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Haematology and pathologic changes associated with *Pseudomonas aeruginosa* isolated from barn swallows around poultry houses in broiler chickens

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Abstract

The impact of vermin found in poultry houses has increased in recent times. This study evaluated the occurrence of *Pseudomonas aeruginosa* in free flying barn swallows found in poultry houses in Ibadan and determined its pathogenicity in broilers. Barn swallows (23) were caught using mist nets, their oral and cloacal microbial culture yielded *Pseudomonas aeruginosa*. One-week-old, thirty-five broilers were divided randomly into infected (n=23) and control (n=12) groups. Each bird in the infected group was inoculated with viable infective dose of 0.5 ml of 8hr broth containing (10⁵) CFU/ml of *Pseudomonas aeruginosa* via oro-nasal route. The infected group showed dullness, rales and bloody diarrhoea. The pack cell volume was consistently lower in the infected group compared to the control group although not statistically significant post-infection. The platelets count was significantly (P <0.05) higher in the infected group on day 7 pi. Total white blood cell was significantly (P <0.05) higher in the infected group on day 14 pi. There was a significant (P<0.05) increase in heterophil count on days 7 and 35 pi. The mean lymphocyte value of the infected chickens was significantly (P <0.05) lower than the control group only on day 35 pi, while the monocyte count in the infected group was significantly (P<0.05) lower on day 28 pi. *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* were sensitive to gentamycin and resistant to oxytetracycline except for *Pseudomonas aeruginosa*. Hepatic congestion, splenomegaly and enteritis were observed in the infected chickens. Barn swallows harbour pathogenic bacteria, causing clinico-pathological changes in chickens and may be partly responsible for transmission of pathogenic bacteria within and between poultry houses due to their free-flying habit. Adequate biosecurity measures including screening nets on poultry houses are recommended to prevent access of these free flying birds into poultry pens.

Keywords: Barn swallow, Broiler, Haematology, Pathology, *Pseudomonas aeruginosa*

Introduction

The Barn Swallow (*Hirundo rustica*), the most widespread species of swallow worldwide, is

commonly found on pastures where livestock graze. These migratory birds closely associate with humans and often build their nests with mud pellets in barns

and feed on insects caught in flight (Gruebler *et al.*, 2010; Brown & Brown, 2019). In Nigeria, barn swallows are commonly seen in poultry houses during the dry season and often perch on open drinkers. Also, their free flying habit allows their frequent movements within and between poultry farms with high possibilities of disease transmission (Saino *et al.*, 2013).

Poultry diseases have been reported to cause considerable economic losses to the industry worldwide and this depends on the severity, prevalence, mortality rates and the mode of disease transmission between birds or flocks (DAHS, 2012; Houghton-Wallace & Lister, 2012). These diseases are spread through various ways: vermins (mainly wild birds, rats and mice), feed droppings, aerosols, and fomites (Fagbohun *et al.*, 2000; Kallapura *et al.*, 2014).

Pathogenic bacteria have been isolated from some poultry facilities associated vermins such as rats, red-headed rock agamas, and wall geckos involved in disease transmission (Ogunleye *et al.*, 2013; Ajayi *et al.*, 2015). The role played by different vermins including wild birds in disease transmission is largely indeterminate and may be ecologically related. This cannot be overemphasized, because these potential vectors co-habit, feed and drink from poultry feed and waterers (Ogunleye *et al.*, 2013; Britni *et al.*, 2017) where biosecurity measures are breached.

Pseudomonas aeruginosa is a gram-negative, aerobic, motile, non-capsulated and non-spore forming bacterium that produces watery soluble green pigment with a fruity odor. It is ubiquitous, often associated with soil, water, humid environments and is the most predominant *Pseudomonas* species associated with respiratory disease, septicaemia and mortality among birds specially chickens (Silby *et al.*, 2011; Elsayed *et al.*, 2016). Generally, it is considered to be an opportunistic organism that causes respiratory infections, septicaemia and other pathologies when exposed to tissues of susceptible birds and the greatest losses occur in very young birds (Fekadu, 2010). Infection with *Pseudomonas aeruginosa* can occur through cutaneous wounds, use of contaminated vaccines, egg dipping and needles, while spread can occur from infected to susceptible flocks under the same environmental conditions (Silby *et al.*, 2011).

