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Original Article

Hematology of layers chickens vaccinated with fowl cholera vaccine and experimentally inoculated with virulent *Pasteurella multocida* serotypes in Zaria, Nigeria

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ABSTRACT

Objective: The objective of this study was to evaluate some hematological parameters in commercial layers inoculated with two virulent *Pasteurella multocida* serotypes.

Materials and Methods: A total of 84 twenty-week-old black Harco layers were randomly assigned to seven groups (A, B, C, D, E, F and G) with 12 birds per group. 1mLof live attenuated fowl cholera (FC) vaccine was administered subcutaneously at 24 weeks of age to groups A and B, emulsified inactivated (killed) FC vaccine was administered dosed at 0.5 mL per bird subcutaneously at 24 weeks of age to groups C and D, groups E and F were not vaccinated, while group G served as control. Groups A, C and E were inoculated with *P. multocida* serotype A:1 and groups B, D and F were inoculated with *P. multocida* serotype A:3. Using McFarland Standard, each bird received a dose of 0.5 mL (0.1 mL intranasally and 0.4 mL intramuscularly) containing 4.5 x 10^8 cfu/bird.

Results: For PCV ($P \leq 0.2692$ and $P \leq 0.7643$) and HB (**(**$P \leq 0.2806$ and $P \leq 0.7266$) on day 2 and 10 post inoculation, there was no significant difference between the vaccinated, non-vaccinated groups and control group G. However, there was a highly significant difference $P \leq 0.05$ in the mean concentrations of ALP between the control group G (67.67±1.453 u/l) vaccinated groups A (80.33±4.98 u/l), B (81.33±2.60 u/l), C (75±6.35 u/l), and D (84±5.132 u/l) and unvaccinated groups E (104±1.528 u/l), and F (78 ±3.512 u/l) post inoculation.

Conclusion The PCV significantly decrease $P \leq 0.05$ in layers vaccinated and inoculated with *P. multocida but* increase in unvaccinated layers inoculated *P. multocida.* The mean serum ALP concentration significantly increase $P \leq 0.05$ in unvaccinated layers inoculated with *P. multocida* when compared to layers vaccinated and inoculated with *P. multocida.*

KEYWORDS

Fowl cholera; *P. multocida*; Layers; Serotype; PCV; HB; ALT; AST; ALP

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INTRODUCTION

Fowl cholera (FC) remains a significant obstacle due to losses to commercial poultry production in most part of tropical Asia and Africa. Fowl cholera usually occurs as a fulminating disease with massive bacteraemia, high morbidity and mortality (OIE, 2008). In Nigeria, FC is a major constraint to poultry production and outbreak could cause high mortalities of up to 80% (Abdu, 1990; Odugbo et al., 2004; Akpavi et al., 2011). FC is caused by *Pasteurella multocida* which is a gram negative, oxidase positive, non-motile, non spore forming facultative anaerobic rod shaped or coccoid bacterium (Petersen et al., 2001). Chickens, turkeys, ducks and geese and all types of birds are susceptible to FC (Glisson et al., 2003).

It was demonstrated that killed FC vaccine protected chickens against homologous challenge. However; in spite of vaccination in the endemic areas outbreak of FC has been reported (Sotoodehnia et al., 1984; Jonas et al., 2001; Kalaydari, 2004). Vaccines have been widely used to prevent FC however; these vaccines generally afforded homologous but not heterologous protection (Petersen et al., 2001). In Nigeria, Dashe et al. (2013) reported *P. multocida* serotypes A:1, A:3 and A:4 and found that *P. multocida* serotype A:1 and A:3 are causing disease in layers . It is believed that Serum biochemical parameters provide valuable information for evaluation of health status of birds and reflect many metabolic alterations of organs and tissues (Kral and Suchy, 2000). The objectives were to evaluate some hematological parameters of layers vaccinated with live and inactivated fowl cholera vaccines and inoculated with two virulent *P. multocida* serotypes.

MATERIALS AND METHODS

Experimental Birds and Housing: Eighty four days old chicks were bought from a hatchery in Ibadan and raised to pullet size in Gusau and brought to Zaria at 20 week old. . All vaccine protocol was followed except FC vaccine which was not administered. The birds were housed at the Poultry Research Unit of the Department of Veterinary Medicine. The facility was washed clean with detergent and then disinfected with formalin at the rate of 40 mL/10 litres of water before arrival of the birds. The birds were allowed to acclimatize for three weeks and were managed on deep litter. Nasal swabs were taken from the birds at 23 weeks to screen for *P. multocida*.

Ethical statement: Ethical consideration was made before commencement of the work, hence the decision to use small dose of inoculums as 4.5 x10^8 cfu/mL of *P. multocida*.

