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https://dx.doi.org/10.4314/nvj.v39i2.6 **SHORT COMMUNICATION**

Continuous Circulation and Mutation at the Hypervariable Region of The VP2 Gene of very Virulent Infectious Bursal Disease Virus in Nigeria

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SUMMARY
Infectious bursal disease (IBD) is a highly contagious viral disease of poultry with huge economic consequences. First reported in Nigeria in 1973, the disease has since become endemic across the country. Inspite of routine vaccine administration, outbreaks are frequently reported in vaccinated flocks which questions the effectiveness of the vaccine to confer adequate protection against the circulating strains of the virus. In this study, suspected IBD outbreaks from 9 poultry farms spanning 2015 – 2016 were investigated. The hypervariable region (HVR) of the VP2 gene of infectious bursal disease virus (IBDV) from these outbreaks was sequenced. Based on the phylogenetic tree and deduced amino acid sequences, the viruses clustered with African very virulent (vv) types (VV2-1) with markers typical for vvIBDV at positions 222A, 242I, 256I, 294I and 299S. Also, unique substitutions were identified at the hydrophilic peak A (215Q→P) and minor peak 2 (280N→Y) in addition to that previously unreported at position 308I→L. These results demonstrate continuous circulation and mutations at the HVR of the VP2 which underscores the need for constant monitoring of IBDV in Nigerian poultry. Further study is required to establish the biological function(s) of these mutations on the pathogenicity and antigenicity of the virus.

Key words: very virulent infectious bursal disease virus, VP2 gene, mutation, poultry, Nigeria.

INTRODUCTION
Since its discovery by Cosgrove (1962), infectious bursal disease (IBD), caused by infectious bursal disease virus (IBDV), has continued to plague the poultry industry worldwide. The disease is acute, highly contagious and affects chickens aged 3-6 weeks, causing severe immunosuppression in infected chickens below 3 weeks of age (Chakman, 2015). Though, evidences of outbreaks in older chickens have been documented (Okoye and Uzoukwu, 1981; Shekaro and Josiah, 2015). The organ in chickens targeted by IBDV is the bursa of Fabricius where it causes a depletion of the B-lymphocytes (Silva *et al.,* 2016). The causative agent is a member of the genus *Avibirnavirus* of the family *Birnaviridae* (Delmas *et al.,* 2012). In nature, only two

serotypes of IBDV are known thus far. The first, serotype 1 is pathogenic in chicken while the second, serotype 2 is apathogenic in chickens (Lasher and Shane, 1994). Infectious bursal disease virus (IBDV) is a non-enveloped, icosahedral bi-segmented RNA virus, with the larger genome segment A (3.3kb) containing two overlapping open reading frames (ORF). A nonstructural protein, VP5, is encoded by the first ORF, while the second ORF encodes a polyprotein that is cleaved proteolytically to produce the viral capsid protein (VP2), ribonucleoprotein (VP3) and viral protease (VP4). Though smaller in size (2.8kb), the second genome segment B encodes a single ORF VP1, an RNA-dependent RNA polymerase (van den Berg, 2000).

Since it was first described in Nigeria in 1973 (Ojo *et al.,* 1973), IBD has ravaged and still continues to wreck havoc on the poultry industry in Nigeria, which has made it achieve an endemic status. There have been several reports of outbreaks of IBD in both vaccinated and unvaccinated poultry chickens in Nigeria (Adamu *et al.,* 2013; Luka *et al.,* 2014; Owolodun *et al.,* 2015; Nwagbo *et al.,* 2016). Previous and recent studies have implicated very virulent (vv) strains of IBD as being responsible for these outbreaks (Owoade *et al.,* 2004; Adamu *et al.,* 2013; Luka *et al.,* 2014; Owolodun *et al.,* 2015; Nwagbo *et al.,* 2016). In addition, a very recent study also implicated reassortant IBDV strains as contributing to the failure of IBD vaccine in protecting chickens against field IBDV infection in Nigeria (Nwagbo *et al.,* 2016). The frequent and relentless outbreaks of IBD, especially in vaccinated poultry flocks have made it mandatory for regular epidemiological studies to be carried out in order to keep track of any genetic changes/variations that may occur in the field. This will enhance the efforts in controlling IBD in Nigeria and also assist in the selection of the right vaccine strain that will be effective in combating IBD outbreaks in the field. In this study, 9 field IBDV isolates obtained from commercial broiler and pullet poultry farms from two states in Nigeria covering 2015 to 2016 were characterized to ascertain the strain(s) responsible for the outbreaks and also to determine if there are any genetic changes or variations.