Pseudomonas infection appears to be a good example of an environment related infection that can cause serious losses in poultry farms. Birds at any age can be infected but young birds are more

vulnerable. Severely stressed or immuno-deficient birds and other co-infection with bacterial infections increase susceptibility of poultry to infection with *Pseudomonas* (Gellatly & Hancock, 2013). The barn swallow is commonly found to fly freely into poultry houses and, as vermin, this migratory bird could play a role in disease transmission of pathogenic organisms within and between farms as they share common feed and open water source with poultry. There is little or no information available on isolation of *P. aeruginosa* from poultry facility associated barn swallows and its pathogenicity studies in Nigeria commercial chickens despite increasing isolation of this organism from necropsied commercial chickens at the Department of Veterinary Pathology, University of Ibadan, Nigeria (Ajayi *et al.*, 2015). Therefore, this study was undertaken to investigate haematology and pathologic changes associated with *Pseudomonas aeruginosa* isolated from barn swallows around poultry houses in broiler chickens.

Materials and Methods

Experimental birds

Twenty-three Barn swallows (*Hirundo rustica*) commonly seen in and around commercial poultry houses especially during the dry season in south west Nigeria, were used as the source of bacterial isolation. They were caught using mist net placed on non-netted free spaces on commercial poultry houses. Thirty-five Isa Brown Day Old Chick (DOC) broilers were obtained from a reputable commercial hatchery in Ibadan, Oyo State. The chicks were brooded together, vaccinated routinely and separated into two groups of 23 inoculated and 12 control groups after a week. All chickens were managed following the international standards of animal care, fed commercial broiler feed and given tested coliform free commercial sachet table water *ad libitum*.

Ethics committee approval

The guidelines for the University of Ibadan Animal Care and Use Research Ethics Committee were strictly followed for this study.

Sample collection and bacterial isolation

Portions of oral, cloaca tissue samples and fresh faecal droppings from cages were collected aseptically for bacteriological cultures. The swab specimens were cultured in nutrient broth tubes and incubated for 24 hours at 37°C. Loop-full of broth was then sub-cultured on to nutrient agar, MacConkey agar, and blood agar plates and

incubated for 24-48 hours at 37°C. Colonies suspected to be *Pseudomonas aeruginosa* were kept in slant agar for further identification of the character of the colony, production of pigment, biochemical reactions (urease, catalase, oxidase, methyl red, Voges-Proskauer, indole and fermentation of glucose, sucrose and maltose). Identification of non-lactose fermenters, which were picked from the MacConkey plates, was carried out on the basis of colony pigmentation, cytochrome oxidase test, indole production, nitrate reduction, gelatin liquefaction, carbohydrate fermentation and haemolysin production (Mansoor *et al.*, 2009).

Antibiotic sensitivity tests

Antibiotic sensitivity test was done using the disc method. Ciprofloxacin (10µg), Chloramphenicol (30µg), Gentamycin (10µg), Trimethoprim (5µg), Ampicillin (10µg), Oxytetracycline (10µg), Ofloxacin (5µg), Pefloxacin, (5µg) and Erythromycin (5µg) were used to determine the sensitivity of isolated organisms to different antibiotics as described by Jorgensen & Turnidge (2007).

Experimental infection and pathogenicity evaluation

The challenge pathogenic bacterium used was isolated from apparently healthy barn swallows. At one week of age, the birds determined to be negative for *Pseudomonas aeruginosa* were divided randomly into two groups: infected group (n=23) and control group (n= 12). Each broiler chicken in infected group was inoculated with viable infective dose of 0.5 ml of 8hr broth containing (10^5) CFU/ml of *Pseudomonas aeruginosa* as described by Ajayi *et al.* (2018) through oronasal route, while each bird in control group (uninfected) received 0.5 mL of phosphate buffered saline (PBS) through the same route as placebo. Both groups were maintained for clinical signs.

At seven days old, pre inoculation, all the chicks were weighed using weighing scale and blood samples were taken for haematological baseline data. Subsequently, body weight and blood samples for haematology were taken weekly post-infection (pi). For gross pathology, three and two chickens were randomly selected from the infected and control groups respectively and humanely sacrificed for tissue/organ pathology at weekly intervals. Gross lesions, when observed were recorded and scored in severity as mild, moderate or severe on days 0, 7, 14, 21, 28 and 35 pi. Tissues from internal organs (spleen and liver) and blood samples were taken

weekly for bacterial isolation and haematological analysis respectively for five weeks.

