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Original Research

Haematological changes induced by experimental *Escherichia coli* infection in layer chicken

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ABSTRACT

This study examined the haematological alterations in layer hens infected with experimental *Escherichia coli* (*E. coli*). Twenty laying chickens (20 weeks old) were acquired and randomly divided into two groups of ten layers each (infected and control). After challenging the infected group with 0.5 milliliters of bacterial aliquot containing 10^9 colony forming units (CFU) of the bacteria intratracheally, 1 ml of blood was collected from each group on days 0, 2, 4, 6, 14, 21, 28, 35, and 42 post-infections (pi) for haematology. The mean PCV of the infected group dropped significantly ($p<0.05$) to its lowest value of 17.0 ± 0.71% on day 4, and then ascended to a peak level of 24.6 ± 1.03% on day 28. In the infected group, the mean Total White Blood Cell count grew considerably ($p<0.05$) from day 2 ($14.81 \pm 3.28 \times 10^9$/L) to day 14 pi, when it peaked at $29.16 \pm 0.81 \times 10^9$/L. From day 2 pi to the lowest value ($1.63 \pm 0.07 \times 10^{12}$L) on day 14 pi, the mean Red Blood Cell count fell; from day 21 to the maximum value ($2.55 \pm 0.08 \times 10^{12}$L) on day 28 pi, it improved. The infected group's mean lymphocyte counts rose from day 4 to a peak level ($21.59 \pm 0.92 \times 10^9$/L) on day 14 pi. After that, it declined, but continued to be significantly ($p<0.05$) higher than the control group up to day 42. In conclusion, *E. coli* infection in layer chickens induced haematological changes.

Keywords: Anaemia, *Escherichia coli*, experimental infection, haematological indices, layer-chicken

INTRODUCTION

The scenario of poultry diseases has changed with emerging and re-emerging diseases including salmonellosis, mycoplasmosis, necrotic enteritis among others, flaring up, thus imposing threats to the poultry industry (Hafeez 2003). Avian colibacillosis caused by *E. coli* is considered as one of the major and principal causes of morbidity and mortality either as primary or as a secondary pathogen, resulting in huge economic loses (LutfulKabir, 2010). Timothy et al. (2008) stated that in the USA, about 30% of the commercial flocks are at any point in time affected by colibacillosis.

Escherichia coli is mostly found in the alimentary tract of healthy birds and animals as a commensal. Usually 10-15% of intestinal *E. coli* are Avian Pathogenic Escherichia coli (APEC) possessing various virulence factors (Barnes *et al.*, 2003). However, infection flares up when bird's defence mechanisms are compromised by various factors such as bad management (Goswami *et al.*, 2004), concurrent infections and immunosuppression.

A reported natural outbreak of colibacillosis in young chickens is noteworthy; however, the concurrent appearance of the illness in chicks across several age groups is exceedingly intriguing, especially due to the diversity of serogroups with varying pathogenicity capacities (Umar *et al.*, 2020). APEC causes significant morbidity and mortality which attributes to multimillion-dollar losses for all facets of poultry industry world-wide (Barnes, 2008). However, there is no report on the association of *E. coli* serogroups with varied pathology seen in natural cases of colibacillosis.

Though numerous studies on different facets of the pathophysiology of colibacillosis has been undertaken across, there is the need to revalidate the currency of existing

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scientific literature on hematological changes associated with different serotypes of *E. coli* under experimental conditions in layer chickens. Hence this study was carried out to study the hematological alteration in *E. coli* infected layer chickens.

MATERIALS AND METHODS

LOCATION OF EXPERIMENTAL STUDY

This study was carried out in the Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State, 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 annual rainfall of this zone ranges from 1,000 to 1,250 mm, while the annual average temperature ranges from 19^0C to 33^0C (Sawa & Buhari, 2011).