MATERIAL AND METHODS

At postmortem, a total of 9 bursae of Fabricius were collected from dead chickens from IBD suspected cases submitted to the Diagnostic division of the National Veterinary Research Institute, Vom, Nigeria. The clinical history and description of the samples are shown in Table I.

To test for the presence of IBDV antigen, all the samples were processed and tested by standard procedure (OIE, 2008). Briefly, 20% (w/v) bursae homogenates from the individual outbreaks were prepared and agar gel immunodiffusion (AGID) test conducted accordingly. In addition, viral RNA was extracted from the bursae homogenates using the QIAamp Viral RNA Mini Kit (Qiagen®, Hilden, Germany) according to manufacturer's instructions. Primers (F: 5´-GCCCAGAGTCTACACCAT-3´ and R: 5´-CCCGGATTATGTCTTTGA-3´) that amplifies a 743bp fragment of the VP2 region of genome segment A was used (Jackwood and Sommer-Wagner, 2005). Using TitanOne RT-PCR kit (Roche, Germany), One-step RT-PCR was performed with the following cycling condition: 42^{o}C for 30mins and 95^{o}C for 2mins followed by 35 cycles of PCR at 95^{o}C for 30 secs, 55^{o}C for 1:30 mins and 72^{o}C for 2 mins. After completion of the 35 cycles, a final extension at 72^{o}C for 7mins was performed. To analyse the PCR products, it was separated using 1.5% agarose gel stained with ethidium bromide (0.5mg/mL) and the bands visualized on gel documentation system (BioStep, Germany).

The amplicons were purified using the QIAquick PCR Purification kit (Qiagen, Germany). To sequence the purified

TABLE I: Description of the field IBDV samples used in this study

Virus isolate	Date	Locality (State)	Age of bird (wks)	Flock type	Field mortality %	Vaccination	Phylogenetic group
IBD-NG889/2015	Jul, 2015	Vom, Plateau	7	Broilers	3.3	1st	VV2-1
IBD-NG499/2016	Jul, 2016	Keffi, Nassarawa	3	Pullets	22.8	ND	VV2-1
IBD-NG511/2016	Aug, 2016	Kuru, Plateau	ND	avian	22.2	ND	VV2-1
IBD-NG513/2016	Aug, 2016	Rayfield, Plateau	5	Pullets	32.8	1st and 2nd	VV2-1
IBD-NG516/2016	Aug, 2016	Kuru, Plateau	4	Broilers	4	ND	VV2-1
IBD-NG580/2016	Oct, 2016	Vom, Plateau	6	avian	8.3	ND	VV2-1
IBD-NG587/2016	Oct, 2016	Durawa, Plateau	ND	avian	9.2	1st and 2nd	VV2-1
IBD-NG590/2016	Oct, 2016	Bukuru, Plateau	ND	avian	2	ND	VV2-1
IBD-NG592/2016	Oct, 2016	Chaha, Plateau	5	Pullets	50	1st and 2nd	VV2-1

ND- No data; 1st – only one dose of IBD vaccine given; 1st and 2nd – two doses of IBD vaccine given; VV – very virulent

amplicons, the two primers used in the RT-PCR amplification were used in both forward and reverse directions by a commercial sequencing service provider (Macrogen® Inc., Korea) on an ABI 3730XL genetic analyzer machine. The Consensus nucleotide sequence was generated for each sample from the two primer set sequences using BioEdit. Alignment of the nucleotide and amino acid sequences including the construction of phylogenetic tree was carried out using MEGA v7.0 (Kumar *et al.,* 2016). The 9 nucleotide sequences generated in this study have been deposited to the GenBank under the accession numbers: MG190860 – MG190868.

RESULTS AND DISCUSSION

All the nine bursae sample tested positive for IBDV antigen both by AGID and the amplification of a 743bp fragment of the VP2 gene. Also, comparison of the nucleotide sequences of the 9 IBD viruses with reference vvIBDV UK661 and variant Del E, showed nucleotide identities that ranged from 96.5% to 97.2% and 93.5% to 94.3% respectively. When compared with each other, the nucleotide sequences of the 9 IBD viruses showed an identity that ranged from 97.2% to 100%. In comparison with some previously published Nigerian IBDV, nucleotide identities ranged from 94% to 99.9% with none of them having a 100% match.

