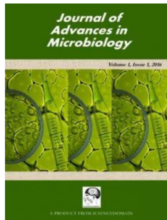

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Genetic Analysis of Infectious Bursal Disease Virus (IBDV) from a Farm in Vom, Plateau State, Nigeria

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Authors' contributions

This study was carried-out in collaboration with all the authors. Author LUE designed the study and wrote the first draft of the manuscript, while authors CIN and DOE performed the statistical analysis. Authors NMS and PEE provided the samples while authors IS and ETO wrote the protocol and author ION managed the literature search. The final manuscript was read and approved by all the authors.

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ABSTRACT

This study describes the genetic analysis of an atypical infectious bursal disease virus (IBDV) detected from an outbreak in a poultry farm in Vom, Plateau State, Nigeria in 2017. The farm with seven thousand, six hundred and eleven (7,611) four weeks old vaccinated pullets had 76.2% mortality (5,796 dead birds) as a result of the outbreak. Thirty-two bursae of Fabricius samples showing lesions of IBD were aseptically collected for IBDV antigen detection and molecular characterisation. All the tested samples were positive by the agar-gel immunodiffusion test (AGDT) for IBDV antigen while the VP2 gene could be detected in 60% of these samples by polymerase chain reaction (PCR). One PCR positive sample was sequenced. Nucleotide and deduced amino acid sequences obtained were compared to sequences from GenBank. The IBDV strain detected (IBDV/VOM/NG/2017) was distinct from the attenuated vaccine strain used on the farm, but formed cluster in the very virulent (vv) IBDV group in the VV2-1 subcluster and was most closely related to previously published IBDV strains from Nigeria (Plateau 104/NG/2010, and more recently

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Bauchi127/NG/2014) with 98% nucleotide identity. Substitution mutations were observed across the VP2 region of the recent vvIBDV strain detected. This finding provides an update on the epidemiology and molecular dynamics of IBDV in Nigeria with implications for control.

Keywords: Very virulent; infectious bursal disease virus; Vom; Nigeria.

1. INTRODUCTION

Infectious bursal disease (IBD), also known as Gumboro disease is an acute highly contagious viral disease of young chickens of 3-6weeks old and characterised by destruction of the lymphoid cells of the bursa of Fabricius with severe immunosuppression and impaired growth of young chickens [1]. The causative agent, the infectious bursal disease virus (IBDV) is a non-enveloped double stranded RNA (dsRNA) virus of the *Birnaviridae* family and *Avibirnavirus* genus [2]. The viral genome of IBDV has two double-stranded RNA segments designated A (3.4kb) and B (2.8kb). The segment A encodes 4 viral proteins the two capsid proteins VP2 (48kDa) and VP3 (32-35kDa) and viral protease VP4 (24kDa) and nonstructural protein VP5 (17-21kDa). The smaller segment B encodes RNA-dependent RNA polymerase VP1 (90kDa) [3]. Strains of IBDV can be grouped into two distinct serotypes. Serotype 1 viruses are pathogenic to chickens while serotype 2 viruses are nonpathogenic. Serotype 1 has been divided into several groups on the basis of antigenic variation and virulence: classical strains, variant strains and very virulent strains [4]. The three IBDV strains currently have a global distribution and occur in most countries with the developed poultry industry. Classical IBDV strains cause bursal damage and lymphoid necrosis resulting in 20-30% mortality [5]. The variant IBDVs are characterised by an antigenic drift caused by point mutations affecting the neutralising epitopes of VP2 [6] These strains emerged in the North American continent and were characterised by causing B-lymphocyte depletion without eliciting an inflammatory response or clinical signs of disease [7]. In the mid-80s very virulent(vv) IBDV strains emerged in Europe and caused devastating outbreaks resulting in 30% and 60-70% mortality in broilers and layers respectively, then spread to Middle East, Asia, Africa and South America [8]. Isolated IBDVs with different traits than the traditional strains have been sporadically reported through the years in different parts of the world [9]. These IBDVs have been generally considered atypical isolates that evolved in restricted geographic regions or during a short period of time under particular conditions. In Nigeria, IBD was first reported in 1973 and since then despite routine vaccination programme, IBDV has assumed an endemic status with vvIBDV being reported throughout the country [10,11,12]. An accurate identification of the field variants circulating in poultry production is essential to understand the epidemiology and control of the disease [13]. This paper describes the molecular characteristics of an IBDV strain detected in a recent outbreak from a poultry farm in Vom, Nigeria.