EXPERIMENTAL ANIMALS AND DESIGN

A total of twenty (20) laying (20 weeks old) chickens vaccinated against endemic vaccinable diseases in the study environment, except against *E. coli* infections, were purchased from a reputable farm in Kaduna State. The birds were housed and managed intensively in the Poultry Research Unit of the Department. Prior to arrival of the birds, the pens were thoroughly washed with water and detergent and sprayed with formalin at a concentration of 4 ml/litre of water. Throughout the experiment, the birds were fed standard commercial layer mash (Hybrid Feeds®) and water was provided to the birds *ad libitum.*

EXPERIMENTAL ESCHERICHIA COLI STRAIN

The *E. coli* strain used in this experiment, APEC serotype O1K1 was obtained from the bacteria bank of the National Veterinary Research Institute, Vom, Plateau State, Nigeria.

GROUPING AND INOCULATION OF BIRDS WITH *E. coli* 01K1

The birds were kept for 4 weeks to acclimatize to the new environment and other handling conditions, after which they were allocated at random to two groups (infected and control) of 10 layer chickens each. The control birds were housed in a pen located far away from the pen in which birds of the infected group were housed. The bacteria from a previously prepared slant were reactivated by sub-culturing on Eosin and methyline blue (EMB). The resulting colonies were then examined for their characteristic features such as colour, morphology and tested for gram stain reaction. On the day of infection (Day 0), bacterial inoculum was prepared using McFarland standards, which were prepared by adding barium chloride to sulphuric acid to obtain a barium precipitate of different turbidity standards. These were used to estimate the number of bacteria present in a liquid suspension (McFarland, 1907). Each of the birds in the infected group was then challenged by inoculating 0.5 ml of bacterial aliquot containing 10^9 colony forming units (CFU) of the bacteria intratracheally (Antao *et al.*, 2008). After inoculation, the bacteria were recovered from the blood of infected birds by following conventional culture, isolation and identification of bacteria by standard procedures as documented in Cowan and Steel (Barrow and Feltham, 2000) and Cheesbrough (2006).

BLOOD SAMPLE COLLECTION

Beginning from day 0 and, subsequently, on days 2, 4, 6, 14, 21, 28, 35 and 42 post-infection, blood samples (1 ml) were collected from the brachial vein of each of 5 birds selected at random from each group, at 08:00 to 09:00 hours of the day, using 23 guage (G) needles. The blood was dispensed into EDTA-coated vacutainer tube and used for haematological evaluations.

HAEMATOLOGICAL EVALUATIONS

Packed cell volume (PCV) was determined using the microhaematocrit method (Feldmann *et al*. 2000), haemoglobin concentration was assayed colorimetrically using cyanmethhaemoglobin method described by Feldmann *et al*. (2000), while red blood cell (RBC) and white blood cell (WBC) counts were determined with improved Neubauer haemocytometer according to Campbell and Ellis (2007). Giemsa blood smear was prepared for differential white blood cell count according to Campbell and Ellis (2007). Erythrocytic indices, including the mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were calculated using the following standard formulae according to Campbell and Ellis (2007):

$$MCV\ (fl) = \frac{PCV(\%)}{RBC(\mu l)} \times 10$$

$$MCHC\ (pg/dl) = \frac{Hb\ (g/dl)}{PCV\ (\%)} \times 100$$

STATISTICAL ANALYSIS

All the data obtained were subjected to statistical analysis including the calculation of the means and standard error of the means using Graph Pad prism version 5.00 for windows, Graph pad Software, San Diego California USA. Differences between groups were evaluated using student t-test and values of $P < 0.05$ were considered significant.

