



Plumage Color Influence on Immune Response and Severity of Fowlpox in The Progeny of Inbred Isa White Chickens

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ABSTRACT

There is limited information regarding chicken's inter and intra-breed resistance to the fowlpox virus (FWPV). Incidentally, it was observed during a natural fowlpox outbreak in a flock of inbred Isa White chickens having different plumage colors that those with predominantly white plumage were most severely affected. Thus, this study assessed the effects of plumage color in progenies of the affected chickens on immune response and severity of fowlpox virus infection. Thirty each of white and colored chickens were selected from the same hatch and challenged experimentally with a field fowlpox virus. Fifteen chickens from each plumage group were vaccinated against fowlpox prior to the challenge while the remaining unvaccinated half were infected with the field virus. Vaccine 'takes', discrete fowlpox lesions and FWPV antibody titer were evaluated. The highest mean vaccine 'takes' in white (9.95 ± 0.34 mm) and colored (10.74 ± 0.41 mm) chickens were observed on day-7 post-vaccination. White chickens had lower mean antibodies to fowlpox virus pre- and post-vaccination (21.83 ± 3.32 and 41.61 ± 6.16 ng/L) than colored chickens (26.89 ± 5.47 and 53.25 ± 7.26 ng/L); however, the difference was not statistically significant ($p > 0.05$). In contrast, mean fowlpox lesions count differed significantly ($p < 0.05$) between white (4.00 ± 0.56) and colored (2.0 ± 0.35) chickens. Immune responses and severity of fowlpox are likely influenced by plumage color in Isa White breed of chickens.

Keywords: Fowlpox, Humoral immunity, Resistance, Susceptibility, Virology.

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INTRODUCTION

Fowlpox is a contagious viral disease of chickens and turkeys caused by the fowlpox virus (FWPV). The disease has two well recognized forms; the cutaneous (dry) form characterized by multifocal lesions of skin particularly around the eyelids, combs, wattle and thigh with low mortality, and the wet form characterized by diphtheritic lesions in digestive and respiratory tracts of birds causing sneezing, dyspnea and relatively high mortality (Tripathy and Reed, 2008; MacLachlan and Dubovi, 2017; Tripathy and Reed, 2020). Chickens may occasionally develop a mixed form, and a third rare systemic form affecting the entire tissues of birds has also been reported (VanRiper and Forrester, 2007).

Fowlpox, an endemic disease in tropical and subtropical countries (Adene and Fatumbi, 2004;

Beytut and Haligur, 2007; OIE, 2016), is one of the most economically important poultry diseases causing a drop in egg production, reduced growth rate in young birds and death (Tripathy and Reed, 2020). It has been ranked as the third most important cause of death in Nigerian indigenous chickens (Abdu *et al.*, 1992). Despite its alarming prevalence, detailed disease epidemiology has not been exploited (Meseko *et al.*, 2012). In high-density, multiple-age farms, the disease may persist for long periods of time despite preventive vaccinations (Ferreira *et al.*, 2018; Sarma *et al.*, 2019). A number of recurrent outbreaks in vaccinated flocks have been reported globally (Odoya *et al.*, 2006; Okwor *et al.*, 2014; Ferreira *et al.*, 2018).

Fowlpox virus is extremely resistant to desiccation; it can survive within scab for a long period under different adverse environmental conditions (Srinivasan *et al.*, 2001). The virus is reported to be

pathogenic in chickens of all ages, sexes and breeds (Idris *et al.*, 20018). Maclachlan and Dubovi (2017) speculated that "under natural conditions, there may be breed differences in susceptibility to fowlpox; chickens with large combs appear to be more affected than those with small combs." However, there is a paucity of information regarding inter and intra-breed levels of susceptibility as well as resistance to fowlpox in chickens. Hence, much research is needed in order to improve or develop newer breeds that are resistant to the disease. The present study was based on an observation following a natural fowlpox outbreak that chickens with predominant white feathers were most severely affected in a flock of inbred Isa White chickens. Progenies from the affected flock were challenged experimentally to assess the possible impact of plumage color on immune response and severity of fowlpox in chickens.

