

## Comparative Study on Diphtheritic, Cutaneous and Systemic Forms of Natural Avipoxvirus Infection in Chickens

Olatunde Babatunde Akanbi<sup>1,2\*</sup>, Amos Gambo Rimfa<sup>1</sup> and Philip Ademola Okewole<sup>1</sup>

<sup>1</sup>Central Diagnostic Laboratory, National Veterinary Research Institute, Vom, Nigeria

<sup>2</sup>Department of Veterinary Pathology, University of Ilorin, Ilorin, Nigeria

\*Corresponding author's Email: Olatunde\_akanbi@yahoo.co.uk

Received: 21 Jul. 2016

Accepted: 22 Aug. 2016

### ABSTRACT

Avipoxvirus of the subfamily Chordopoxvirinae is known to cause fowl pox infection in chickens. The disease manifest as Cutaneous, diphtheritic, systemic and oncogenic forms in birds. The former two being the most frequent forms of the infection and occurring in chickens. Twelve cases of fowl pox virus infection in chicken flocks over a 5-year period were reviewed to describe the pathologies and the forms of pox virus infection observed in Bauchi and Plateau States in Nigeria. Three forms (cutaneous, diphtheritic and systemic) of fowl pox virus infection were investigated in indigenous and commercial backyard chicken breeds at different ages and with infection during different period of the year using gross- and histo-pathological features. Our findings showed that the cutaneous form was most common in Bauchi and Plateau States in north-eastern and north-central Nigeria respectively. Rather than the mixed Cutaneous and diphtheritic form previously reported, we observed a new co-occurrence of a mixed Cutaneous and systemic form of fowl pox virus infection in a young cockerel chicken. Also, there seems to be no seasonal variation in the occurrence of fowl pox virus infections in the chicken flocks in the study area, a notion responsible for fowl pox virus vaccine demands in the country. Therefore, present study suggest a routine fowl pox vaccination program for susceptible chicken flocks as all the flocks reported in this study had a history of unvaccinated status with the exception of the indigenous chicken which is rarely vaccinated against any infectious or contagious disease in the country.

**Key words:** Comparative, Fowl pox virus, Infection, Chicken

### INTRODUCTION

Fowl pox virus infections in chickens are caused by avipoxvirus belonging to the subfamily chordopoxvirinae in the family poxviridae affecting a wide range of vertebrate hosts (Quinn et al., 2011, Lawson et al., 2012, Meseko et al., 2012 and Bwala et al., 2015). The avipoxvirus genus contain fowl pox virus infecting fowls, turkey pox virus infecting turkeys and pigeon pox virus in pigeons which are closely related and are not strictly host-specific (Quinn et al., 2011). Avian pox disease affects both domestic and free living birds in nature which results in varying morbidity and mortality (Afonso et al., 2000). Avian pox virus infection is said to be characterized by Cutaneous (dry pox), diphtheritic (wet pox), systemic and oncogenic manifestation (Tsai et al., 1997, Lawson et al., 2012). Although only the cutaneous and diphtheritic forms have been documented in chickens to be caused by fowl pox virus (Tripathy and Reed, 2008). The systemic form has been reported in other avian species (Tripathy and Reed, 2008). Cutaneous form of pox in chicken is characterized by local epithelial hyperplasia that

includes epidermis and underlying feather follicles (Tripathy and Reed, 2008), resulting in the formation of nodules, papules, vesicles and eventual formation of scabs (Tripathy and Reed, 2008). The diphtheritic form is reported to be more severe, causing significant mortality and economic losses in affected flocks (Singh et al., 2003), and it is characterized by the formation of white opaque nodules or yellowish patches which develop on the mucous membranes of the oral cavity, tongue, oesophagus or upper trachea (Tripathy and Reed, 2008). Nodules rapidly increase in size and often coalesce to become a yellow, cheesy, necrotic, pseudo diphtheritic, or diphtheritic membrane. A mixed cutaneous and diphtheritic forms are said to be common with development of lesions on the comb and wattles as well as diphtheritic lesions in the mouth and/or respiratory tract of the same bird. The systemic form of avian pox virus infection has been documented, whereby the liver had single to multiple soft white to yellow nodules ranging in size from 0.2- 0.5cm in diameter (Tripathy and Reed, 2008). In chickens, fowl