ETHICAL APPROVAL

Ethical approval was obtained from the Ethical Committee on Animal Use and Care of Ahmadu Bello University, Zaria with approval number: ABUECAUC/2017/026

RESULTS

EFFECT OF *E. coli* INFECTION ON THE HAEMATOLOGICAL VALUES OF LAYER CHICKENS

PACKED CELL VOLUME (PCV)

The mean PCV in the *E. coli*-infected and control groups are presented in Figure I. A progressive and significant (p<0.05) decrease in the mean PCV was observed in the infected group from day 2 to a lowest value (17 ± 0.71%) on day 4 pi. Thereafter, the mean PCV in the infected group gradually rose to its peak level (24.6±1.03%) on day 28 pi. Following this, the mean PCV stabilized, with non-significant fluctuations until termination of the experiment

RED BLOOD CELL (RBC) COUNTS

The mean RBC count in the *E. coli* infected and control layers are presented in Figure II. The mean red blood cell count (RBC count) steadily decreased beginning from day 2 pi (1.92 ± 0.04 x 10^{12}/L) to the lowest value (1.63 ± 0.07 x 10^{12}/L) on day 14 pi, that differed significantly (p< 0.05) from that (2.21 ± 0.04 x 10^{12}/L) of the control group. A sharp increase was observed in the mean RBC count from day 21 (1.76 ± 0.12 10^{12}/L) to the highest (2.55 ± 0.08 10^{12}/L) on day 28 pi. Thereafter, the mean RBC count slightly decreased and stabilized until termination of the experiment. The mean RBC count in the control group remained fairly unchanged throughout the experiment.

Figure II: Mean (± SEM) red blood cell count in *E. coli*-infected and control groups in layers

HAEMOGLOBIN (Hb) CONCENTRATION

The mean Hb concentration in the *E.* coli-infected and control layers is presented in Figure III. The mean Hb concentration progressively decreased in the infected group beginning from day 2 pi (7.11 ± 0.47g/dl) to a lowest value (5.67 ± 0.24g/dl) on day 4 pi that differed significantly (p<0.05) from the corresponding value in the control group. Afterwards, it gradually increased to reach levels comparable with those of the control on days 28, 35 and 42 pi.

MEAN CORPUSCULAR VOLUME (MCV)

The mean MCV of erythrocytes in the *E. coli*-infected and control layers is presented in Figure IV. Following the infection with *E.* coli, no significant (p>0.05) change in the mean MCV was observed until day 14 when it rose in the infected group to a significantly (p<0.05) higher value (127.54 ± 12.26 fl) than that of the control group. It then stabilized to levels comparable with those of the control up to termination of the experiment.

MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION (MCHC)

The mean MCHC of erythrocytes in the *E. coli*-infected and control layers is presented in Figure V. The mean MCHC profile following the infection remained fairly unchanged and comparable with those of the control group, except for the non-significant (p>0.05) fluctuations throughout the experiment.

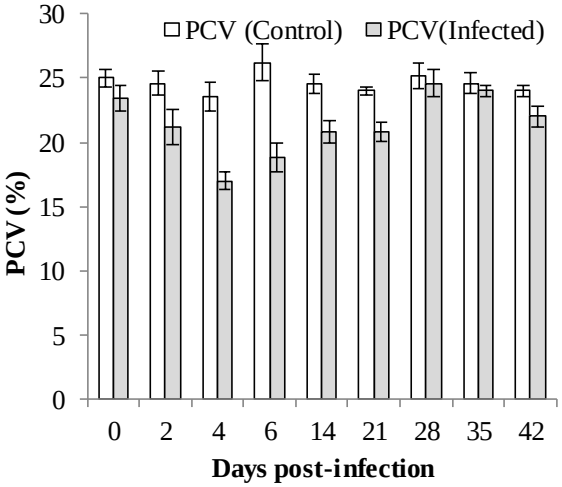

Figure I: Mean (± SEM) packed cell volume (PCV) of *E. coli* infected and control groups

TOTAL WHITE BLOOD CELL (WBC) COUNTS

The mean total WBC count in the *E. coli*-infected and control layers is presented in Figure VI. The mean total WBC count rapidly increased in the infected group from day 2 (14.81 ± 3.28x 10^9/L) to a peak level (29.16 ± 0.81 x 10^9/L) on day 14 pi. Following this, the mean value of this parameter in the *E. coli*-infected group showed progressive decline up to the end of the experiment. The values of the WBC count during the infection were significantly (p< 0.05) higher in the infected than that in the control group.

