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SHORT COMMUNICATION

Fowlpox Virus from Backyard Poultry in Plateau State Nigeria: Isolation and Phylogeny of the P4b Gene Compared to a Vaccine Strain

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SUMMARY

Fowlpox is a viral infection that cause nodular and diphtheritic lesions on the skin and mucous membrane of the digestive and respiratory tracts of birds. It is a disease of economic importance in domestic and wild birds especially in tropical and subtropical countries. Despite its endemicity, there is limited literature on the genetic diversity of field virus circulating in Nigeria. In this study, cutaneous tissue samples were aseptically collected from suspected poultry flocks through passive surveillance conducted in Plateau state Nigeria from 2009 to 2013. Fowlpox virus was isolated in chorion allantoic membrane of embryonated chicken eggs and identified with Agar Gel Immuno Diffusion test using homologous reference antigen and antisera. Genomic DNA amplification and cycle sequencing of the P4b gene locus of seven selected field isolates along with a local vaccine strain was done and analysed alongside published sequences of FPV P4b gene using MEGA 5 software. Nucleotide analysis of our isolates and a vaccine strain showed 100% similarity and also shared 72 – 100% homology with selected sequences from the GenBank while clustering on the phylogenetic tree in clade A, subclade A1. This study represents the first available fowl poxvirus sequences (KP987207-KP987214) from the West and Central Africa regions in the GenBank. Full genome sequences and comparative molecular analysis of circulating field and vaccine strain is critical for the design and implementation of target oriented control programme.

Key words: Fowlpox, Isolation, Nigeria, Phylogeny, Virus.

INTRODUCTION

Fowlpox is a viral infection that cause nodular and diphtheritic lesions on the skin and mucous membrane of the digestive and respiratory tracts of birds with the proliferative lesions mainly on unfeathered areas of the skin (Woodruff and Goodpasture, 1931). It is a disease of economic importance listed by the World Animal Health Organization. It is common in domestic and wild birds especially in tropical and subtropical countries (Adene and Fatumbi, 2004; OIE, 2016). Fowlpox has been controlled in developed countries. However, it is still a problem in most developing countries largely due to poor hygiene, contaminated instruments/ surfaces, and vector transmission by mosquitoes and other flies (Tripathy and Ree, 2008). The Avianpox viruses shows considerable divergence phylogenetically and are categorized into three major groups viz Fowlpox or Fowlpox-like virus; Canary or Canarypox-like virus, and the psittacinepox viruses (Jarmin *et al*., 2006). The prototype Fowlpox virus causes disease in chickens and turkey globally resulting in mortality and production losses (Hess *et al*., 2011).

Though fowlpox is widespread, in Nigeria, it is more common in free range and backyard poultry flocks, affecting mainly chickens and turkeys (Adene *et al.*, 2004; Meseko *et al*., 2012). Despite its endemicity there is limited literature on the genetic diversity of field virus circulating in the country. While vaccines are effective and are routinely used to reduce morbidity and mortality, yet there are evidence of infections in some vaccinated flocks (Odoya *et al*., 2006). This study was therefore designed to isolate, and identify the relationship of circulating field virus through the phylogenetic analysis of the P4b gene.

MATERIALS AND METHODS

Cutaneous tissue samples were aseptically collected through passive surveillance from suspected poultry flocks from 2009 to 2013 in Jos north, Jos south and Bassa local government areas of Plateau state, Nigeria. The samples were stored in ultralow refrigerator (-80^{o}C) until processed. Virus isolation was carried out according to standard procedures previously described by Odoya *et al*., 2010; and OIE, (2016). Pox lesions and scarified tissues were homogenised in a combination of antibiotics solution and clarified under cold and slow centrifugation at 1000 -1500 rpm for 10 to 15 minutes. Thereafter, 0.2ml of 20% tissue suspensions was inoculated onto chorio-allantoic membranes (CAMs) of 9-12-day-old chicken embryo from specific antibody negative flock. Inoculated eggs were incubated for 5-7 days at 37^{0}C in a humidified incubator and examined daily for embryo viability or deaths. At the end of incubation, the eggs were examined for focal proliferative, white pock lesions or generalized thickening of the CAMs before they were harvested and held at -80^{o}C for thirty minutes for each cycle of three freeze-thawing. Virus identification was carried out with Agar Gel Immuno Diffusion (AGID) test using homologous reference antigen and antisera from Charles River Laboratory, (USA) as previously described by Meseko *et al*., (2012).

