs typical of fowl typhoid; namely weakness, ruffled feathers, huddling together, somnolence, greenish-yellow diarrhea, weight loss, drop in egg production, decrease in feed and water consumption and mortality rate (50%). There were, however, macrocytic hypochromic anaemia, leuckocytosis and heterophilia. In conclusion, the experimental Salmonella Gallinarum infection induced acute anaemia, leukocytosis, heterophilia and lymphopenia.

Key Words; Fowl typhoid, Salmonella, Inoculum, Leukocytosis

INTRODUCTION

Fowl typhoid caused by Salmonella Gallinarum is recognized worldwide as a disease of social and economic significance (Shivaprasad, 1997). In Africa, it has been reported in many countries including Tanzania, Uganda (Okoj, 1993), Senegal (Arbelot et al., 1997), Nigeria (Sa'idu et al., 1994) and Morocco (Bouzoubaa et al., 1987). It is a septicaemic disease that affects primarily chicken and turkey, although natural infections in many other avian species have been reported (Wray et al., 1996; Shivaprasad, 1997). Although Salmonella Gallinarum infection is frequently considered a problem of adult and grower chicken, chicks are often affected. The outbreak of fowl typhoid in young chicks may be associated with vaccination against fowl typhoid practiced by most breeders

which leads to vertical transmission of the disease (Jordan and Pattison, 1992; Roa, 2000). Efforts at controlling fowl typhoid through the application of a co-ordinated policy of hygienic measures, together with serological testing and slaughter of positive reactors, have led to the seemingly eradication of Salmonella gallinarum in many developing countries (Barrow, 1999). However, fowl typhoid remains a leading disease of the poultry industry in many areas of the world (Okwori et al., 2013). Acute form of the disease manifests as respiratory distress and depression with a characteristic clinical sign of greenish- yellow diarrhea, there may be enlarged and congested liver, spleen and kidney. Liver may have white foci of 2-4mm in diameter (Beyaz et al., 2010). In acute to sub acute cases, there is multifocal necrosis of hepatocytes with accumulation of fibrin and infiltration of heterophils mixed with a few lymphocytes and plasma cells can be seen in the liver (Kokosharov et al., 1997; Hossain et al., 2006). In acute to sub acute cases, there is multifocal necrosis of hepatocytes with accumulation of fibrin and infiltration of heterophils mixed with a few lymphocytes and plasma cells can be seen in the liver (Kokosharov et al., 1997; Hossain et al., 2006). In sub-acute outbreaks, sporadic mortality over a long period is experienced while in chronic cases, especially in cases where there are large nodules in the heart, the liver will have congestion with interstitial fibrosis. The spleen may have severe congestion or fibrin deposits and severe hyperplasia (Chishti et al., 1985). The transmission of Salmonella Gallinarum can be through fecal droppings of infected birds, bird carcasses and laid eggs. The infection could be introduced by importation of live infected chickens and hatched eggs. Mechanical spread may be by humans, wild birds, mammals, flies, ticks, feed sacks, etc. (Steigh and Duguid, 1989). For the past few decades, poultry production has become increasingly organized, specialized and integrated into an industry of major national and international importance (Mai et al., 2004; Khan et al., 2007). As a result, poultry diseases are every poultry farmer's nightmares. The economic losses attributed to these infections are enormous and in most cases unquantifiable. In Nigeria, early detection of the disease in any locality can help reduce/eliminate the losses that may occur in the event of the disease outbreak (Okwori et al., 2013). This study evaluated the haematological changes in layers experimentally infected with Salmonella gallinarum in Zaria, Kaduna State, Nigeria.

MATERIALS AND METHODS

Area of Study

The study was carried out in Zaria, Kaduna State, which is located within the Northern Guinea Savannah Zone of Nigeria, between latitude 7^0 and 11^0N, and longitude 7^0 and 44^0E; the average rainfall of this zone ranges from 1,000 to 1,250 mm, and the average temperature ranges from 17^0C to 33^0C (Sa'idu et al., 1994).

Experimental Birds

A total of twenty 18-week old ISA Brown layers were purchased from kujama farm in Kaduna. These birds were duly vaccinated against endemic infectious diseases except fowl typhoid. On arrival, they were housed and managed intensively in washed, cleansed and disinfected poultry research pens of veterinary teaching hospital Ahmadu Bello University, Zaria. From the day of arrival and throughout the experiment, the birds were fed on standard commercial layer mash (Hybrid Feed®) and water was provided ad libitum. The birds were acclimatized for a period of four weeks to get used to all the handling conditions.