HETEROPHIL COUNTS

The mean heterophil count in the *E. coli*-infected and control layers is presented in Figure VII. The mean heterophil count showed a rapid and progressive increase in the infected group from day 2 (2.41 ± 0.77x 10^9/L) to a peak level (5.08 ± 0.76 x 10^9/L) on day 6 pi that was significantly (p< 0.05) higher than the corresponding value in the control group. Following this, the value progressively decreased in the infected but remained significantly (p< 0.05) higher than in the control up to the termination of this experiment.

LYMPHOCYTE COUNTS

The mean lymphocyte count in the *E. coli*-infected and control layers is presented in Figure VIII. The mean lymphocyte counts progressively increased starting from day 4 pi (16.47±1.78x 109/L) to the highest value (21.59 ± 0.92 × 10^9/L) on day 14 pi that was significantly (p< 0.05) higher

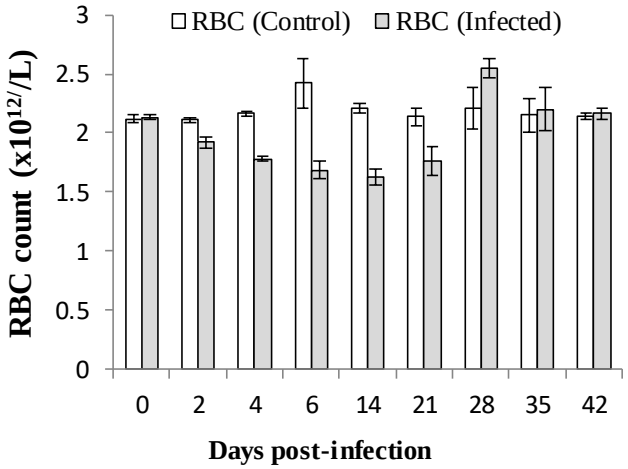

Figure II: Mean (± SEM) red blood cell count in *E. coli*-infected and control groups in layers

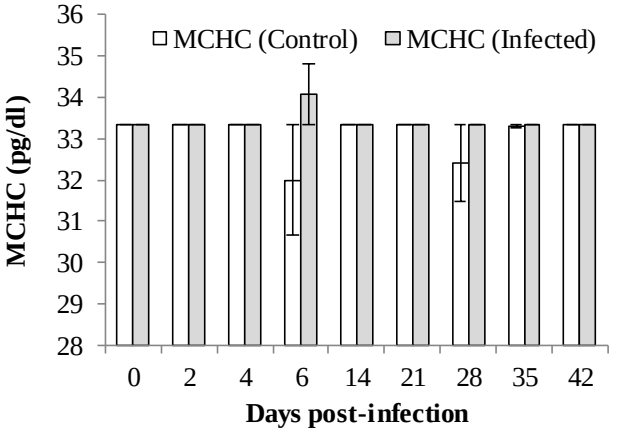

Figure V: Mean (± SEM) Mean corpuscular haemoglobin concentration of erythrocytes in the *E. coli*-infected and control groups of layers.

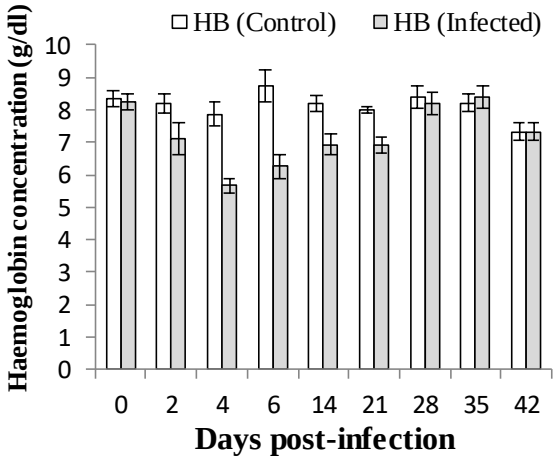

Figure III: Mean (± SEM) Haemoglobin concentration in the *E. coli*-infected and control groups of layers.