Genomic DNA amplification and sequencing of the P4b gene of the isolates was carried out based on the P4b locus described by Jarmin *et al*., (2006). Briefly, Viral DNA was extracted from CAMs homogenate using DNA extraction kits (QIAGEN, Germany) according to manufacturer's instruction. PCR gene amplification targeting the 578bp P4b gene fragment was carried out according to Manarolla *et al.*, (2009) with slight modifications. A 25µl PCR reaction buffer

containing 1.5 mM of $MgCl_2$, 12 µl dNTPs, 6 pmol of each primers, 200 uM of each dNTPs and 1.25 U Taq polymerase was constituted. The primers were based on P4b sequence of fowlpox virus strain HP444 (Forward primer: 5'-CAGCAGGTGCTAAACAACAA-3': reverse primer: 5'-CGGTAGCTTAACGCCGAATA-3') as previously described by Lee and Lee (1997) and also used by Manarolla et al., 2010. An initial denaturing step of 94^0C for 2 minutes was followed by 35 amplification cycles consisting of annealing at 60 ^{0}C for 1 minute, and 72 ^{0}C for 1 minute and final extension at72 ^{0}C for 2 minutes. The amplicons obtained were thereafter separated by gel electrophoresis using 2% Agarose gel, stained with ethidium bromide. Trans-illuminator visualization of expected band size of 578bp was carried out and results documented.

Cycle sequencing of the Pb4 gene locus of seven selected field isolates along with a local vaccine strain was carried out using the two primers directly after purification by a commercial sequencing facility (Inqaba Biotech, Pretoria, South Africa). Analysis of the sequences generated was performed along with published sequences of FPV P4b gene obtained from the GenBank with MEGA 5 software (Tamura *et al*., 2011).

RESULTS AND DISCUSSION

Over the study period between 2009 and 2013, only fifty pox lesions collected from adult chickens by passive surveillance were tested by CAM inoculation out of which fifteen showed lesions on CAM and were confirmed by AGID. However, only seven were shipped for PCR and gene sequencing. Two of the isolates on CAM (NGA-VRD090273 and NGAFPV-13-03) were from farms with a history of vaccination. Nucleotide sequencing analysis of the P4b gene of isolates and a vaccine virus strain (Baudette) showed 100% nucleotide identity. The isolates also shared 72 – 100% homology with selected sequences from the GenBank. Furthermore, isolates from Nigeria clustered on the phylogenetic tree in clade A, subclade A1 with other fowlpox virus from Europe, Asia, America and other parts of Africa (Figure I). All sequences obtained in this study were deposited in the GenBank database under accessions KP987207 – KP987214 (Table I). The P4b is a conserved region of the fowl pox genome, which is less likely to undergo frequent mutation, and this may explain the high degree of homology observed and in the clustering on the phylogenetic tree. Our study is similar to observation in a turkey flock in Brazil by Kunert – Filho et al, (2016) where all four APV P4b fragment sequences showed 100% homology. This may infer that only a single or similar

TABLE I: Recent field isolates of fowlpox virus from Plateau State Nigeria with sequences deposited in the GenBank according to accession numbers

S/N	LGA	Year	Sample Identity	GenBank Accession No
1	Jos North	2009	NGA_VRD09_172	KP987207
2	+Jos North	2009	NGA_VRD09_273	KP987209
3	Jos South	2009	NGA_VRD09_477	KP9872010
4	Jos South	2013	NGA_FPV_13_01	KP9872011
5	Jos South	2013	NGA_FPV_13_02	KP9872012
6	+Bassa	2013	NGA_FPV_13_03	KP9872013
7	Jos South	2013	NGA_FPV_13_04	KP9872014
8	Vom FPVvac	2012	NGA_FPVV_03_12	KP987208

+ Isolates from vaccinated flock; FPV vac = Fowl pox vaccine

Recent field isolates of fowlpox virus from Plateau State Nigeria with sequences

deposited in the GenBank according to accession numbers

Avipox strain is responsible for the observed clinical cases. Our study also corroborates similar observations by Lee and Lee, (1997) and Manarolla et al, (2010) that the PCR amplification of the P4b gene is a valuable diagnostic method for fowlpox infection.