Haematological analysis

The chickens were properly restrained and 2mls of blood were collected from the brachial vein using 5ml syringe and dispensed into heparinised tubes. Packed Cell Volume (PCV) was determined by spinning about 75µl of each blood sample in heparinised capillary tube in a haematocrit centrifuge for about 5 minutes and read on haematocrit reader as described by Benson *et al.* (1989), while erythrocyte (RBC) and leucocyte (WBC) counts were determined using haemocytometer method as described by Lamb (1981). The differential white blood counts (neutrophils, eosinophils, basophils, lymphocytes and monocytes) were determined as described by Lamb (1981).

Statistical analysis

The data generated were analysed using the Statistical Package for Social Sciences (SPSS 20). Student t-test was used to compare variables between the infected and control groups. Values of $P < 0.05$ were considered significant.

Results

*Bacterial isolates from oral and cloaca tissues of barn swallow (*Hirundo rustica*)*

Bacteriological culture of the oral and cloaca tissues from the live barn swallows revealed three distinct bacterial colonies. The first suspected colony was *Pseudomonas aeruginosa* which appeared large, irregular, and translucent which produced a greenish diffusible pigment and characterized by the ability to grow at 42°C with a characteristic fruity smell. On blood agar, the colony produced beta haemolysis suggestive of haemolysis. The organism is a gram-negative motile rod. Another colony observed was *Staphylococcus aureus* which appeared typically as raised, smooth medium to large colonies that were slightly translucent with creamy-yellow pigmentation. The third organism identified as *Escherichia coli* was found to produce bright pink colonies on MacConkey agar. The rough colonies were flat, dry and spreading, with a cut-glass appearance. These organisms were individually scooped separately into bijoux bottles and stored for use.

Antibiotics sensitivity test

The antibiogram of bacterial isolates from the barn swallows associated with poultry houses in Ibadan, southwest Nigeria (Table 1). The *Staphylococcus*

aureus isolate was sensitive to Ciprofloxacin (10µg), Perfloxacin (5µg), Gentamycin (10µg), Ampicillin (10µg), and Erythromycin (5µg), but resistant to Ofloxacin (5µg), Chloramphenicol (30µg), Trimethoprim (5µg) and Oxytetracycline (10µg). *Escherichia coli* isolate was sensitive to Chloramphenicol (30µg), Erythromycin (5µg), Gentamycin (10µg) and Ofloxacin (5µg) but resistant to Ciprofloxacin (10µg), Perfloxacin (5µg) and Oxytetracycline (10µg). *Pseudomonas aeruginosa* isolate was sensitive to Ciprofloxacin (10µg), Ofloxacin (5µg), Trirnethoprim (5µg) and Oxytetracycline (10µg) but resistant to Ampicillin (10µg), Erythromycin (5µg), Perfloxacin (5µg), Gentamycin (10µg) and Chloramphenicol (30µg).

Pathogenicity study

Clinical observations: Varying degrees of dullness, rales and bloody diarrhoea progressive from 5-day post-infection (5 dpi) were observed in all chickens (23/23) in the infected group throughout the experiment while sitting on the hock (5/23) and subcutaneous pectoral haematoma (1/23) were also observed in the infected chickens on 7 dpi. On 14 dpi, one chicken was found dead, while another developed periorbital swelling/oedema on 35 dpi. The control chickens showed no observable clinical signs.

Bodyweight: At 7 days post-infection (dpi), the average body weight of the infected and control

groups was 264.4g and 310.7g, respectively indicating a significant decrease (P <0.05) in weight between the infected and control groups of chickens (Table 2).

Haematology: The mean (±SD) values of the PCV, RBC, Hb, platelet and plasma protein are shown in table 3. The PCV of the infected group was lower than the control group pre-infection and was consistently lower compared to the control group throughout the experiment. The RBC of the infected group was significantly (P<0.05) higher only on day 14 pi, while the Hb concentration was significantly (P<0.05) lower in the infected group on day 28 pi. The platelet and plasma protein values were significantly (P<0.05) higher in the infected group on day 7 and 28 pi, respectively.