2. MATERIALS AND METHODS

2.1 Case History and Sample Collection

An outbreak of a suspected case of IBD was reported on the 3rd of May, 2017 in a poultry farm which lies between Longitude 8°C 48'E and Latitude 9°C 43' N with an altitude of 1,273.2 m located in Vom, Jos South local government area of Plateau State, Nigeria. The farm had seven thousand, six hundred and eleven (7,611) four weeks old pullets which hitherto had been vaccinated against IBD using an indigenous IBD vaccine. By 8th May, 2017, the farm had lost five thousand, seven hundred and ninety six (5,796) birds (76.2% mortality). Typical signs of IBD were evident on clinical examination. Post mortem examination was performed on freshly dead birds from the affected farm with the outbreak of suspected IBD after onset of mortality; thirty-two bursae from the necropsied birds were aseptically collected and kept in the refrigerator at -20°C for further laboratory tests.

2.2 Agar-gel Immunodiffusion Test (AGIDT)

Twenty percent bursal homogenate was prepared by weighing 1 g each of the bursa into mortar and pestle and grinded into paste. 4ml of phosphate buffer saline (PBS) (pH= 7.2) was added with 1 mg/ml of streptomycin sulphate, 0.4 mg/ml of gentamycin sulphate, and 1000 UI/ml of penicillin. Using reference IBD serotype 1 antiserum, and known reference positive and negative bursal homogenates antigen as control. The test was performed according to the standard protocol as described by OIE [13].

2.3 Reverse-transcription Polymerase Chain Reaction (RT-PCR)

Viral RNA was extracted from the supernatant of the homogenised bursae using Qiagen RNeasy extraction kit (Qiagen, Stanford, CA, USA), following the manufacturer's protocol. The extracted RNA was stored at -80°C until analysed. The VP2 region of IBDV genome segment A was amplified using a one-step RT-PCR kit (Mannheim, Germany) as described previously [14]. The oligonucleotide primer sequence used was IBD_VP2F:5'-GCCCAGTCTACACCAT-3'and IBD_VP2R:-5' – CCCGGATTATGTCTTTGA-3' (Macrogen Inc. Seoul, Korea) that amplifies 743bp fragment of the genome segment. The reaction mixture of 22 µl containing 5 µl of 5X reaction mixture, 0.5 µl of dNTPs, 1 µl of $MgCl_2$, 0.5 µl of RNase inhibitors, 0.5 µl of each of IBD_VP2F primer (20 pmol/µl) and IBD_VP2R primer (20 pmol/µl), 13.5 µl of nuclease free water and 0.5 µl of Titan^{TM} one enzyme mix was added to 3 µl RNA template. The thermal profiles for the amplification were as follows: 42°C for 30 mins and 95°C for 2 mins followed by 40 cycles, a final extension at 72°C for 7 mins. The PCR products were analysed on a 1.5% agarose gel electrophoresis in Tris acetate- EDTA (TAE, 0.04M Tris acetate, 1mM EDTA), stained with ethidium bromide (0.5 mg/ml) and bands were visualised on a gel documentation system (BioStep, Germany). A 1Kbp DNA ladder (Invitrogen, Germany) was used to comparatively determine the molecular size of the PCR amplicon.