Source of bacterial organism

Salmonella Gallinarum obtained was obtained from the Department of Veterinary Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. Sub-culture of Bacterial organism and preparation of McFarland standards. The bacterium from the previously prepared slant was reactivated by sub-culturing on MacConkey agar (MCA). The resulting colonies were then examined for their characteristic features, color and morphology and tested for the gram stain reaction (Gram negative). McFarland turbidity standards were made in the laboratory by preparing a 1% solution of anhydrous Barium Chloride and 1% solution of sulfuric acid and they were mixed to obtain a barium precipitate. The volumes of the two reagents were adjusted to prepare standards

of different turbidities that represent different concentrations of bacterium. The standards were used to visually compare the turbidity of a suspension of bacteria.

Pre-infection bacteriological monitoring of experimental birds

During the period of acclimatization, all birds were checked to ensure they were free from Salmonella spp. Individual cloacal swabs were collected and then immersed in buffered peptone water, and then followed by plating them in MacConkey agar (MCA) and blood agar (BA). Both cloacal swab and plates were incubated in a bacteriological oven at 37^0C for 24 hours according to the standard laboratory methods (Wigley et al., 2001; Parmer and Davies, 2007).

Challenge of the birds with Salmonella Gallinarum

At 22 weeks old, the chickens were allocated into two groups at random (infected and control) of 10 birds each. Few colonies were scooped from the cultured plate and inoculated into a sterile test tube, each containing 20 ml of 0.5% normal saline, until the turbidity was equivalent to 9 x 10^8 CFU/ML. At 26 weeks old, after reaching their peak point of lay, each of the birds in the infected group was challenged by oral administration of 0.5 ml inoculum containing $9x10^8$CFU/ML of Salmonella Gallinarum, while the birds in control group which were uninfected with the bacterium, but given distilled water only.

Clinical Observation

Following inoculation of the birds with the Salmonella Gallinarum, the infected group was observed daily for clinical signs of fowl typhoid and findings were recorded.

Determination of Haematological Parameters

Blood samples of 0.5 ml each was collected from the infected and control groups via wing vein, using 25 gauge needle and syringe on days 0, 2, 4, 7, 14, 21, 28, 35, and then 42 post infection. The blood was dispensed into (EDTA) as anticoagulant and used for haematological evaluations.

Haematological Evaluation

Red blood cell count, packed cell volume and haemoglobin concentration were measured according to standard methods. The mean corpuscular volume and the mean corpuscular haemoglobin concentration were calculated. Total white blood cell count and differential leukocyte count were determined by the method (Feldman et al., 2000) using Natt and Herrick solution as diluent (Natt and Herrick, 1952).

Bacteriological Isolation

At post-mortem, tissues from the ovary, liver, kidney and spleen were aseptically taken for isolation of Salmonella Gallinarum using standard laboratory methods (Wigley et al., 2001; Parmer and Davies, 2007).

Statistical Analysis

Data obtained were subjected to statistical analysis including the calculation of the mean and standard error of the mean. Data between groups were evaluated by student t-test and values of $P<0.05$ were considered significant using Graph Pad Prism Version 5.00 for Windows, GraphPad Software, San Diego California USA.

RESULTS

All the infected groups showed clinical signs of fowl typhoid starting at day 7 post-infection, which include: depression and huddling, ruffled feathers, somnolence, greenish-yellow diarrhea, loss of weight, a decrease in feed and water consumption, decreased egg production and sudden death, while the control Group showed no sign of disease. There was mortality in the infected group, with mortality rates of 50% among experimentally infected layers while no abnormal signs or gross lesions were observed in normal control layers during the experiment.

Bacterial recovery from infected birds

Salmonella Gallinarum was isolated from the liver, spleen, kidney and ovary of the infected layers from day 9 post-infection and throughout the experimental period. Biochemical test revealed indole negative, urea negative, catalase and citrate positive and it produces hydrogen sulphide (H_2S) in triple sugar iron agar TSI.

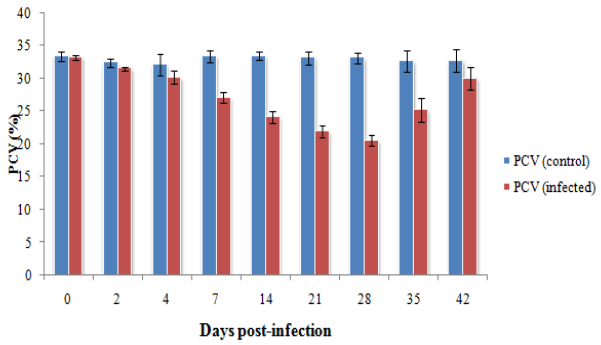

Figure 1: Mean (± SEM) packed cell volume (PCV) in *Salmonella Gallinarum* Experimentally-infected and control layers.