MATERIALS AND METHODS

Ethical Consideration:

The care for chickens used in this study was with professional standards as approved by the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC/2019/11).

Experimental Chickens:

Embryonated chicken eggs (ECE) weighing 45 to 50 g from healthy inbred Isa White breeder hens, sourced from Virology Unit, Department of Veterinary Microbiology, ABU Zaria-Nigeria, were used for the study. The eggs were hatched using an egg incubator (Brinsea Ova easy 380, USA) at 37.5 °C. The chicks were fed twice daily using commercial poultry feed (Hybrid Feeds Ltd. Kaduna, Nigeria) and water was provided *ad-libitum*.

Fowlpox Virus Vaccine and Antigen:

A commercial 1,000 dose live attenuated fowlpox vaccine (ABIC® Biological Laboratories Ltd. Israel, lot no. 20511046A) was used in this study. Fowlpox virus antigen was prepared from the infected chorioallantoic membrane (CAM) in accordance with OIE (2016) Guidelines. Briefly, eight local field outbreaks of fowlpox were identified, scabs were then collected and 20% homogenate was prepared using antibiotics containing normal saline as a diluent. This was followed by two cycles of freezing and thawing, centrifugation and inoculation of supernatants onto the CAM of 10-day-old ECE as described by Gilhare (2012). Afterward, the infected CAMs with characteristic pock lesions were subjected to the same procedure as described for scabs to obtain the supernatant used as FWPV antigen for the challenge.

Experimental Design:

A total of 60 chickens comprising 30 each of white and colored (black, brown-black and mottled) were used for the study. The chickens were randomly assigned into four groups of 15 each based on their plumage colors as groups A, B, C and D. Groups A and C had white plumage while B and D were colored. Groups A and B were vaccinated at 8-week of age with 0.01 mL of live attenuated fowlpox vaccine via the right wing-web and later challenged with 0.1 mL of field FWPV isolate at 3-week post vaccination via the left wing-web site. Groups C and D were infected at 11-week of age without prior vaccination.

Sample Collection:

The Vaccinated chickens (groups A and B) were examined for the presence and size of vaccine "takes" at the site of inoculation (from 5th to 10th-day post-vaccination) using a Vernier caliper. Scab or swelling at the site of vaccine application was considered a positive reaction (takes). The presence and number of discrete fowlpox lesions were evaluated on day 18 and day 22 post-challenge. The average of the two readings for each chicken was considered as the number of lesions per bird.

Blood was collected into sterile plain vacutainer tubes pre-vaccination, 3-weeks post-vaccination, and 3-weeks post-challenge in groups A and B, while for groups C and D pre-infection and 3-weeks post-infection. Sera were harvested from all the collected blood samples and used for FWPV antibody quantification using indirect enzyme-linked immunosorbent assay (ELISA) following the manufacturer's instruction (KTE220001, Abbkine, Inc, China).

Data Analyses

Data were analyzed using SPSS version 16.0 statistical software. Data were expressed as mean ± standard error of mean (mean ± SEM), subjected to statistical analyses using Independent t-test (vaccine takes diameters and FWPV antibody titers) and Mann Whitney U-test (fowlpox lesions count). Values of $p < 0.05$ were considered significant.

RESULTS

Isolation and Propagation of Field Fowlpox Virus Isolates:

The infected CAMs revealed hemorrhages, edema, and generalized thickening with opaque-white pock lesions of about 3 to 5 mm in diameter at first passage (Plate I). More severe pock lesions were seen following three repeated passages (Plate II). There was high embryo mortality (30%) in inoculated eggs with a foul odor.