Platelet showed a statistically significant increase at 7 dpi. Table 4 shows the mean values of total White Blood Cells (WBC) count and the absolute leucocytes count of the infected and control group. The mean total WBC count of the infected (16.05 ± 1.66 × 10³/µl) became significantly higher than in the control group (14.21 ± 2.64 × 10³/µl) at 14 dpi. Heterophil count was significantly higher in the infected group on 7 and 35 dpi. The mean monocyte value of the infected was significantly (P < 0.05) lower on 28 dpi. The mean lymphocyte value of the infected chickens was significantly lower than in the control group on 35 dpi.

Table 1: Antibiogram of bacteria isolates from barn swallows (*Passer domesticus*) around poultry houses in Ibadan, Oyo State

S/N	Antibiotic	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>E. coli</i>
1	Ciprofloxacin (10 µg)	+	+	-
2	Perfloxacin (5 µg)	-	+	-
3	Erythromycin (5 µg)	-	+	+
4	Ofloxacin (5 µg)	+	-	+
5	Chloramphenicol (30 µg)	-	-	+
6	Trimethoprim (5 µg)	+	-	+
7	Oxytetracycline (10 µg)	+	-	-
8	Gentamycin (10 µg)	-	+	+
9	Ampicillin (10 µg)	-	+	-

Table 2: Weekly Mean (±SD) value body weights of infected and control groups

Parameters	Pre-infection		7 dpi		14 dpi		21 dpi		28 dpi		35 dpi	
	Infected (n=22)	Control (n=12)	Infected (n=22)	Control (n=12)	Infected (n=18)	Control (n=10)	Infected (n=15)	Control (n=8)	Infected (n=12)	Control (n=6)	Infected (n=9)	Control (n=4)
Weight (g)	130.91 ± 9.62	134.83 ± 7.88	262.41 ^a ± 21.8	310.67 ^a ± 22.20	407.00 ^a ± 50.04	577.00 ^a ± 86.42	778.67 ^a ± 102.39	1075.00 ^a ± 107.83	940.00 ^a ± 135.91	1440.0 0 ^a ± 110.27	1417.78 ^a ± 144.38	2130.00 ^a ± 60.00

aa: Superscript with a similar letter on the same row are significant (P<0.05); SD: Standard Deviation; dpi: Day Post Infection

Table 3: Weekly Mean (\pm SD) value of packed cell volume, red cell parameters, haemoglobin Platelet and Plasma protein of the infected and control groups

Parameters	Pre-infection		7 dpi		14 dpi		21 dpi		28 dpi		35 dpi	
	Infected (n=20)	Control (n=13)	Infected (n=12)	Control (n=10)	Infected (n=10)	Control (n=10)	Infected (n=10)	Control (n=8)	Infected (n=9)	Control (n=6)	Infected (n=8)	Control (n=4)
PCV (%)	25.12 \pm 2.49	25.46 \pm 1.20	27.3 \pm 2.79	28.33 \pm 2.57	27.3 \pm 2.66	28.4 \pm 1.43	28.7 \pm 2.16	28.88 \pm 3.14	28.78 \pm 1.99	30.5 \pm 2.26	28.13 \pm 2.95	29.75 \pm 1.71
RBC ($\times 10^6/\mu\text{l}$)	3.10 \pm 0.58	2.92 \pm 0.42	3.29 \pm 0.42	3.04 \pm 0.36	3.53 \pm 0.40	3.12 \pm 0.42 ^a	3.86 \pm 0.38 ^a	3.92 \pm 0.75	3.66 \pm 0.33	3.77 \pm 0.12	3.53 \pm 0.20	3.77 \pm 0.17
HB (g/dl)	8.6 \pm 0.78	8.34 \pm 0.38	9.1 \pm 0.62	8.75 \pm 1.1	8.97 \pm 0.57	8.94 \pm 0.75	9.26 \pm 0.78	9.19 \pm 1.25	4.74 \pm 0.32 ^a	5.33 \pm 0.62 ^a	8.65 \pm 1.05	9.25 \pm 0.99
Platelet ($\times 10^9/\mu\text{l}$)	16.0 \pm 82.0	16.9 \pm 78.8	13.4 \pm 29.5 ^a	12.1 \pm 18.3 ^a	13.0 \pm 18.3	15.0 \pm 34.2	10.2 \pm 19.1	12.0 \pm 87.9	17.0 \pm 40.7 ^a	18.4 \pm 24.2 ^a	21.6 \pm 69.8	20.3 \pm 13.6
Plasma protein (g/dl)	3.3 \pm 0.1	3.3 \pm 0.2	3.5 \pm 0.1 ^a	3.3 \pm 0.2 ^a	3.9 \pm 0.2	3.9 \pm 0.3	3.2 \pm 0.3	3.4 \pm 0.3	5.3 \pm 0.3 ^a	4.7 \pm 0.6 ^a	5.5 \pm 0.6	5.1 \pm 0.4