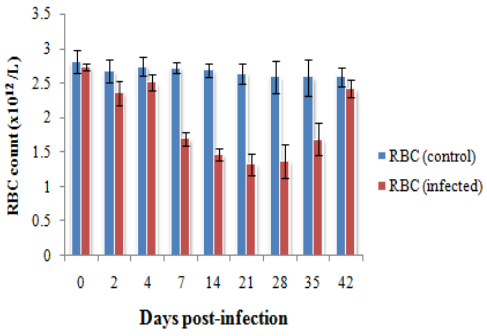

Figure 2: Mean (±SEM) total red blood cell count in *Salmonella Gallinarum* experimentally-infected and control layers.

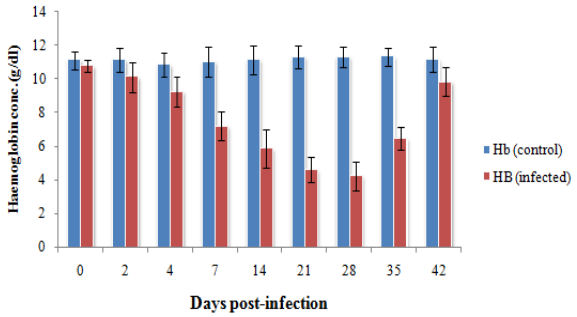

Figure 3: Mean (±SEM) haemoglobin concentration in *Salmonella Gallinarum* experimentally-infected and control layers.

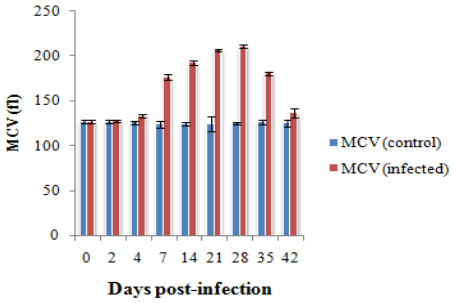

Figure 4: Mean (±SEM) corpuscular volume in *Salmonella Gallinarum* experimentally-infected and control layers.

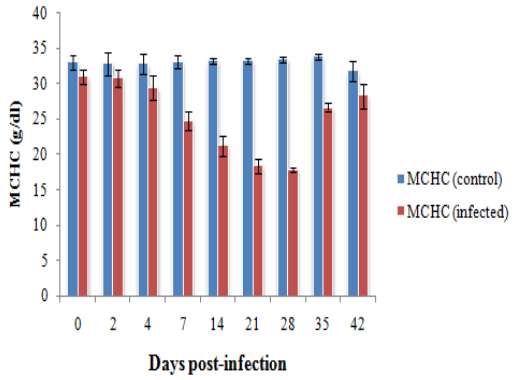

Figure 5: Mean (±SEM) corpuscular haemoglobin concentration in *Salmonella Gallinarum* experimentally-infected and control layers.

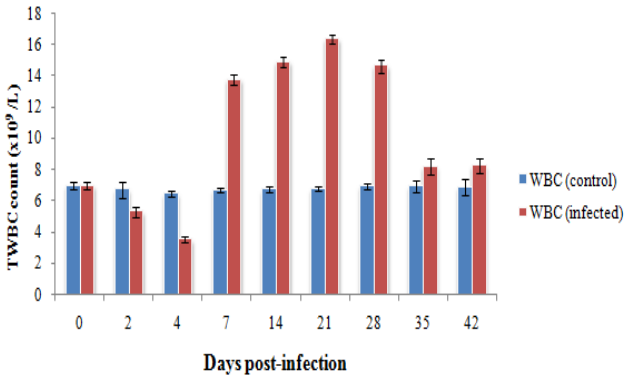

Figure 6: Mean (±SEM) Total White Blood Cell Count in *Salmonella Gallinarum* experimentally- infected and control layers.

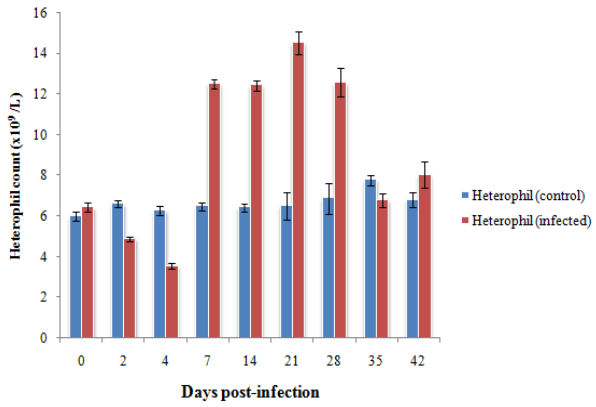

Figure 7: Mean (±SEM) heterophil Count in *Salmonella Gallinarum* experimentally-infected and control layers.