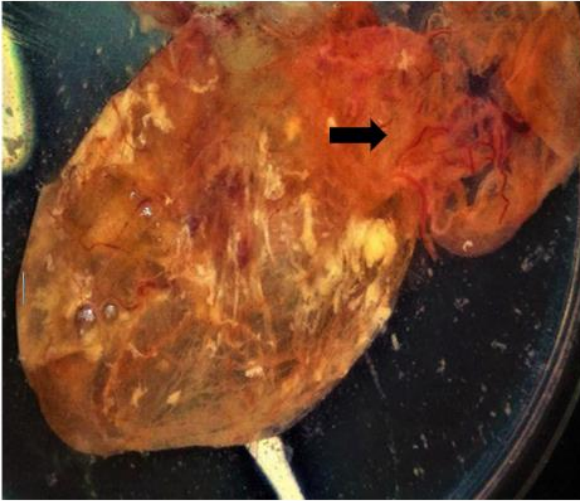


Plate I: Edematous & hemorrhagic (black arrows) chorioallantoic membrane inoculated with a field fowlpox virus isolate at first passage.

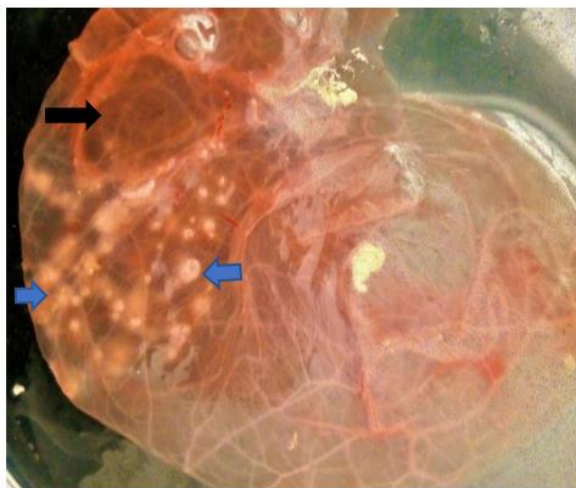


Plate II: Generalized hemorrhages (black arrow) and characteristic prominent opaque-white pock lesions (blue arrows) in chorioallantoic membrane infected with a field fowlpox virus isolate at third passages.

Vaccine ‘Takes’ Diameter:

At 5-days post-vaccination (dpv), group A chickens had 93.3 % vaccine “takes”, whereas group B chickens had 100% “takes”. However, at 6 to 10-dpv, all vaccinated chickens (group A and B) showed 100% “takes” even though some of these “takes” were very small. Overall, the highest vaccine “takes” diameter recorded per individual chicken was 13.2 mm on 6-dpv in group A, while the lowest was 2.7 mm on 10-dpv in group B. No vaccine “take” was detected in the unvaccinated chickens. From 5 to 7-dpv, group B chickens had higher mean vaccine “takes” diameters of 7.33 ± 0.47 , 9.15 ± 0.52 , and 10.74 ± 0.41 mm than the corresponding group A chickens (6.11 ± 0.53 , 9.12 ± 0.57 and 9.95 ± 0.34 mm) until when a peak was

reached at 7-dpv, however, the differences were not statistically significant ($p > 0.05$). The “takes” regressed from 8 to 10-dpv in an inverse trend with mean values of 9.31 ± 0.33 , 6.50 ± 0.35 , 5.08 ± 0.30 for group A, and 7.99 ± 0.41 , 5.62 ± 0.32 , 4.42 ± 0.31 for group B. However, the differences were equally not statistically significant ($p>0.05$) (**Fig. 1**).