aa: Superscript with a similar letter on the same row are significant ($P < 0.05$); SD: Standard Deviation; dpi: Day Post Infection; PCV: Packed Cell Volume; RBC: Red Blood Cell; Hb: Haemoglobin

Table 4: Weekly Mean (\pm SD) Value of Total WBC and absolute differential leucocytes count of the infected and control groups

Parameters	Pre-infection		7 dpi		14 dpi		21 dpi		28 dpi		35 dpi	
	Control (n=13)	Infected (n=20)	Control (n=10)	Infected (n=12)	Control (n=10)	Infected (n=10)	Control (n=8)	Infected (n=10)	Control (n=6)	Infected (n=9)	Control (n=4)	Infected (n=8)
Total WBC count ($\times 10^3/\mu\text{l}$)	13.51 \pm 3.45	13.30 \pm 4.06	26.13 \pm 40.48	12.33 \pm 3.57	14.21 ^a \pm 2.64	16.05 ^a \pm 1.66	28.13 \pm 38.00	17.72 \pm 2.23	22.94 \pm 2.24	21.98 \pm 5.00	22.88 \pm 3.99	22.65 \pm 7.22
Heterophil ($\times 10^3/\mu\text{l}$)	3.42 \pm 1.27	3.80 \pm 1.48	3.08 ^a \pm 12.44	8.44 ^a \pm 1.56	3.00 \pm 1.73	3.68 \pm 2.46	3.79 \pm 3.94	2.96 \pm 1.59	6.67 \pm 2.10	6.79 \pm 3.82	5.45 ^a \pm 3.23	9.13 ^a \pm 4.75
Eosinophil ($\times 10^3/\mu\text{l}$)	0.33 \pm 0.13	0.30 \pm 0.17	0.80 \pm 1.22	0.32 \pm 0.24	0.23 \pm 0.09	0.31 \pm 0.17	0.80 \pm 1.17	0.55 \pm 0.22	0.92 \pm 0.68	0.84 \pm 0.85	0.50 ^a \pm 0.60	1.06 ^a \pm 0.59
Basophil ($\times 10^3/\mu\text{l}$)	0.00 \pm 0.00	0.03 \pm 0.06	0.00 \pm 0.00	0.01 \pm 0.03	0.03 \pm 0.07	0.02 \pm 0.05	0.17 \pm 0.43	0.02 \pm 0.06	0.08 \pm 0.13	0.02 \pm 0.07	0.00 ^a \pm 0.00	0.11 ^a \pm 0.16
Monocyte ($\times 10^3/\mu\text{l}$)	0.35 \pm 0.14	0.20 \pm 0.18	0.61 \pm 1.29	0.30 \pm 0.25	0.37 \pm 0.10	0.27 \pm 0.17	0.75 \pm 0.69	0.46 \pm 0.10	0.62 ^a \pm 0.21	0.41 ^a \pm 0.15	0.72 \pm 0.18	0.79 \pm 0.44
Lymphocyte ($\times 10^3/\mu\text{l}$)	9.47 \pm 2.36	8.99 \pm 2.99	16.28 \pm 25.58	8.76 \pm 2.96	10.59 \pm 2.33	11.83 \pm 2.10	22.73 \pm 31.82	13.70 \pm 2.63	14.65 \pm 2.69	14.28 \pm 4.66	15.64 ^a \pm 3.10	11.53 ^a \pm 2.86

aa: Superscript with similar letter on the same row are significant ($P < 0.05$), dpi: Day Post Infection; WBC: White Blood Cell

Table 5: Gross pathological changes observed in *Pseudomonas aeruginosa* infected broiler chickens