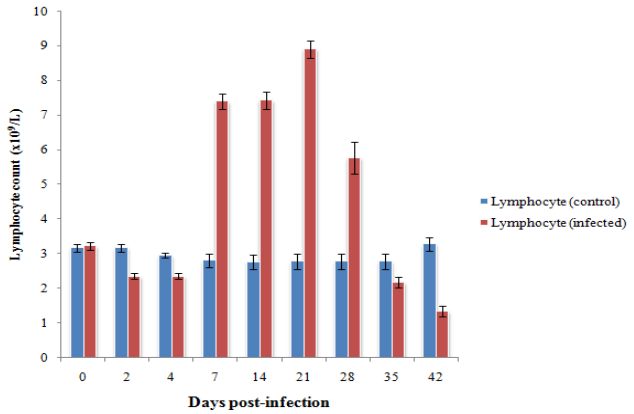

Figure 8: Mean (±SEM) Lymphocyte Count in *Salmonella Gallinarum* experimentally-infected and control layers.

DISCUSSION

The clinical signs observed in the Salmonella gallinarum-infected layers in this study, which included depression, ruffled feathers, huddling, loss of body weight, drop in egg production, somnolence and greenish-yellow diarrhoea were consistent with findings in previous reports (Shivaprasad, 2000; Freitas Neto et al., 2007; Ezema et al., 2009; Garcia et al., 2010). The 50% mortality in the layers recorded in this study was in the range (10-100%) reported, previously (Shivaprasad, 1996; Uzzau et al., 2000; Oliveira et al., 2005; Paiva et al., 2009), in chickens. The haematological changes in the Salmonella Gallinarum-infected layers in this study presented significant decreases ($P<0.05$) in mean packed cell volume (PCV), haemoglobin concentration and red blood cell (RBC) count corresponded with the observations in the acute phase of fowl typhoid in which anemia has reported (Assoku and Penhale, 1978; Prasanna and Paliwal, 2002). Christensen et al., (1996) surmised that the modification of the erythrocytes is associated directly with the cytopathic effect of Salmonella Gallinarum lipopolysaccharide/ outer membrane proteins or indirectly by induction of antibodies or both, to the number of bacteria present in the tissues. Earlier, Assoku and Penhale (1978) had suggested that the anemia associated with acute fowl typhoid in chicken may be due to increased ability of the reticuloendothelial cells to take up erythrocytes hence destruction of erythrocyte is extravascular. In addition, the hepatic dysfunction caused by the organism and the intestinal disturbances may lead to deficiency of vitamin B_{12} due to interference with its absorption in intestine and its storage in liver (Feldman et al., 2000). The significant increase ($P<0.05$) in mean corpuscular volume (MCV) and significant decrease ($P<0.05$) in mean corpuscular haemoglobin concentration (MCHC) in the infected group, when compared with the corresponding values in the control group, was due to hemolysis of erythrocytes and subsequent bone marrow response with resultant reticulocytosis (Feldman et al., 2000). The macrocytic and hypochromic erythrocytic changes in the infected chickens, observed in this study, however conflicts with findings in the reports of Christensen et al. (1996) and Mdegela et al.(2002), who observed microcytic hypochromic anaemia during acute phase of fowl typhoid infection. The initial significant decreases ($P<0.05$) in total white blood cell, heterophil and lymphocyte counts in the infected group, especially on days two to four post-infection may have been caused by the cytopathic effect of Salmonella Gallinarum lipopolysaccharides (LPS) on leukocytes of the infected layers. This finding agreed with the reports of Lam and Munn (2002) in which following the mixing of heterophils with Salmonella Typhimurium, changes in heterophil morphology and fast disappearance of the cell type were observed. The authors attributed the heterophil disappearance may be due to contact with the Salmonella typhimurium lipopolysaccharide which caused heterophil degranulation. On the other hand, the significant increase ($P<0.05$) in total white blood cell, heterophil and lymphocyte counts observed on day seven post-infection was similar to the one reported by Berchieri (2000), who attributed the increase in leukocyte count may be due to fast multiplication of Salmonella Gallinarum inside the phagocytes, with subsequent cell lysis and release of the bacterium into the extracellular compartment, which evoked strong immune response. The increase in heterophil count in the Salmonella Gallinarum-infected layers may also be due to the fact that heterophils are the cells

that respond most in bacterial infection (Feldman et al., 2000). The leukocytosis recorded in this study coincided with the period of manifestation of the clinical signs (depression, somnolence, anorexia, ruffled feathers and greenish -yellowish diarrhea) of fowl typhoid in the infected group. This finding conformed with reports of Berchieri (2000) and (Freitas Neto et al., 2007). In addition, the possible bacterial invasion of the target organs, such as the liver, spleen, kidneys, and ovarian follicle, may cause increase in peripheral blood leukocytes as an inflammatory response. The lymphopenia observed on days 35 and 42 post-infection in the infected group may be due to stress of infection with Salmonella Gallinarum which induced adrenal gland's release of cortical hormones that destroy the lymphocytes (De Groot and Morris, 1950).

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