Amino acid sequences deduced from the nucleotide sequences of the hvVP2 region of the nine viruses from aa 210 – 442 revealed amino acids consistent with vvIBDV at positions 222A, 242I, 256I, 294I and 299S. Comparison of the amino acid sequences of the nine viruses from position 211 – 353 with reference vvIBDV UK661, OKYM, HK46, variant IBDV Del E, some previously published Nigerian IBDV and African IBDVs showed amino acid

substitutions in 3 out of the 9 viruses studied at aa positions 215Q→P (IBD-NG513/2016), 280N→Y (IBD-NG590/2016), and 308I→L (IBD-NG587/2016) (Table II). Two of the observed mutations occurred at major peak A loop P_{BC} (aa 215) and minor peak 2 loop P_{FG} (aa 280) respectively while the third mutation occurred outside the loop at aa

TABLE II: Comparison of genome segment A amino acids substitutions at selected positions between the recent Nigerian IBD viruses and other published IBD viruses from the GenBank

ISOLATE	AMINO ACID SUBSTITUTIONS AT POSITIONS												
	215	219	222	237	242	254	269	272	278	280	299	300	308
UK661	Q	Q	A	I	I	G	T	I	A	N	S	E	I
IBD-NG899/2015	.	.	.	.	.	S	.	.	.	.	.	A	.
IBD-NG499/2016	.	.	.	.	.	S	.	.	.	.	.	A	.
IBD-NG511/2016	.	.	.	.	.	S	.	.	.	.	.	A	.
IBD-NG516/2016	.	.	.	.	.	S	.	.	.	.	.	A	.
IBD-NG513/2016	P	.	.	.	.	S	.	.	.	.	.	A	.
IBD-NG580/2016	.	.	.	.	.	S	.	.	.	.	.	A	.
IBD-NG587/2016	.	.	.	V	.	S	.	.	.	.	.	A	L
IBD-NG590/2016	.	.	.	.	.	S	.	.	.	Y	.	A	.
IBD-NG592/2016	.	.	.	.	.	S	S	.	.	.	.	A	.
KP266324_BAUCHI58/NG/2012	.	T	.	.	.	S	S	.	.	.	.	Q	.
KP266333_PLATEAU78/NG/2011/1	.	T	.	.	.	S	.	.	.	.	.	Q	.
KP152258_UYO201/NG/2014	.	.	.	.	.	S	.	.	.	.	.	A	.
KP152270_BENIN142E/NG/2013	.	.	.	.	.	.	.	T	N	.	.	.	.
KP152271_ABUJA93/NG/2013	.	.	.	.	.	S	.	.	.	.	.	A	.
JX424059.1_IBDV33/Abuja.NG/2011	.	.	.	.	.	S	.	.	.	.	.	A	.
JX424070.1_IBDV63/Kaduna.NG/2009	.	.	.	.	.	S	.	.	.	.	.	A	.
AJ586955.1_IBDV/Oyo.NIE/99/015/c	.	.	.	.	.	S	.	.	.	.	.		.
AJ586939.1_IBDV/Oyo.NIE/97/102/c	.	.	.	.	.	S	.	.	.	.	.	A	.
AJ586951.1_IBDV/Oyo.NIE/98/058/	.	.	.	.	.	.	.	.	.	.	.		.
AJ586924.1_IBDV/Osun.NIE/96/076/c	.	.	.	.	.	.	.	.	.	.	.	A	.
EF363551.1_Bk	H	.	.	.	.	S	.	.	.	.	.		.
AJ001941.1_88180_IVORY_COAST	.	.	.	.	.		.	.	.	.	.	Q	.
KM870811_BF36-9	.	.	.	.	.	S	.	.	.	.	.	A	.
HQ231797.1_86-2006Eth	.	.	.	.	.	S	.	.	.	.	.	Q	.
AB200982_KMRG-40_Tanzania	.	.	.	.	.	S	.	.	.	.	.	A	.