pox is said to affect all ages, all sexes and all breeds (Weli and Tryland, 2011). Fowl pox infection is a slowly spreading disease and an economically important disease of chickens and turkeys as it can cause egg production losses and even mortality, especially in commercial poultry (Tripathy and Reed, 2003). The disease is mostly seen in poultry kept in free-range holdings (Bwala et al., 2015) in South Africa, although it is reported to be widespread in backyard and intensively reared poultry flocks in Nigeria (Adene and Fatumbi, 2004). The disease is reported to be spread by biting arthropods, which included mosquitoes and mites (Proctor and Owens, 2000), and through infective aerosols, contaminated feed or water, and skin trauma resulting from pecking by other birds (Bwala et al., 2015). The aim of this work is to report a mixed cutaneous and systemic form of fowl pox infection observed at the Nigerian National Veterinary Research Institute diagnostic laboratory.

## MATERIALS AND METHODS

Over a 5-years period, 2011 to 2015, thirty-nine unvaccinated mixed sex backyard, commercial and indigenous chickens of different breeds and varying ages from 12 suspected cases of fowl pox infection (Table 1) presented to the Central Diagnostic Laboratory of the National Veterinary Research Institute, Vom Nigeria were diagnosed by gross- and histo-pathology. Carcasses of the affected chickens were necropsied and the tissues including lung, trachea, oral mucosa, liver, facial and nasal scabs were removed and fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 µm, mounted on clean glass slides, and stained with Hematoxylin and Eosin (H&E) stains for histopathological examination using low and high powered field of Carl Zeiss® binocular microscope.

### Ethical Approval

This study was evaluated and followed the ethical guidelines of the Ethics Committee of the National Veterinary Research Institute, Vom, Nigeria.

## RESULTS

### Cases

Ten of the analyzed cases exhibited the cutaneous form of fowl pox virus infection, while in one case, both the Cutaneous and systemic forms were seen and the remaining one case showed the diphtheritic form (Table 1). The cutaneous form of the disease was found to be the most common, and affected all types (indigenous and commercial) and breed (pullet, broiler, cockerel and layers) of chicken examined cutting across

all ages from 9-52 weeks old chickens. The cutaneous form also occurred at both the dry (October to January) and during the raining season (June-September) in the study area in Plateau and Bauchi states of Nigeria. The systemic form, which co-occurred with the cutaneous form of fowl pox, occurred in a young, 9 weeks old cockerel chicken and during the dry season of the year. The only case of the diphtheritic form occurred in 2 years old indigenous laying hen during the hamattan season of December.

### Gross and histopathology

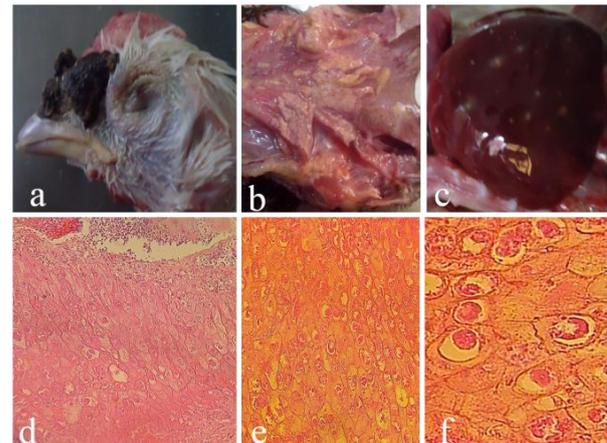
The cutaneous form of fowl pox infection in the majority of the cases in chickens are frequently characterized by pale to yellow, often times discolored combs with multifocal 0.05-0.5 cm in diameter nodule formation on the combs, face, peri-orbital and ocular areas, as previously described. Occasionally, the lesion is characterized by papule formation which thickens and coalesced to form large dark brown scabs which often occludes the nares (Figure 1a). The only case that exhibited the diphtheritic form (Figure 1b), occurred in an adult indigenous laying hen. In this hen, there was an extensive raised yellowish patchy, necrotic, diphtheritic membrane which covers the mucous membranes of the oro-pharynx and the proximal 1/8<sup>th</sup> of the trachea. In one of the cases that showed the cutaneous form of fowl pox infection, a systemic form was also observed, wherein the liver had tiny multifocal soft white to yellow nodules ranging in size from 0.05-0.1cm in diameter (Figure 1c). At histopathology, a 9 weeks old pullet with large facial and nasal scabs (Figure 1a) showed hyperplasia of the stratified squamous cells exhibiting acanthosis with severe inflammatory exudation of heterophils, macrophages and lymphocytes (Figure 1c) and often times with haemorrhagic ulcers. Also, there were several small to medium sized eosinophilic intracytoplasmic and intra-keratinocytic inclusions, identified as Bollinger bodies (Figure 1d) which at higher magnification, are central to eccentric eosinophilic intracytoplasmic bollinger bodies typical of fowl pox infection (Figure 1e).