Source of FC vaccines: The live attenuated (local) FC vaccine was obtained from the National Veterinary Research Institute (NVRI) Vom and the vaccine serotype is *P. multocida* (aviseptica) serotype A:1 (NVRI, 2002). The batch number of the vaccine was 11/2014 and expiration date was 10/2014. Emulsified inactivated FC vaccine (IZOVAC FC®) containing serotypes A:1, A:3 and A:4 manufacture by IZO SURL Italy. The vaccine had batch number 02201 and manufactured date was 4/14 while date of expiration was 4/2016.

Source, culture and biochemical test of *P. multocida* serotype A:1 and A: The bacterium *P. multocida* (serotypes A:1 and A:3) was obtained from the Bacteriology Unit, NVRI Vom, Plateau State, Nigeria . The *P. multocida* serotypes were subcultured on blood agar, colonies were examined for colonial morphology (non-hemolytic, moderately sized round colonies) and tested for gram reaction (Gram negative). Biochemical test carried out as described by CLSI (2009) showed that the *P. multocida* were oxidase positive, indole positive, catalase positive and did not grow on MacConkey agar.

Inoculation of birds with *P. multocida* serotype A:1 and A:3: *P. multocida* was cultured on blood agar plate and incubated at 37°C for 24 h, several colonies of serotype A:1 and A:3 were scooped separately and inoculated into separate test tube, each containing 20 mL of 0.5% normal saline, until the turbidity was equivalent to 4.5 x10^8 cf/mL which is standard 3 from McFarland standard . Each of the birds in the experimental group was inoculated with dose of 0.5 mL containing 0.1 mL solution of *P. multocida*, which was administered intranasally (Arsov, 1965) and 0.4 mL was inoculated through the breast muscle intramuscularly to each bird using an insulin syringe (Choudhury et al., 1987) .

Blood collection: About 2 mL of blood was collected from the birds via the wing vein using a 23 guage sterile needle and syringe. 1 mLof the blood was collected in (EDTA) sample bottle and the other 1mLinto plain sample bottle which are allowed to clot at room temperature from which sera were obtained and stored at -20^{0}C until used for enzyme biochemistry. Blood was collected from the birds in each group twice before inoculation and twice after inoculation with *P. multocida* at weekly interval.

Determination of Packed Cell Volume: Packed cell volume (PCV) was determined using the microhematocrit method (Sirois, 1995).

Determination of total plasma protein concentration: Total plasma protein was determined using hand refractometric method (Sirois, 1995).

Determination of Hemoglobin Concentration: Blood hemoglobin concentration was assayed colorimetrically as cyanomethhemoglobin (Drabkin, 1945).

Biochemical Assays: Three serum samples were taken from each group (A to G) once at day 43 post inoculation and Serum enzymes biochemistry was carried out at the Clinical Pathology Laboratory of Ahmadu Bello University Teaching Hospital Shika, Nigeria, using laboratory kits obtained from Randox Laboratory Ltd., United Kingdom. The absorbance was read using a UV-VIS Spectrophotometer (DREL 300 HACH). Parameters that were determined were Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP).

Data analyses: All results obtained were expressed as means±standard error of the mean. One way analysis of variance (ANOVA) with Turky's Multiple comparison post-hoc test using Graph Pad Prism® version 5.3 for Windows® (Graph Pad software, San Diego, California, USA) was used to compare the level of significance between groups. Values of $P \leq 0.05$ were considered significant at 95 % confidence interval.

RESULTS AND DISCUSSION

There was no significant difference in PCV $P \leq 0.1938$ among all the groups on day 1pre inoculation. On day 2 and 10 post inoculation, there was no significant difference $P \leq 0.2692$ and $P \leq 0.7643$ between the vaccinated groups A, B, C, D and non-vaccinated group E and F and control group G. There was an observable increase in PCV on day 2 post inoculation in all inoculated groups higher than observed on day 1 before inoculation while at day 10 post inoculation there were decrease in PCV in all inoculated groups with no decrease in group G (**Table 1**). For the mean total plasma protein concentration no statistical significant difference $P \leq 0.5309$ in all groups on day 1 pre inoculation. On day 2 and 10 post inoculation there was no statistical significant difference $P \leq 0.4513$ and 0.7613 among the groups (**Table 2**). There was no statistical significant difference in hemoglobin concentration $P \leq 0.2439$ in all groups on day 1 pre inoculation. On day 2 and day 10 post inoculation there was no statistical significant difference in all the groups with $P \leq 0.2806$ and $P \leq 0.7266$ respectively.