Only few FPV sequences from Africa are available in the GenBank and none from West and Central Africa until this study. Offerman *et al.*, (2013) reported that Isolates circulating in South Africa belong to subclade A2 and A3, which are different from the isolates in our study (Figure I). The limited number of isolates sequenced in this study necessitate further investigation to identify more fowlpox sequences in other West and Central Africa countries to better understand the molecular epidemiology of the virus in these regions.

The fowlpox isolates from Nigeria all clustered on the phylogenetic tree with the baudette vaccine strain (NGA-FPVV-03-2012) obtained from Kabete in Kenya by the National Veterinary Research Institute in the 1970s. It is now over 40 years since that strain had been in use for fowlpox vaccine production in Nigeria without much modification. Genomic and antigenic variation in circulating field and vaccine strains could lower vaccine efficacy requiring close monitoring especially where outbreaks in vaccinated flocks are observed as reported by Singh *et al*., (2000) and Odoya *et al*., (2006). This study represents the first available fowl poxvirus sequences (KP987207-KP987214) from the West and Central Africa regions. Full genome sequences and comparative molecular analysis of circulating field and vaccine strain is critical for the design and implementation of target oriented control programme.

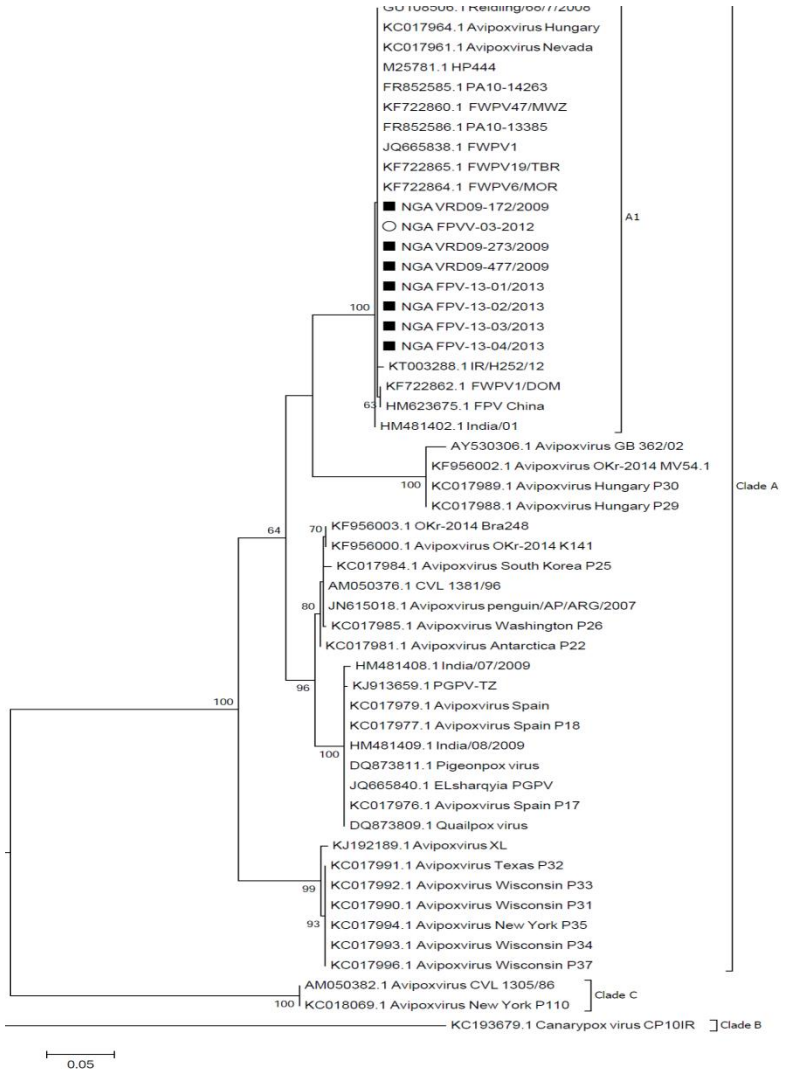

Figure I: Phylogenetic tree of fowlpox P4b gene of isolates from Nigeria with representatives of clade A, B, and C retrieved from the GenBank. Maximum Likelihood tree reconstruction was performed using MEGA 5. Scale bar represent substitution per site