## DISCUSSION

During the study period, 2011-2015 only 12 cases were reported for the purpose of diagnosis at the Central Diagnostic Laboratory of the National Veterinary Research Institute, Vom Nigeria. The cases involved 39 chickens cutting across different ages, sexes and breeds as previously been observed in fowl pox infections (Weli and Tryland, 2011), either in poultry kept in free-range holdings (Bwala et al., 2015) or in backyard (Akanbi et al., 2015) and to some extent commercially reared poultry flocks (Adene and

Fatumbi, 2004). With eleven occurrences of these 12 cases (91%) in this study, the cutaneous form of fowl pox disease was found to be the most common as have been reported elsewhere (Lawson et al., 2012), and affected all types (indigenous and commercial) and breeds (pullet, broiler, cockerel and layers) of chickens. Only one case of the diphtheritic form was observed in this study and rather than a mixed cutaneous and diphtheritic form said to be common (Tripathy and Reed, 2008), only a mixed cutaneous and systemic form was observed in a young, 9 weeks old cockerel chicken (8%). The systemic form does not seem to have been documented in domestic chicken as far as our literature search was concerned. But we found this form to co-occur in this cockerel. The only diphtheritic form seen in this study occurred in a 2 years old indigenous laying hen. It is not clear whether, this case was as a result of virus reactivation due to stress or immunosuppression (Lawson et al., 2012) as there was a history of previous infection with pox virus in the flock. The pathology of the cutaneous and diphtheritic forms of the disease in these chickens are consistent with earlier findings (Tripathy and Reed, 2008), although our pathological findings in the systemic form in chicken vary slightly, as multifocal soft white to yellow nodules similar to the findings in the Andean condor (Tripathy and Reed, 2008) was only seen on the liver of the mixed cutaneous and systemic case. The only case of the diphtheritic form that occurred in the indigenous hen was severe and fatal, consistent with previous report (Singh et al., 2003). It was hypothesized that the poxvirus infection or its sequelae was the main contributory cause of death in these cases as no other pathology was observed. As it has been reported previously (Lawson et al., 2012) that susceptibility to Avipoxvirus infection varies among host species and in relation to host age with juveniles being most susceptible, the pox viral infection in this study were characterized by morbidity and severe mortality in mainly young birds and cocks. Therefore the effect of the disease on egg production was not observed as earlier reported in commercial poultry (Tripathy and Reed, 2003). As observed in this study, there seems to be no seasonal variation in the occurrence of fowl pox virus infections in the chicken flocks in this study over the 5-years period as the disease was recorded during the wet (Jun-Sept) and dry (Oct-Jan) seasons of the year. The notion of seasonal variation in the infection patterns of fowl pox has been responsible for fowl pox virus vaccine demands in Nigeria (O. Asala [Viral vaccine production, National Veterinary Research Institute, Vom, Nigeria], pers. comm., 18 January 2016). This may also be responsible for the unvaccinated status of all the cases in this study with the exception of the indigenous chickens which are

rarely vaccinated against any infectious or contagious disease. In view of our findings, we therefore suggest a routine fowl pox vaccination program for susceptible chicken flocks



**Figure 1.** **a:** chicken, 9 weeks old pullet with large facial and nasal scabs; **b:** 2 years old indigenous layer chicken with large oro-pharyngeal and tracheal diphtheritic membrane; **c:** chicken, 9 weeks old pullet with multifocal tiny, up to 0.05cm in diameter nodules on the liver; **d:** scab tissue, hyperplasia of stratified squamous cells exhibiting acanthosis and hetero-lymphocytic infiltration X 10, H&E stain; **e:** facial tissue, showing small to medium sized eosinophilic intrakeratinocytic inclusions (Bollinger bodies) X10 H&E stain; **f:** higher magnification of eosinophilic intrakeratinocytic inclusions (Bollinger bodies) typical of fowl pox infection X40, H&E stain.