2.4 DNA Sequencing

One of the RT-PCR products was purified using QIA quick PCR purification kit (Qiagen). PCR amplicon was sequenced by dideoxy sequencing using BigDye Terminator Sequencing kit using the forward and reverse primers used in the amplification procedure by a commercial sequencing service provider (Macrogen® Inc. Korea) on an ABI 3730 XL genetic analyzer machine (Applied Biosystem). Phylogenetic analysis of the VP2 sequence obtained was performed and comparison with 33 reference sequences from the GenBank using MEGA 7.0 software [15]. The neighbour joining method [16] was used and the distance between nucleotide sequences was computed using Tamura- Nei model [15] and bootstrap values of the phylogenetic nodes were calculated out of 1000 replicates [17]. The deduced amino acid alignment of the sequence was also compared with reference sequences for possible mutation.

3. RESULTS

IBDV antigen was detected in all the samples tested by AGIDT with evidence of distinct lines of precipitation. The 743bp fragment of the hypervariable VP2 region was amplified in 60% of samples that were positive by AGIDT. Genetic relationships of the IBDV detected in Vom, Nigeria was determined by phylogenetic analysis of the VP2 sequence data with 33 reference IBDV strains from the GenBank (Fig. 1). The results show that IBDV/VOM/NG/2017 clustered in the vvIBDV group within the VV2-1 African lineage, and was most closely related to some previously published IBDV strains (Plateau104/NG/2010 and more recently Bauchi127/NG/2014) from Nigeria with 98% nucleotide identity (nt). The present IBDV strain also shares ancestral relationships (97% nt) with other strains from Nigeria including UYO206/NG/2014, KWARA151NG/NG/2014, BF 31-2, BF 36-9, IBDV33/Abuja.NG/2011 and IBDV63/Kaduna.NG/2009. The nucleotide similarity between IBDV/VOM/NG/2017 and the variant DelE and very virulent UK661ranged was 90% and 95% respectively. The phylogenetic tree further showed that the current IBDV detected (IBDV/VOM/NG/2017) is not a vaccine virus (vaccine virus is an intermediate variant strain AJ586964_NVRI-VOM). Sequences of the deduced amino acid of the IBDV strain detected in this study (IBDV/VOM/NG/2017) was compared with 33 reference IBDV strains retrieved from Genbank which include strains from Nigeria, Africa, Non-African countries and IBD vaccines (from intermediate variant strain of virus AJ586964_NVRI-VOM). The conserved amino acids (virulence markers) at positions 222A, 242I, 256I, 294I and 299S typical with the vvIBDVs were present in IBDV/VOM/NG/2017. When compared to the reference strains, four unique amino acid substitution mutations were observed across the VP2 region of IBDV/Vom/NG/2017 (residues 221Q→L, 232A→S, 255L→F and 288N→Y) (Table 1). Two of the mutations 221Q→L, and 288N→Y occurred on the major hydrophilic peak A and minor peak 2 respectively while 232A→S occurred outside the loop between major hydrophilic peak A and minor hydrophilic peak 1 with 255L→F occurring next to minor hydrophilic peak 1.

Fig. 1. Midpoint-rooted neighbor-joining tree showing relationships between the 2017 IBDV strain from Vom, Plateau State based on the VP2 sequence. VP2 sequences of additional IBD viruses available in GenBank were included in the analysis

4. DISCUSSION

Currently, IBDV is one of the most important contagious immunosuppressive diseases of poultry in Nigeria. The irreversible immune suppression caused by IBDV in young chickens increases their susceptibility to a multitude of opportunistic avian pathogens that are normally non-pathogenic in healthy flocks [18]. Phylogenetic analysis indicates that IBDV/VOM/NG/2017 is a vvIBDV strain due to its clustering with reference vvIBDVs used for comparison. The vvIBDV strains have been reported in vaccinated and unvaccinated poultry flocks in some parts of Nigeria [10,11,19]. For ease of classification and identification of the probable origin and relatedness of some vvIBDVs from Africa, African vvIBDVs are divided into three; VV-1, consists of IBDV from Ethiopia, Nigeria and Zambia, VV-2, consists of IBDV from Nigeria, Tanzania and Zambia, while VV-3, collectively consists of IBDV from Africa, Asia, Europe and some other countries [20]. IBDV/VOM/NG/2017 clustered with the VV-2 group of African vvIBDVs and specifically within the subcluster VV2-1. The nucleotide sequence of IBDV/VOM/NG/2017 shared a 98% similarity with previously published Nigerian IBDV strains from Bauchi and Plateau states, while it shared a 97% nucleotide similarity with previous published IBDVs from four other regions (Uyo, Kwara, Abuja, Kaduna) in Nigeria. It will be pointed out here that Plateau state where IBDV/VOM/2017 was detected shares boundary with Bauchi State. It could be assumed that the movement and trade in poultry birds between the two states may be instrumental to the spread of the virus as previously postulated (Owolodun et al. 2015).