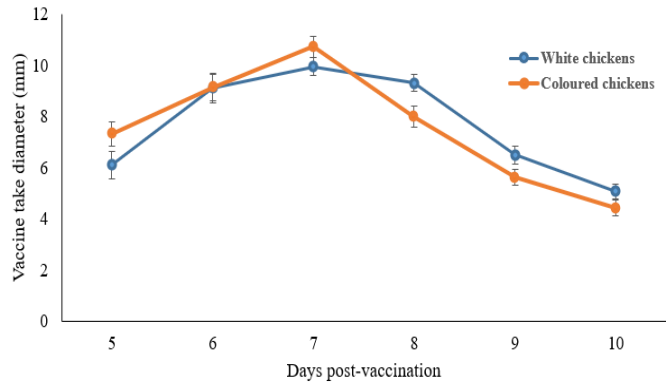


Fig.1: Mean vaccine “takes” diameters in the progeny of inbred Isa White chickens having different plumage colors vaccinated with live attenuated fowlpox virus vaccine at 8-weeks old.

Enzyme-Linked Immunosorbent Assay:

Pre- and post-vaccination, the FWPV antibody titers in group A (21.83 ± 3.32 ; 41.61 ± 6.16 ng/L) were lower ($p > 0.05$) compared to group B (26.89 ± 5.47 ; 53.25 ± 7.26 ng/L). All vaccinated chickens were protected, therefore, showed no clinical sign or lesion post-challenge. Interestingly, the antibody titer level in group B, 3-weeks post-challenge was slightly lower than that recorded in A; however, the difference was not statistically significant ($p > 0.05$) (**Fig. 2**). In unvaccinated-infected chickens, group D chickens had higher post-infection mean antibody titer (53.29 ± 12.41 ng/L) than group C (34.92 ± 7.54 ng/L). However, the difference was not statistically significant ($p > 0.05$) (**Fig. 3**).

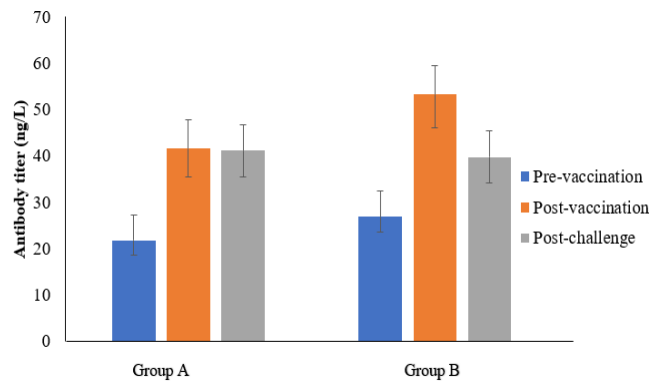


Fig. 2: Mean (± SEM) fowlpox virus antibody titers in the progeny of inbred Isa White chickens vaccinated at 8-weeks old and challenged with a field fowlpox virus isolate at 11-weeks old.

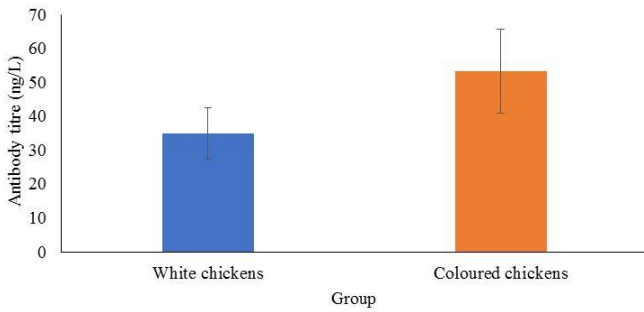


Fig. 3: Mean (\pm SEM) fowlpox virus antibody titer in the unvaccinated progeny of inbred Isa White chickens infected with a field fowlpox virus isolate at 11-weeks old.

Fowlpox Scab Lesions Count:

All group C chickens showed classical cutaneous fowlpox lesions particularly on the featherless skin of their head, beak, eyelids and wings. In contrast, two black chickens in group D had no lesions throughout the study. The highest number of discrete fowlpox lesions per individual bird recorded was eight as seen in group C, while the lowest was one as observed in both groups. Overall, the mean lesions count observed in group C (4 ± 0.562) chickens was significantly higher ($p < 0.05$) than that of group D (2 ± 0.345) (Fig. 4).