**Table 1.** Cases of different forms of fowl pox virus infection in unvaccinated mixed sex commercial and indigenous chickens in Bauchi and Plateau States of Nigeria during 2011-2015

Cases	Age in weeks (wks)/ sex	Onset	No. of Samples	Pox form
Jos 1	9 wks/ pullet	Jan	1 Carcass	Cutaneous
Toro 1	9 wks/ cockerel	Sept	7 Carcasses	Cutaneous
Toro 2	9 wks/ cockerel	Sept	5 Live	Cutaneous/ Systemic
Jos 2	9 wks/ pullet	Oct	6 Carcasses	Cutaneous
Jos 3	9 wks/ pullet	Oct	13 Carcasses	Cutaneous
Jos 4	22 wks/ layer	June	1 Carcass	Cutaneous
Jos 5	52 wks /indigenous layer	July	1 Carcass	Cutaneous
Jos 6	Adult/ indigenous cock	July	1 Carcass	Cutaneous
Jos 7	9 wks broiler	Sept	1 Live	Cutaneous
Jos 8	17 wks/ cockerel	Nov	1 Carcass	Cutaneous
Jos 9	46 wks/ layer	June	1 Carcasses	Cutaneous
Langtang 1	104 wks/ indigenous hen	Dec	1 Carcass	Diphtheritic

## CONCLUSION

It is concluded that routine vaccination of chicken flocks be instituted, especially small-holder flocks to prevent the losses due to morbidity and mortality associated with fowl pox infection.

### Acknowledgements

We thank Sunday Atam, Andrew Zal, Gyang Dung, Timothy Davou for assistance rendered during the documentation of the cases and Pwajok Tobias, Chris and Mrs Schuparis for the processing of the tissues for microscopy.

### Competing interests

The authors declare that there are no significant personnel, professional or financial competing interest that might have influenced the presentation of the results of the study described in this manuscript.

## REFERENCES

- Adene DF and Fatumbi OO (2004). Case Review and Lesions on Poultry Disease Control in South West Nigeria. Poultry Health and Production: Principles and Practice, StirlingHorde Publishers, Oyo, Nigeria.
- Afonso CL, Tulman ER, Lu Z, Zsak L, Kutish GF and Rock DL (2000). The genome of fowlpox virus. *Journal of Virology*, 74: 3815–3831.
- Akanbi OB, Odita CI, Okewole PA, Bot CJ, Obalisa A, Pam EG, John DN, Shallmizhili J, Ijale GO and Alim B. (2015). Clinical and Pathological Investigation on Turkey Diseases in North-central City of Jos, Nigeria, 2009-2014. *J. World's Poult. Res.* 5(4): 115-121.
- Bwala DG, Fasina FO and Duncan NM (2015). Avian poxvirus in a free-range juvenile speckled (rock) pigeon (*Columba guinea*)', *Journal of the South African Veterinary Association*, 86:1, Art #1259.
- Lawson B, Lachish S, Colvile KM, Durrant C, Peck KM, et al. (2012). Emergence of a Novel Avian Pox Disease in British Tit Species. *PLoS ONE* 7(11): e40176. doi:10.1371/journal.pone.0040176
- Meseko CA, Shittu IA and Akinyede O (2012). Seroprevalence of Fowl Pox Antibody in Indigenous Chickens in Jos North and South Council Areas of Plateau State, Nigeria: Implication for Vector Vaccine. *International Scholarly Research Network Article ID 154971*.
- Proctor H and Owens I (2000). Mites and birds: Diversity, parasitism, and co-evolution, *Trends in Ecology and Evolution*, 15:358–364.
- Quinn PJ, Markey BK, Leonard FC, FitzPatrick ES, Fanning S and Hartigan PJ (2011). "Poxviridae" in *Veterinary Microbiology and Microbial Disease*, pp. 593-602.
- Singh P, Kim TJ and Tripathy DN (2003). Identification and characterization of fowlpox virus strains using monoclonal antibodies. *Journal of Veterinary Diagnostic Investigation*, 15: 50–54.
- Tripathy DN and Reed WM (2008). Pox, in *Diseases of Poultry*, Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK, Swayne DE. Eds., pp. 291–307, Blackwell Publishing, Oxford UK 12th edition, 2008.
- Tsai SS, Chang TC, Yang SF, Chi YC, Cher RS, Chien MS and Itakura C (1997). Unusual lesion associated with avian pox virus infection in rose-faced love birds (*Agapornis roseicollis*). *Avian Pathology*, 26: 75-82.
- Weli SC and Tryland M (2011). Avipoxviruses infection biology and their use as vaccine vectors. *Virology Journal*, 8: doi:10.1186/1743-422X-8-49.