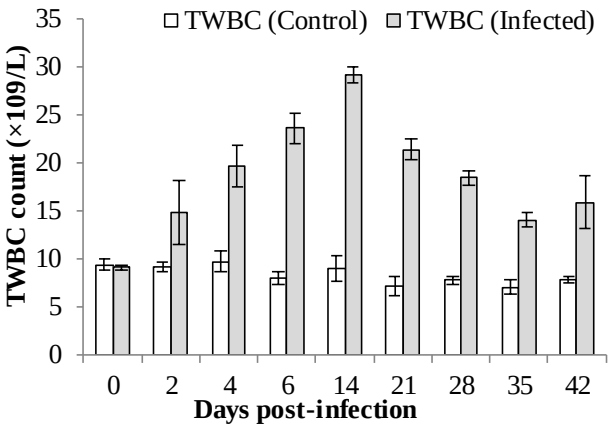

Figure VI: Mean (± SEM) total white blood cell count in the *E. coli*-infected and control groups of layers.

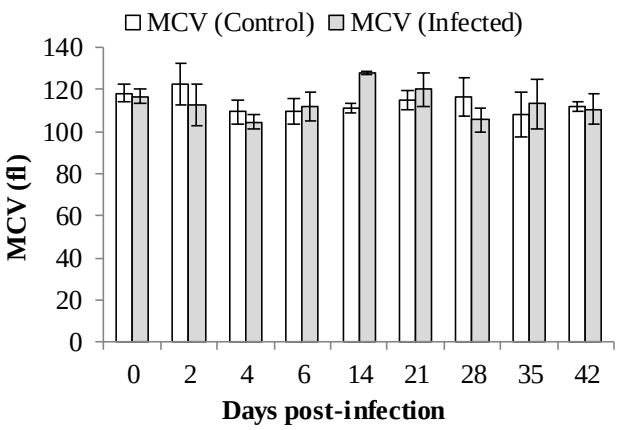

Figure IV: Mean (± SEM) corpuscular volume (MCV) of erythrocytes in the *E. coli*-infected and control groups of layers.

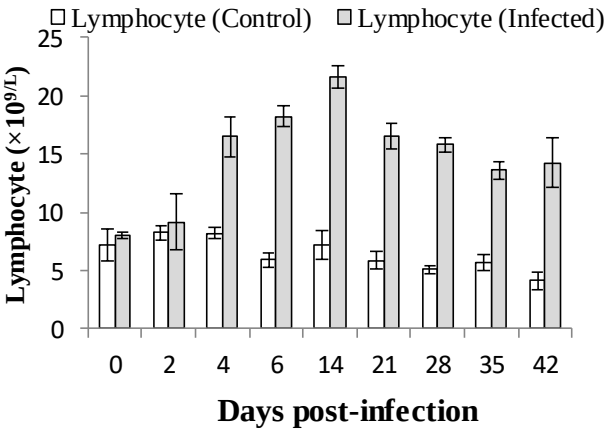

Figure VIII: Mean (± SEM) lymphocyte count in the *E. coli*-infected and control groups of layers.

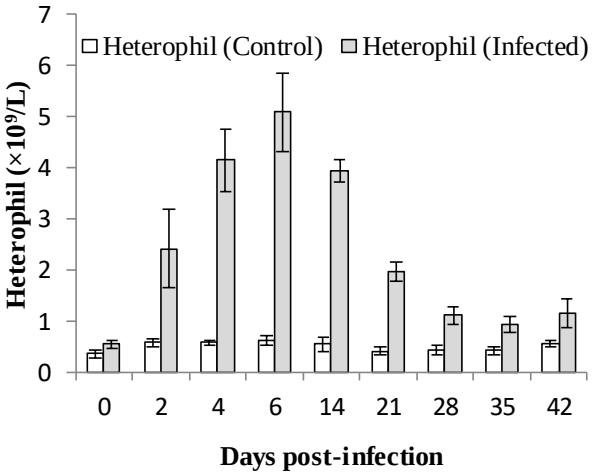

Figure VIII: Mean (± SEM) lymphocyte count in the *E. coli*-infected and control groups of layers.

than the control group. The mean lymphocyte count remained significantly higher than that in the control group, up to day 42 pi when the experiment was terminated.