Table 1. Comparison of segment A amino acid substitutions at selected positions between IBDV/VOM/NG/2017 and other strains of IBDV from Genbank

Strain/Isolate	Amino acid substitution at positions											
	221	222	232	242	253	254	255	256	279	284	288	300
UK_661_A	Q	A	A	I	Q	G	L	I	D	A	N	E
DelE	.	T	.	V	.	S	.	V	N	.	.	.
IBDV/VOM/NG/2017	L	.	S	.	.	S	F	.	.	.	Y	A
JX424059.1_IBDV33/Abuja.NG/2011	.	.	.	.	.	S	.	.	.	.	.	A
AJ586955.1_IBDV/Oyo.NIE/99/015/c	.	.	.	.	.	S	.	.	.	.	.	.
KM870810_BF31-2	.	.	.	.	.	S	.	.	.	.	.	A
AJ586951.1_IBDV/Oyo.NIE/98/058/c	.	.	.	.	.	.	.	.	.	.	.	.
KP152264_BAUCHI127/NG/2014	.	.	.	.	.	S	.	.	.	.	.	A
KP152231_UYO206/NG/2014	.	.	.	.	.	S	.	.	.	.	.	Q
KP152284_PLATEAU104/NG/2010	.	.	.	.	.	S	.	.	.	.	.	A
AJ001941.1_88180_IVORY_COAST	.	Q	.	.	.	.	.	.	.	.	.	Q
AJ586964_NVRI-VOM	.	P	.	V	H	.	.	V	N	T	.	E
KF573194.1_GEORGIA_VACCINE	.	.	.	.	.	.	.	.	.	.	.	E
HQ231797.1_86-2006Eth	.	.	.	.	.	S	.	.	.	.	.	Q
AB200982_KMRG-40_Tanzania	.	.	.	.	.	S	.	.	.	.	.	A
AJ878903_BD3	.	.	.	.	.	S	.	.	.	.	.	A

Ironically, Akwa Ibom (Uyo) and Kwara States do not share any close boundary with Plateau State and interstate trade in poultry birds could be a factor for the spread.

Alignment of the deduced amino acid sequences show that virulence markers (residues 222A, 242I, 256I, 294I and 299S) typical with the vvIBDV [21] were observed in vvIBDV strain detected in this study. Substitution mutations occurred in the hypervariable region of VP2 (the major hydrophilic peak A, minor peak 2 and outside the loop between peak A and peak 1). Substitution mutation on any of the four hydrophilic loops of IBDV can cause a drift in the antigenicity of the virus thereby enabling it to escape neutralisation [9,12] and capable of causing IBD vaccines used in the field to be inefficacious. IBDV control has only been possible through the use of efficacious vaccines, but vaccination efforts are complicated by the fact that frequent viral genetic mutations, reassorting of genome segments and recombination can potentially increase virulence and alter antigenicity, rendering vaccines and vaccine protocols less effective [9].

5. CONCLUSION

The genetic findings further confirm the continuing evolution of vvIBDV in Nigeria resulting in persistent strains that may escape neutralisation and cause mortality in vaccinated poultry flocks. Adequate farm biosecurity and continuous surveillance is recommended for improved disease control.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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