A dot indicates identical sequences

position 308 (Table II). As previously reported, amino acid changes occurring in the hydrophilic peaks can cause an antigenic drift (Jackwood and Sommer-Wagner, 2011). It is well

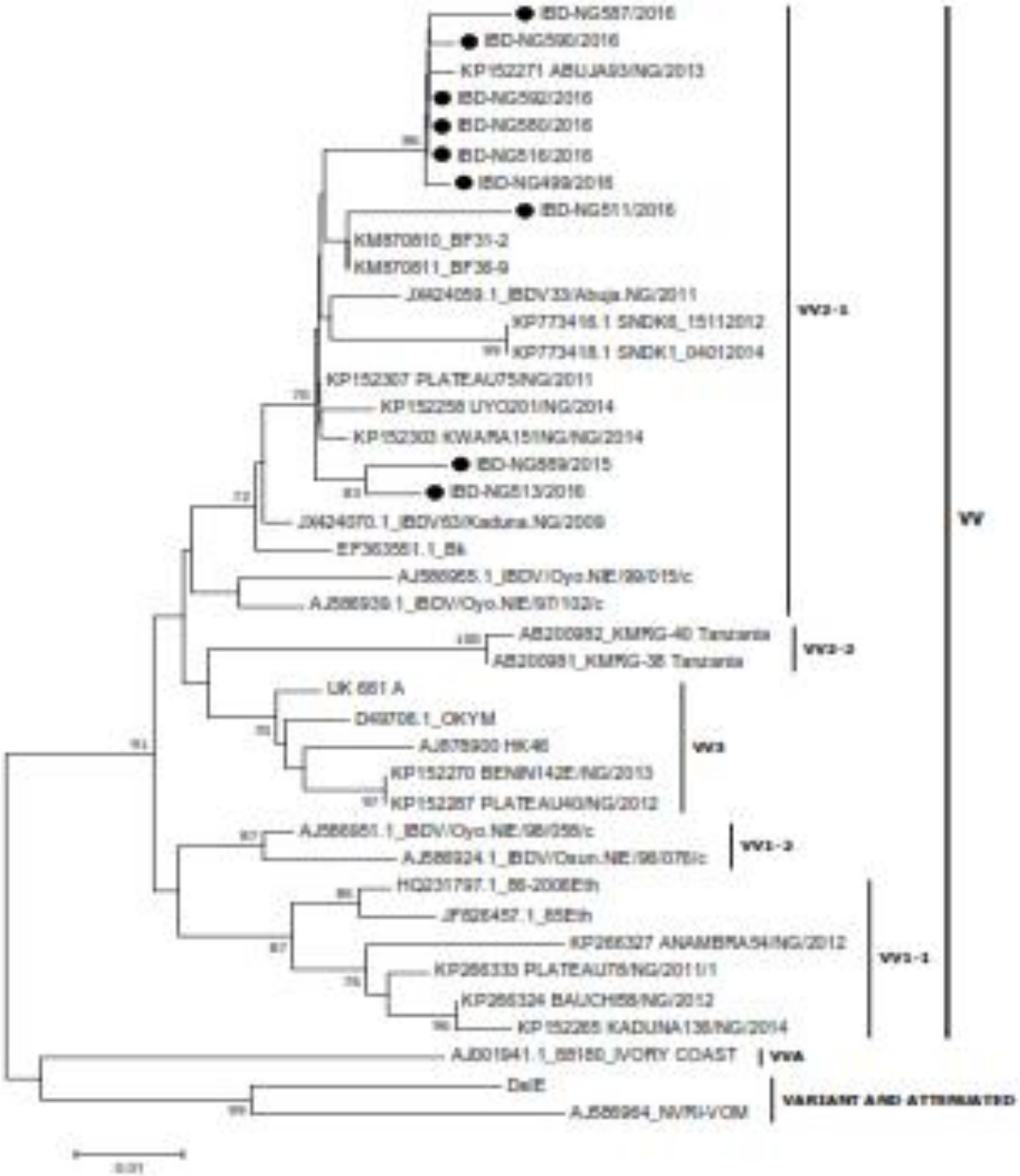

FIGURE I: Phylogenetic analysis of the nucleotide sequences of the hvVP2 of IBDV used in this study with reference IBDV from GenBank using the neighbor joining method with 1000 bootstrap replication. IBD viruses used in this study are identified by shaded circles and reference strains are identified by GenBank accession number followed by name. Scale bar represent substitution per site

documented that viruses especially RNA viruses which are error-prone use the mechanism of mutation to escape neutralization by vaccines which ensures