DISCUSSIONS

The decrease in PCV, RBC count and haemoglobin concentration agreed with findings in the reports of Saini (2004), who observed fall in packed cell volume, haemoglobin level and red cell count within 24 hours post-inoculation of birds with *E. coli*. Many factors could have contributed to the reduction in the haematological parameters. It has been suggested that septicemia produced by *E. coli* (Christie and Halliday, 1979) could produce haemodilution which is reflected as reduction in the haematological parameters. Also, these observed changes may be due to reduction in supply of nutritional factors necessary for sustenance of normal erythropoiesis (Esievo, 2017; Ogungbemi *et al.*,2017) because of anorexia and diarrhoea (Feldman *et al.*, 2000) observed in the *E. coli*-infected group.

The significantly higher mean corpuscular volume recorded in the *E. coli*-infected layers in this study on day 14 pi signified a possible macrocytic anaemia. Since the diarrhoea observed in the *E. coli*-infected birds in this study was not haemorrhagic, it is possible that a deficiency of some vitamins and minerals, such as B_{12}, folic acid and cobalt that are essential for normal erythropoiesis may be responsible for the macrocytosis (Ogungbemi *et al.*, 2017). This may be due to reduced feed intake and possible impairment of intestinal absorption as a result of diarrhoea. The observed non-significant difference in MCHC between infected and control groups is at variance with the report of Haq *et al.* (2015), who reported decrease in MCHC in *E. coli* infected pigeons. Low PCV with enlarged red corpuscles and normal haemoglobin concentration indicate normochromic, macrocytic anaemia (Haq *et al.*, 2015). These results support the earlier suggestion that nutritional deficiency could be responsible for the abnormal erythrocyte parameters observed in the infected group.

The significant increase in mean WBC, heterophil and lymphocyte counts in the early phase of the infection is in line with the report of Honda *et al.* (2016). Some of the most common causes of increase in WBC count are localized or generalized infections, tissue necrosis, acute haemorrhage and haemolysis (Morgulis, 2002; Esievo, 2017). As such, the significant increase in the WBC count in infected layers from day 2 up to 42 pi could be attributed to the effects of the *E. coli* infection on heterophil and lymphocyte counts.

The early significant increase in mean heterophil count in infected layers agree with the role of heterophils as first line of defence against infections. These cells carryout phagocytosis and killing of pathogens by producing reactive oxygen species, bactericidal substances and proteolytic enzymes involved in the process of oxidative burst and degranulation (He *et al.*, 2008; Genovese *et al.*, 2013). Heterophils are known to increase in circulation in both local and generalized infections caused by *E. coli* bacteria (Morgulis, 2002). Migration of heterophils to sites of bacterial infection or inflammation was thought to induce bone marrow haematopoietic tissues to increase the production of heterophil precursor cells (Juul-Madsen *et al.*, 2008).

The significant increase in mean lymphocyte count from day 4 to 42 pi suggests that infection with *E. coli* stimulates lymphocytosis. Mitchell and Johns (2008) reported that an increased presence of lymphocytes in peripheral circulation was common in birds with infectious diseases. Infection with *E. coli* is therefore associated with significant antibody production (Campbell, 2004).

CONCLUSION

It could be concluded from this study that, experimental infection with *Escherichia coli* in layers caused significant decrease in packed cell volume, relative to the control, with erythrocyte morphology being macrocytic normochromic. This may be associated with nutritional deficiency due to decrease feed intake caused by the infection.

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CONFLICT OF INTEREST

There was no conflict of interest among authors.

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