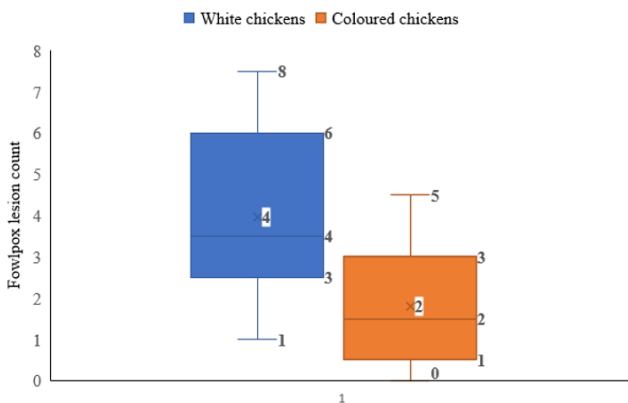


Fig. 4: Comparative fowlpox scab lesions count in the unvaccinated progeny of inbred Isa White chickens experimentally infected with a field fowlpox virus isolate at 11-weeks old.

DISCUSSION

The characteristic pock lesions observed when field FWPV isolates were propagated in CAMs of ECE in the present investigation correlate well with the findings of other research (Weli *et al.*, 2004; Haligur *et al.*, 2009; Khan, 2014; Masola *et al.*, 2014) which independently reported localized pock lesions (of

average size 3-6 mm in diameter) restricted to the dropped portion of CAMs in 9-12 days old ECE at first passage. While the enhanced cytopathology seen in CAMs during repeated passages of the field FWPV isolates signify increased ease of propagation, virulence and adaptation of the viruses in developing chicken embryos as previously reported (Gilhare *et al.*, 2015). Islam *et al.* (2008) documented that, albeit the development of pock lesions on infected CAM of ECE during FWPV propagation is a highly consistent finding, it is always unpredictable about the number of passages required for adaptation. In this study, the field FWPV isolate produced distinct pock lesions following three repeated passages. Hence, it can be a suitable candidate for developing new vaccines. High embryo mortality observed in the current trial contradicts Gilhare, (2012) who reported zero embryo mortality when isolating field FWPV. The difference might be due to variation in the virulence of field isolates involved.

The appearance of 100% vaccine "takes" in vaccinated chickens of the present study denotes a high level of protection against subsequent field challenge by virulent FWPV. This important finding correlates well with the 100% vaccine "takes" reported by Wambura and Mzula (2017) when using similar live attenuated FWPV vaccine (Biovac Vir 102TM, Biovac Ltd, Israel), but differs with the 90-95% "takes" observed by two other studies (Islam *et al.* 2008; El-Mahdy and Mikheal, 2014). The differences observed might be most likely due to variation in FWPV vaccine strains, immune status, age and breed of the chickens used. The success of vaccination programs against FWPV is usually indicated by the presence of a protective immune response in over 80% of vaccinated chickens (OIE, 2010; Ha *et al.*, 2013). Therefore, the presence of 100% vaccine "takes" reactions with absolute protection against pathogenic field FWPV isolate 3-weeks post-vaccination proved the potency of the FWPV vaccine used. Interestingly, vaccine "takes" might be a collection of lymphocytes (Siccardi, 1975) and possible circulating macrophage-derived immune mediators like interferons and serum nitrite; hence, consistently strong stimulation of these inflammatory reactions following FWPV vaccination as seen in chickens with white plumage implies high susceptibility with conceivable severe lesions development when exposed to a field FWPV isolate. This had vividly been underpinned by significantly higher lesion counts recorded in unvaccinated white chickens when infected with a virulent field FWPV isolate. Therefore, though the difference in the mean vaccine "takes" diameters between white and colored chickens were not statistically significant, the tide remains clinically relevant.