their continuous survival (Pikula et al., 2017). Likewise, the bi-segmented nature of IBDV encourages the sharing and exchange of genetic materials between the two segments of IBDV thereby giving rise to homologous recombination and reassortant IBDV strains which favors variation of IBDV in nature (Qin and Zheng, 2017). Sequence analysis of the VP2 region spanning from aa positions 206 – 350 have been used for molecular characterization of IBDV by Wu *et al.* (2007). This region is the hot zone for mutation, tissue culture adaptation and viral virulence (Brandt *et al.*, 2001). Comparison of the nine viruses and previously published Nigerian IBDV from GenBank showed that this mutation was unique to the 3 samples. A previous Nigerian IBDV isolate (EF363551.1_BK) had mutation Q→H at position 215 but in this study, amino acid in this position was substituted with a P (proline). Amino acid position 215 of Nigerian IBDV have undergone two successive mutations so far from Glutamine (polar) in UK661 to Histidine (basic polar) and Proline (nonpolar). As reported by Mohamed *et al.* (2014), changes in the charges on the amino acids may affect the topography of the epitopes on the VP2 responsible for neutralization which can lead to vaccination failure. Further comparison of the nine viruses with some IBDV and reference IBDV from GenBank showed these mutations were unique to the recent IBDV from Nigeria (data not shown).

A phylogenetic tree constructed based on the nucleotide sequences comprising of the nine recent Nigerian isolates, previous Nigerian IBDV, IBDV from some African countries, vaccine strain, variant and reference vvIBDV showed three major clusters; very virulent, variant and vaccine. The nine isolates used in this study were found in the first cluster that comprised of vvIBDVs but 6 out of 9 formed a unique cluster with a previous Nigeria IBD virus (KP152271 ABUJA93/NG/2013) and sub clusters of their own within the group (Figure I). Very virulent IBD viruses in the African continent are divided into three; VV1, VV2 and VV3. VV1 consists of IBD viruses from Ethiopia, Nigeria and Zambia, for VV2, IBDV from Nigeria, Tanzania, Zambia and recently Senegal while VV3 consists of IBDV from Africa, Asia, Europe, South America and others (Nwagbo *et al.*, 2016; Badji *et al.*, 2016). On the tree, the nine viruses clustered with the African very virulent type VV2 specifically in the VV2-1 lineage (Figure I). The VV2-1 lineage was unique to IBD viruses from Nigeria but has recently been joined by IBDV from Senegal. Grouping of IBDV in Africa into lineages and sublineages has helped to further elucidate the genetic relatedness and or divergence of African IBDV strains from one country to another thereby showing the dynamics of IBDV in Africa.

In conclusion, this study has further implicated the very virulent strain of infectious bursal disease virus as the cause of the outbreaks being experienced in vaccinated poultry flocks in Nigeria and also reflects the continuing genetic instability of IBD virus in the country. In view of this report, urgent review of the vaccination strategy is recommended to curb the incessant outbreak of IBDV in Nigeria.

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REFERENCES

ADAMU, J., OWOADE, A. A., ABDU, P. A., KAZEEM, H. M. and FATIHU, M. Y. (2013). Characterization of field and vaccine infectious bursal disease viruses from Nigeria revealing possible virulence and

regional markers in the VP2 minor hydrophilic peaks. *Avian Pathology* 42: 420–433.

BADJI, A., DIALLO, A. A., DUCATEZ, M., LO, F. T., MBENGUE, M., DIOUF, M., SAMB, Y., DIOP, M., LO, M. M., THIONGANE, Y., GUERIN, J. L. and ALAMBEDJI, R. B. (2016). Genetic evolution of infectious bursal disease virus in Senegal. *Journal of Veterinary Medicine and Animal Health* 8: 237–243.

VAN DEN BERG, T. P. (2000). Acute infectious bursal disease in poultry: A review. *Avian Pathology* 29: 175–194.

BRANDT, M., YAO, K., LIU, M., HECKERT, R. A. and VAKHARIA, V. N. (2001). Molecular Determinants of Virulence, Cell Tropism, and Pathogenic Phenotype of Infectious Bursal Disease Virus. *Journal of Virology* 75: 11974–11982.

CHAKMAN, S. (2015). Epidemiology of infectious bursal disease in broiler birds of three districts in Bangladesh. *Asian Journal of Medical and Biological Research* 1: 59–64.

COSGROVE, A. (1962). An Apparently New Disease of Chickens: Avian Nephrosis. *Avian Diseases* 6: 385–389.

DELMAS, B., MUNDT, E., VAKHARIA, V. N. and WU, J. L. (2012). Birnaviridae. In: Virus taxonomy: ninth report of the International Committee on Taxonomy of Viruses., (eds) A. M. Q. King, E. Lefkowitz, M. J. Adams and E. B. Carstens, (9th edition). Academic press, San Diego, California.