There was increase in hemoglobin concentration on day 2 post inoculation in all inoculated groups while at day 10 there was decrease from all inoculated groups with no decrease in control group G (**Table 3**). There was no statistical significant difference in ALT concentrations in all groups post inoculation with $P \leq 0.113$. Group E had the highest value of ALT concentrations when compared to other groups. There was no statistical significant difference in mean enzyme concentrations in all groups post inoculation with p value of $P \leq 0.2384$ Group E had the highest value of serum AST concentration when compared to other groups. There was a statistical significant difference in mean serum concentrations of ALP in all groups post inoculation with $P \leq 0.0008$. Group E had the highest value of ALP with control group (G) having lower value when compared to other groups (**Table 4**).

Although it has been generally accepted that fowl cholera is a septicemic infection, bacteria can only be isolated in large numbers from the blood of birds very late in infection, and it has been proposed that this late re-emergence of blood-borne bacteria is due to the rupture of liver and spleen phagocytes Pabs-Garnon and Soltys (1971) so as result of this rupture of liver and spleen, values of PCV and HB will be affected following inoculation with *P. multocida* as it seen in this study. There was an increase in PCV and HB concentration on day 2 post inoculation in layers in vaccinated groups and non-vaccinated but inoculated groups. This suggests that birds had probably developed hemoconcentration due to inappetance and poor water intake after infection. This is similar to report of Samour (2013). On day 10 post inoculation, there was a decreased in PCV and HB concentrations for vaccinated groups and non-vaccinated but inoculated groups. This decrease could be attributed to hemolytic effect of the *P. multocida* endotoxin as reported by Diallo and Frost (2000). This drop in PCV values is in agreement with report of Mohammed (2009), Akpavi et al. (2011) and Ficken and Barnes (1989) that infection with *P. multocida* causes decrease PCV. For total protein, there was no significant difference between all groups before and after inoculation. This finding is in agreement with reports of Mohammed (2009). There was no statistical significant difference in ALT concentration in all groups post inoculated with *P. multocida*. This is in agreement with report of Mohammed (2009). There was no statistical significant difference in AST concentration in all groups post inoculation with *P. multocida*. These findings disagreed with the report of Mohammed (2009) who reported increase in AST concentrations when chickens were inoculated with *P. multocida*. There was statistical significant difference in ALP concentration between vaccinated and unvaccinated but inoculated with

Table 1: Changes in mean packed cell volume of chickens vaccinated with fowl cholera vaccine and inoculated with *P. multocida* serotype A:1 and A:3.

Group	A	B	C	D	E	F	G
Serotype	A:1	A:3	A:1	A:3	A:1	A:3	
Dose of inoculum	$4.5x10^8$ cfu/b	$4.5x10^8$ cfu/b	$4.5x10^8$ cfu/b	$4.5x10^8$ cfu/b	$4.5x10^8$cfu/b	$4.5x10^8$cfu/b	
			Packed cell volume				
Day 1 Pre-inoculation	27.33±4.216	23.33±1.978	27.83±1.138	24.83±2.548	20.33±1.838	24±0.8165	30.83 ±1.447
Day 2 Post-inoculation	33±3.792	40±1.826	37.5±1.875	41.83±0.7032	37±1.095	38.33±1.382	30.33±1.174
Day 10 Post-inoculation	27.17±3.208	29.67±1.476	29.5±2.884	32.17±1.249	30.67±0.9189	30.33±1.801	30.17±1.537

A-B Vaccinated with live attenuated FC vaccine at 24 week and inoculated with serotype A:1(A) and (B) A:3 at 30 week of age.
C-D Vaccinated with inactivated FC vaccine at 24 week and inoculated with serotype A:1(C) and (D) A:3 at 30 week of age.
E-F Positive control non-vaccinated but inoculated with serotype A:1(E) and (F) A:3 at 30 week of age.
G- Negative control non-vaccinated and non inoculated.

Table 2: Changes in mean total plasma protein concentration of chickens vaccinated with fowl cholera vaccine and inoculated with *Pasteurella multocida* Serotype A:1 and A:3

Group	A	B	C	D	E	F	G
Serotype	A:1	A:3	A:1	A:3	A:1	A:3	
Dose of inoculum	$4.5x10^8$cfu/b	$4.5x10^8$cfu/b	$4.5x10^8$cfu/b	$4.5x10^8$cfu/b	$4.5x10^8$cfu/b	$4.5x10^8$cfu/b	
			Total protein	concentration			
Day 1 Pre inoculation	6.167±0.2275	6.067±0.588	5.7± 0.3454	6.5±0.3786	6.13±6.384	6.033±0.3323	6.867± 0.2044
Day 2 post+inoculation	6.767±0.804	5.5±0.5627	6.2±0.5292	6.267 ±0.1606	6.167± 0.1085	6.633±0.348	5.7±0.2864
Day10 post-inoculation	6.1± 0.728	6.367±0.7419	5.833±0.3242	6.1±0.177	5.267±0.2348	6±0.4789	5.767±0.1585

A-B Vaccinated with live attenuated FC vaccine at 24 week and inoculated with serotype A:1(A) and (B) A:3 at 30 week of age.
C-D Vaccinated with inactivated FC vaccine at 24 week and inoculated with serotype A:1(C) and (D) A:3 at 30 week of age.
E-F Positive control non-vaccinated but inoculated with serotype A:1(E) and (F) A:3 at 30 week of age.
G- Negative control non-vaccinated and non-inoculated.