Gross lesions	7 dpi		14 dpi		21 dpi		28 dpi		35 dpi	
	Control	Infected								
Subcutaneous haematoma	-	+(1/3)	-	-	-	-	-	-	-	-
Muco-haemorrhagic enteritis/diarrhoea	-	-	-	-	-	+(2/3)	-	++(2/3)	-	+++ (1/3)
Splenomegaly	-	-	-	-	-	-	-	++(2/3)	-	+++ (2/3)
Hepatomegaly/hepatic congestion	-	-	-	-	-	-	-	-	-	+(3/3)
Renomegaly	-	-	-	-	-	-	-	-	-	+(3/3)

dpi: Day Post Infection; Mild (+); Moderate (++); Severe (+++).

Post mortem findings: The gross pathological changes observed in the infected chickens are shown in Table 5. At 21 and 28 dpi gross lesions of muco-haemorrhagic enteritis (Plate I) were observed in two of the three sacrificed chickens while the control showed no grossly visible enteric lesions.

The infected group also consistently showed lesions of marked splenomegaly (Plates II) in 2/3 of the necropsied birds at 28 and 35 dpi. Mild hepatic congestion and hepatomegaly (Plate III) were observed in 3/3 of the infected chickens at 35 dpi.



Plate I: Muco-haemorrhagic enteritis in the *Pseudomonas aeruginosa* infected broiler chicken at 21, 28 and 35 dpi



Plate III: Normal liver (A) from the control while (B) shows mild congestion and enlargement of liver from *Pseudomonas aeruginosa* infected broiler at 35 dpi

Pseudomonas aeruginosa pure colony re-isolated from spleen of chicken on centrimide agar is presented on Plate IV.

Discussion

In this study, three significant pathogenic bacterial species were isolated from the free-flying barn swallow (*Hirundo rustica*). This supports the findings of Reed *et al.* (2003), who reported that wild birds harbour emerging zoonotic pathogens and serve as a reservoir hosts. In particular, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* which were isolated from the barn swallow, are among the pathogenic bacteria of economic importance in poultry (Lutful, 2010). The isolation of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in the barn swallows in Ibadan, Nigeria is a significant finding and appears to be the first report in Nigeria and a pointer to the role these species



Plate II: Normal spleen (A) from the control group and severe splenomegaly (B) in *pseudomonas aeruginosa* infected chicken at 35 dpi



Plate IV: *Pseudomonas aeruginosa* pure colony re-isolated from spleen of chicken on centrimide agar following experimental infection at 35 dpi (Arrow)

likely play as a vermin and vector in poultry production.

Antibiogram test revealed that the isolated organisms were highly sensitive to Norfloxacin, Chloramphenicol, Erythromycin, Ofloxacin and Perfloxacin but resistant to Trimethoprim, Oxytetracycline and Ampicillin. An earlier report by Ibrahim *et al.* (2019) similarly showed isolated *E. coli* to be resistant to Trimethoprim among other antibiotics. These probably suggest a widespread circulation of this resistant strain in poultry. The *Pseudomonas aeruginosa* isolate in this study, showed resistance to Erythromycin, Perfloxacin, Chloramphenicol and Ampicillin which are the commonly used antibiotics against infectious diseases in poultry production in Nigeria. This finding is in tandem with report by Hebat-Allah (2004) and Ogunleye (2012) that isolated *P. aeruginosa* from poultry that died of septicaemia. The associated

resistance of the bacterium to Ampicillin, Streptomycin, Chloramphenicol and Gentamycin is consistent with our antibiogram observations in this study. This may indicate persistent circulation of this organism among the poultry population in this area. Following experimental infection with the isolated *Pseudomonas aeruginosa* in broiler chicken, the observed significant and progressive lower weight gain in the infected chicken's group maybe as a result of poor feed conversion rate sequel to enteritis resulting in mal-absorption and subsequently reduced weight gain as compared to the control group. Similar observation was reported by Hebat-Allah *et al.* (2004) in experimental infection of *P. aeruginosa* in broilers.