JACKWOOD, D. J. and SOMMER-WAGNER, S. E. (2005). Molecular Epidemiology of Infectious Bursal Disease Viruses: Distribution and Genetic Analysis of Newly Emerging Viruses in the United States. *Avian Diseases* 49: 220–226.

JACKWOOD, D. J. and SOMMER-WAGNER, S. E. (2011). Amino acids contributing to antigenic drift in the infectious bursal disease Birnavirus (IBDV). *Virology* 409: 33–37.

KUMAR, S., STECHER, G. and TAMURA, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution* 33: 1870–1874.

LASHER, H. N. and SHANE, S. M. (1994). Infectious bursal disease. *Worlds Poultry Science Journal*. 50: 133–166.

LUKA, P. D., YAKUBU, B., JAMBOL, A. R., AUDU, B. J., DOGONYARO, B. B. and OWOLODUN, O. A. (2014). Detection and differentiation of infectious bursal disease virus from the outbreaks in two layer farms by PCR-RFLP in Jos, Nigeria. *Veterinary World* 7: 30–33.

MOHAMED, M. A., ELZANATY, K. E. S., BAKHIT, B. M. and SAFWAT, M. M. (2014). Genetic Characterization of Infectious Bursal Disease Viruses Associated with Gumboro Outbreaks in Commercial Broilers from Asyut Province, Egypt. ISRN *Veterinary Science* 2014: 1–9.

NWAGBO, I. O., SHITTU, I., NWOSUH, C. I., EZEIFEKA, G. O., ODIBO, F. J. C., MICHEL, L. O. and JACKWOOD, D. J. (2016). Molecular characterization of field infectious bursal disease virus isolates from Nigeria. *Veterinary World* 9: 1420–1428.

OIE. (2008). Infectious bursal disease. In: Manual of Standards for Diagnostic Tests and Vaccines for Terrestrial Animals, (5th edition). OFFICE INTERNATIONAL DES EPIZOOTICS, Paris, France: 549–565.

OJO, M. O., ODUYE, O. O., NOIBI, L. M.

and IDOWU, A. L. (1973). Gumboro-like disease in Nigeria. *Tropical Animal Health and Production* 5: 52–56.

OKOYE, J. O. and UZOUKWU, M. (1981). An outbreak of infectious bursal disease among chickens between 16 and 20 weeks old. *Avian diseases* 25: 1034–8.

OWOADE, A. A., MULDERS, M. N., KOHNEN, J., AMMERLAAN, W. and MULLER, C. P. (2004). High sequence diversity in infectious bursal disease virus serotype 1 in poultry and turkey suggests West-African origin of very virulent strains. *Archives of Virology*149: 653–672.

OWOLODUN, O. A., YAKUBU, B., JAMBOL, A. R., AUDU, B. J., DOGONYARO, B. B. and LUKA, P. D. (2015). Further evidence for very virulent infectious bursal disease virus in vaccinated chickens in Nigeria. *Tropical Animal Health and Production* 47: 1437–41.

PIKULA, A., DOMAŃSKA-BLICHARZ, K., CEPULIS, R AND ŚMIETANKA, K. (2017). Identification of infectious bursal disease virus with atypical VP2 amino acid profile in Latvia. *Journal of Veterinary Research* 61: 145-149.

QIN, Y AND ZHENG, S.J. (2017). Infectious Bursal Disease Virus-Host Interactions: Multifunctional Viral Proteins that Perform Multiple and Differing Jobs. *International Journal of Molecular Science* 18: 1-13.

SHEKARO, A. and JOSIAH, I. E. (2015). Infectious bursal disease outbreak in fifteen weeks old pullets in Kaduna, Nigeria. *Journal of Animal Production and Advances* 5: 636–644.

SILVA, M. S. E, BERTRAN, K., MORESCO, K., JACKWOOD, D. J. and SWAYNE, D. E.(2016). Infection with Some Infectious Bursal Disease Virus Pathotypes Produces Virus in Chicken Muscle Tissue and the Role of Humoral Immunity as a Mitigation Strategy. *Avian diseases* 60: 758–764.

WU, J., YU, L., LI, L., HU, J., ZHOU, J. and ZHOU, X. (2007). Oral immunization with transgenic rice seeds expressing VP2 protein of infectious bursal disease virus induces protective immune responses in chickens. *Plant Biotechnology Journal* 5: 570–578.