Table 3: Changes in mean hemoglobin concentration of chickens vaccinated with fowl cholera vaccine and inoculated with *Pasteurella multocida* serotype A:1 and A:3

Group	A	B	C	D	E	F	G
serotype	A:1	A:3	A:1	A:3	A:1	A:3	
Dose of inoculum	4.5x10^{8}cfu/b	4.5x10^{8}cfu/b	4.5x10^{8}cfu/b	4.5x10^{8}cfu/b	4.5x10^{8}cfu/b	4.5x10^{8}cfu/b	
			Hemoglobin	concentration			
Day1 Pre inoculation	8.9±1.409	7.733±0.652	9.117 ±0.3833	8.117 ±0.8085	6.683 ±0.6343	7.967 ±0.3029	9.117±0.5102
Day 2 post- inoculation	11.73 ±1.245	13.23±0.607	12.28 ±0.5659	13.73± 0.2679	12.18±0.34	12.57 ±0.4341	9.28±0.3745
Day10 post-inoculation	8.95±1.021	9.783±0.4475	9.617±0.9765	10.62±0.3563	10.18±0.3092	10.02±0.6129	9.9±0.5453

A-B Vaccinated with live attenuated FC vaccine at 24 week and inoculated with serotype A:1(A) and (B) A:3 at 30 week of age.
C-D Vaccinated with inactivated FC vaccine at 24 week and inoculated with serotype A:1(C) and (D) A:3 at 30 week of age.
E-F Positive control non-vaccinated but inoculated with serotype A:1(E) and (F) A:3 at 30 week of age.
G- Negative control non-vaccinated and non-inoculated.

Table 4: Changes in mean serum concentration of ALT, AST and ALP of chickens vaccinated with fowl cholera vaccine and inoculated with *Pasteurella multocida* Serotype A:1 and A:3

Group	A	B	C	D	E	F	G
Serotype	A:1	A:3	A:1	A:3	A:1	A:3	
Dose of inoculum	4.5x10^{8}cfu/b	4.5x10^{8}cfu/b	4.5x10^{8}cfu/b	4.5x10^{8}cfu/b	4.5x10^{8}cfu/b	4.5x10^{8}cfu/b	
			Enzyme concentration	Post inoculation			
ALT	50.67±3.756	48.67±2.028	48±4.359	55.33±4.807	64.67±1.856	50.67±4.41	50±5.292
AST	45.67±45.67	44.33±1.764	44.67±2.906	49.67±4.372	54±2.517	48±3.786	43.33±1.764
ALP	80.33±4.978^{ae}	81.33±2.603^{be}	75±6.351^{ce}	84±5.132^{de}	104±1.528	78±3.512^{ef}	67.67±1.453^{eg}

Superscript with the same alphabet are significantly different (P<0.05)
A-B Vaccinated with live attenuated FC vaccine at 24 week and inoculated with serotype A:1(A) and (B) A:3 at 30 week of age.
C-D Vaccinated with inactivated FC vaccine at 24 week and inoculated with serotype A:1(C) and (D) A:3 at 30 week of age.
E-F Positive control non-vaccinated but inoculated with serotype A:1(E) and (F) A:3 at 30 week of age.
G- Negative control non-vaccinated and non inoculated

P. *multocida* with group E having higher value of 104 iu/l. This high value of ALP in group E could be due to hepatocellular damage or degenerative changes induced by *P. multocida* or its endotoxins as reported by Mohammed (2009). It is believed that Serum biochemical parameters provide valuable information for evaluation of health status of birds and reflect many metabolic alterations of organs and tissues.

CONCLUSION

The PCV significantly decrease $P \leq 0.05$ in layers vaccinated and inoculated with *P. multocida but* increase in unvaccinated layers inoculated *P. multocida*. The mean serum ALP concentration significantly increase $P \leq 0.05$ in unvaccinated layers inoculated with *P. multocida* when compared to layers vaccinated and inoculated with *P. multocida*.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

AUTHORS' CONTRIBUTION

This study is a component of MSc thesis of the major author, YML and my supervisors. AMW, PAA, MAR, PHM participated in proposing and designing the experiment. YML carried out the experiment, collected data, analyzed the data, and drafted the manuscript. AMW, PAA, MAR, PHM read and corrected the manuscript. The other authors assist me in drafting the final manuscript.

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