Haematological parameters are reliable health indicators (Katayev *et al.*, 2010; Ozarda *et al.*, 2019). The initial decrease in plasma protein in the infected chickens by 21dpi, followed by an increase at 28 and 35 dpi can be attributed to haemorrhage and progressive dehydration, respectively, observed in the infection over the study period. This finding agrees with the reports from Ajayi *et al.* (2018) who observed the same trend of the haematological result. The significant increase in total WBC count on day 14dpi and a decrease in lymphocyte counts on 35dpi in the infected group compared to the control group suggest inflammatory response and stress, respectively. The marked reduction in the mean value of lymphocytes of the infected at 35 dpi may reflect the overwhelming nature of *Pseudomonas aeruginosa* at compromising the defensive mechanism. This is because the major function of the white blood cell is inflammatory response/phagocytosis to prevent infection (Blann, 2014). The increase in the mean count of heterophils at 14, 28 and 35 dpi in the infected could reflect the possibility of heterophils inflammatory response to curtail the infection by phagocytosis in the acute phase of the disease. The basophil and eosinophil both have some relative increases in their mean value at 35 dpi in the infected group and this could point to their involvement in the defensive mechanism as phagocytes. The pattern of platelet distribution in the infected and control group is similar and this supports the findings by Ajayi *et al.* (2018).

The clinical signs of rales (dyspnea) and bloody diarrhoea which may be as a result of sinusitis and enteritis respectively are similar to the reports of Walker *et al.* (2002) who observed enteritis, sinusitis and diarrhoea in chickens infected with *Pseudomonas aeruginosa*. The post mortem lesions

showed marked splenomegaly, renomegaly, hepatic congestion and hepatomegaly. These lesions are consistent with the septicaemic nature of *Pseudomonas aeruginosa* similar to that reported by Hebat-Allah *et al.* (2004) and Ajayi *et al.* (2018).

The re-isolation of *Pseudomonas aeruginosa* from tissues with lesions in the infected group at 35 dpi confirms that the organism is indeed infective and responsible for the observed clinical signs and pathology, hence, pathogenic to broiler chicken.

This study has shown that free flying barn swallows as vermin in Nigeria poultry houses carry pathogenic bacteria including *P. aeruginosa*, that cause various clinical, haematological, and gross pathological lesions in broilers and may be responsible for the transmission (vector) of these pathogenic bacteria between poultry houses and farms due to their free-flying habit.

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Conflict of Interest

The authors declare that there is no conflict of interest.

References

- Ajayi JO, Anise NH & Ogunleye AO (2018). Clinico-pathological study of broiler chickens experimentally infected with *Proteus mirabilis* and *Pseudomonas aeruginosa* isolated from red-headed rock agamas (*Agama agama*) co-habiting with poultry in Oyo State, Nigeria. *Journal of Veterinary Medicine and Animal Health*, **10**(1): 34-44.
- Ajayi JO, Ogunleye AO, Happi AN & Okunlade AO (2015). Bacteria isolated from the oral and cloaca swabs of lizards co-habiting with poultry farms in Ibadan, Oyo State, Nigeria. *Africa Journal of Biomedical Research*, **18**(3): 211-215.
- Benson HJ, Gunstream SE, Talaro A & Talaro KP (1989). *Anatomy and Physiology Laboratory Textbook*. Win. C. Brown Publisher Dubuque IOWA
- Blann A (2014). Routine blood test, functions and diseases of red and white cells. *Nursing Times*, **110**(8): 16-18.
- Britni B, Ashwini K & Jim A (2017). Prevalence of *Trichomonas*, *Salmonella*, and *Listeria* in