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REFERENCES

ADENE, D. F. and FATUMBI, O. (2004). Case Review and Lesson on Poultry Diseases Control in South West Nigeria. In Poultry Health and Production, Principles and Practice. Stirling Horden Publishers (Nig) Ltd, Ibadan, Oyo State, Nigeria. 191-200.

HESS, C., MAEGDEFRAU-POLLAN, B., BILIC, I., LIEBHART, D., RICHTER, S., MITSCH, P. and HESS, M. (2011). Outbreak of cutaneous form of poxvirus on a commercial turkey farm caused by the species fowlpox. Avian Dis. 55: 714–718.

KUNERT-FILHO, H.C., CIBULSKI, S.P., FINKLER, F., GRASSOTTI, T.T., JAENISCH, F.R.F., DE BRITO, K.C.T., CARVALHO, LOVATO, M. and DE BRITO, B.G. (2016). First phylogenetic analysis of Avipox (APV) in Brazil. Pesq. Vet. Bras. 36 (5): 357-362.

LEE L.H. and LEE. K.H. (1997). Application of the polymerase chain reaction for the diagnosis of fowl poxvirus infection.. J. Virol. Methods 63, 113–119.

JARMIN, S., MANVELL, R., GOUGH, R.E., LAIDLAW, S.M., and SKINNER, M.A. (2006). Avipoxvirus phylogenetics: identification of a PCR length polymorphism that discriminates between the two major clades. J. Gen. Virol. 87: 2191–2201.

MANAROLLA, G., PISONI, G., SIRONI, G. and RAMPIN, T. (2010). Molecular biological characterization of avian poxvirus strains isolated from different avian species. Vet Microbiol. 140:1-8.

MESEKO, C. A., SHITTU, I.A. 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. ISRN Vet. Sci. doi: 10.5402/2012/154971:1–4.

ODOYA, E. M., ABEGUNDE, A., OMOTAINSE, S.O., GWANKAT, E. and OKPARA. U.G. (2006). Outbreak of turkey pox disease in fowl pox vaccinated poults in Vom Plateau State of Nigeria. African J. Clin. Exp. Microbiol. 7: 136–138.

OFFERMAN, K., CARULEI, O., GOUS, T.A., DOUGLASS, N. and WILLIAMSON A.L. (2013). Phylogenetic and histological variation in avipoxviruses isolated in South Africa. J. Gen. Virol. 94: 2338–2351.

OIE. Fowl pox. In: Manual of Diagnostc Tests and Vaccines for Terrestrial Animals. 2016.http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.03.10_FOWLPOX.pdf.

SINGH, P., KIM, T.J. and TRIPATHY, D. (2000). Re-emerging fowlpox: evaluation of isolates from vaccinated flocks. Avian Pathol. 29: 449–455.

TAMURA, K., PETERSON, D., PETERSON, N., STECHER, G., NEI, M. and KUMAR, S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol. Biol. Evol. 28: 2731–2739.

TRIPATHY, D. N. and REE, W.M. (2008). Pox, In: Saif, Y.M., Fadly, A.M., Glisson, J.R., McDougald, L.R., Nolan, L.K., Swayne, D.E. (Eds), Diseases of Poultry, twelveth ed Iowa State University Press, Ames, IA: 291–307.

WOODRUFF, A. M. and GOODPASTURE, E.W. (1931). The Susceptibility of the Chorio-Allantoic Membrane of Chick Embryos to Infection with the Fowl-Pox Virus. Am. J. Pathol. 7: 209–222.