The relatively higher mean ELISA antibody titers observed in colored chickens pre- and post-vaccination might have been responsible for better protection against subsequent challenge with the field FWPV isolate. Conversely, the decreased FWPV antibody titer of the colored chickens at 3-weeks post-challenge could result from viral neutralization and better immunogenic response as compared to the white chickens that maintained relatively same titer despite exposure. Also, the higher antibody titer observed in the unvaccinated colored chickens posts experimental infection might imply a longer-lasting active immune response.

It is worth noting that genetically dissimilar birds have been reported to have different susceptibility rates to viral infections (**Thiruvankandan et al., 2011**). For instance, it has been documented that differences in genetic background influenced the induction of innate and acquired immune responses in chicken lines, hence their susceptibility to virulent infectious bursal disease virus (vIBDV). Likewise, when genetically different chickens were challenged with vIBDV, White-leghorn chicks showed the most severe clinical disease, higher mortality rate, higher bursal lesion scores and IBDV-antigen load relative to other infected chicks (**Bumstead et al., 1993; Aricibasi et al., 2009**). Furthermore, interferon genes, particularly IFN- α and IFN- β , have been reported to confer resistance to birds against fowlpox among several other diseases (**Thiruvankandan et al., 2011; Biobaku and Ami, 2018**). Therefore, chickens that do not express the above unique genes or mutations to the genes would lead to marked susceptibility to FWPV.

Moreover, the mean antibody titers recorded at different stages of this study using an indirect ELISA were relatively higher than some published data (**Wang et al., 2006; Ohore et al., 2007; Stuke et al., 2017**). The discrepancy could be attributed to the different FWPV vaccine strains, vaccination method, virulence of the challenge viruses, age and breeds of the birds used, and sensitivity of the detection and quantification techniques employed. The assertion that age might have influenced the relative increase in FWPV antibody titers observed is in line with other parallel studies (**Siddiky et al., 2004; Wang et al., 2006; Wambura and Mzula, 2017**) which established higher geometric mean titers for chickens vaccinated with live attenuated fowlpox vaccines at 6 weeks-old than those vaccinated at day-old. In addition, of the detection techniques used, ELISAs have generally been reported to detect humoral FWPV antibodies much earlier, faster and easier than the commonly employed agar gel immunodiffusion test (AGID) or passive hemagglutination test (PHA) following vaccination or infection (**Buscaglia et al., 1985; Mockett et al., 1987; Pathak, 2016**).

The absence of fowlpox lesions observed in the two colored (precisely black) chickens post-infection could be attributed to a possible enhanced cell-mediated immune response (T-cell responses and cytokine gene expression) to control the fowlpox viral replication faster before the onset of lesions. This owes to the fact that cell-mediated immunity which develops earlier than the humoral immune response (**Tripathy and Reed, 2020**) is primarily responsible for recovery from FWPV infection (**Morita, 1973; Gerlach, 1994**).

CONCLUSION

Evaluation of humoral immune response, vaccine “takes” and fowlpox lesion counts provided circumstantial evidence that variation in plumage color influenced the susceptibility vis-a-vis resistance of chickens to fowlpox. This has been well demonstrated by the inferior immune response to vaccination and significantly higher fowlpox lesions post experimental infection seen in progenies of inbred Isa White chickens having white plumage. However, extensive research on genetics, cell-mediated immune responses, and other factors (such as the preference of mechanical vectors to birds) that might influence susceptibility to FWPV in chickens should be carried out.

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Author's contribution

BN. Umar performed the experiments and drafted the manuscript; J. Adamu conceived, designed, and participated fully in the study; while H.M. Kazeem revised the article critically and approved its intellectual content, all authors reviewed and approved the final version of the manuscript.

Declaration of Conflicting Interests

The authors declare that there is no conflict of interest.

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