- wild birds from Southeast Texas. *Avian Diseases*, **61**(3): 347.
- Brown MB & Brown CR (2019). Barn Swallow (*Hirundo rustica*), version 2.0. In: *The Birds of North America* (PG Rodewald, Editor). Cornell Lab of Ornithology, Ithaca, NY, USA.
- DAHS (2012). Dupont Animal Health solution – Europe. Poultry Production Biosecurity.
- Elsayed MSA, Ammar AMA, Shehri ZS & Abd-El Rahman NA (2016). Virulence Repertoire of *Pseudomonas aeruginosa* from some poultry farms with detection of resistance to various antimicrobials and plant extracts. *Cellular and Molecular Biology*, 10.4172/1165-158X.1000124.
- Fagbohun OA, Oluwayelu DO, Owoade AA & Olayemi FO (2000). Survey for antibodies to newcastle disease virus in cattle egrets, pigeons, and Nigerian laughing doves. *African Journal of Biomedical Research*, **3**(3): 193-194.
- Fekadu K (2010). Pseudomonas infection in chickens. *Journal of Veterinary Medicine and Animal Health*, **2**(4): 55-58.
- Gellatly SL & Hancock RE (2013). *Pseudomonas aeruginosa*: New insights into pathogenesis and host defenses. *Pathogens and Disease*, **67**(3): 159-173.
- Gruebler MU, Korner-Nievergelt F & Von Hirschheydt J (2010). The reproductive benefits of livestock farming in Barn Swallows *Hirundo rustica*: Quality of nest site or foraging habitat. *Journal of Applied Ecology*, **47**(6): 1340–1347.
- Hebat-Allah AHM (2004). Some studies on Pseudomonas species in chicken embryos and broilers in Assiut governorate. *Assiut University Bulletin of Environmental Research*, **7**(1):23-30.
- Houghton-wallace J & Lister S (2012). Backyard poultry, husbandry and general management. *In Practice*, **34**(3): 136-145.
- Ibrahim RA, Cryer TL & Lafi SQ (2019). Identification of *Escherichia coli* from broiler chickens in Jordan, their antimicrobial resistance, gene characterization and the associated risk factors. *BMC Veterinary Research*, doi.10.1186/s12917-019-1901-1.
- Jorgensen JH & Turnidge JD (2007). Susceptibility test methods: dilution and disk diffusion methods In: *Manual of Clinical Microbiology* PR Murray, EJ Baron, JH Jorgensen, ML Landry, MA Pfaller, editors), ninth edition. ASM Press, Washington, D.C. Pp 1152–1172.
- Kallapura G, Morgan MJ, Pumford NR, Bielke LR & Wolfenden AD (2014). Evaluation of the respiratory route as a viable portal of entry for Salmonella in poultry via the intratracheal challenge of *Salmonella enteritidis* and *Salmonella typhimurium*. *Poultry Science*, **93**(2): 340-346.
- Katayev A, Balciza C & Seccombe DW (2010). Establishing reference intervals for clinical laboratory test results. *American Journal of Clinical Pathology*, **133**(2): 180–186.
- Lamb GM (1981). *Manual of Veterinary Laboratory Techniques in Kenya*. Ministry of Livestock Development/CIBA GEIGY, Basle, Switzerland. Pp 96-107.
- Lutful KSM (2010). Avian colibacillosis and salmonellosis: A closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. *International Journal of Environmental Research and Public Health*, **7**(1): 89–114.
- Mansoor T, Musani MA, Khalid G & Kamal M. (2009). *Pseudomonas aeruginosa* in chronic suppurative otitis media: sensitivity spectrum against various antibiotics in Karachi. *Journal of Ayyub Medical College Abbottabad*, **21**(2):120-123.
- Ogunleye AO (2012). Identification of GyrA mutations conferring fluoroquinolone resistance in *Pseudomonas aeruginosa* isolated from poultry in Ibadan, Oyo state Nigeria. *African Journal of Microbiology Research*, **6**(7): 1249-1254.
- Ogunleye AO, Ajuwape ATP & Adetosoye AI (2013). *Salmonella enterica* serotype Pullorum isolated from a lizard co-habiting with poultry. *African Journal of Research*, **7**(14): 1215-1221.
- Ozarda Y, Higgins V & Adeli K (2019). Verification of reference intervals in routine clinical laboratories: Practical challenges and recommendations. *Clinical Chemistry and Laboratory Medicine*, **57**(1): 30-37.
- Reed KD, Meece JK, Henkel JS & Shukla SK (2003). Birds migration and emerging zoonoses: West Nile virus, lyme disease, influenza and enteropathogens. *Clinical Medicine and Research*, **1**(1): 5-12.
- Saino N, Romano M & Caprioli M (2013). Molt, feather growth rate and body condition of

- male and female barn swallows. *Journal of Ornithology*, **154**(2): 537-547.
- Silby MW, Winstanley C, Godfrey SA, Levy SB & Jackson RW (2011). Pseudomonas genomes: diverse and adaptable. *FEMS Microbiology Review*, **35**(4): 652-680.
- Walker SE, Sander JE, Cline JL & Helton JS (2002). Characterization of *Pseudomonas aeruginosa* isolates associated with mortality in broiler chicks. *Avian Diseases*, **46**(5): 1045 –1050.