MOLECULAR CHARACTERIZATION OF FIELD INFECTIOUS BURSAL DISEASE VIRUS FROM CHICKENS IN SOME STATES OF NIGERIA

BY

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A DISSERTATION SUBMITTED TO THE DEPARTMENT OF APPLIED MICROBIOLOGY AND BREWING, FACULTY OF BIOSCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D) IN MEDICAL MICROBIOLOGY OF NNAMDI AZIKIWE UNIVERSITY AWKA, NIGERIA.

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APRIL, 2017

CERTIFICATION

I certify that the research work titled "Molecular characterization of field infectious bursal disease virus from chickens in some states in Nigeria" was carried out by Nwagbo Ijeoma Okwuchukwu (*NAU/PG/PhD/2009487001F)* under my supervision in the Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. The work embodied in this research is original and has not been submitted in part or full for any other diploma or degree of this or other university.

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APPROVAL

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DEDICATION

This work is dedicated to the glory of God Almighty.

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ABSTRACT

Infectious bursal disease (IBD) is an economically important, acute, highly contagious and immunosuppressive viral disease of poultry that affects young birds between the ages of 3 to 6 weeks. The disease targets lymphoid tissues with a special predilection for the Bursa of Fabricius. Infectious bursal disease virus (IBDV) can cause high mortality and morbidity in chickens aged 3 weeks upwards depending on the virulence of the infecting strain. In the field, despite the massive vaccination program designed to curb IBD virus, outbreaks are still being reported in vaccinated flocks. This study investigated the underlying factor(s) responsible for the constant and persistent post vaccinal outbreaks of IBD with high mortalities and morbidities recorded in poultry flocks in Nigeria, by studying the genes of IBD viruses obtained from outbreaks in the field. Bursae samples obtained from chickens suspected to have died of IBDV were first screened using Agar gel immunodiffusion (AGID) test followed by isolation attempts of field IBD viruses using embryonated eggs and chicken embryo fibroblast (CEF) cell culture. Reverse transcriptase-polymerase chain reaction (RT-PCR) was used to detect the genome of IBDV. Reverse transcriptase-polymerase chain reaction/Restriction fragment length polymorphism (RT-PCR/RFLP) was used to study the number and size of the restriction fragments obtained following digestion of RT-PCR products with restriction endonucleases. The hypervariable (hv) VP2 (viral protein 2) and VP1 (viral protein 1) regions of segments A and B genomes of the field IBDVs were sequenced, respectively. Nucleotide, amino acid and phylogenetic analysis were carried out on the VP2 region for accurate strain identification/characterization and on the VP1 (viral protein 1) region to identify reassortant strains of IBDV. Out of the139 bursae initially screened, 93 (66.9%) were positive by AGID. At second passage of the field viruses, IBDV antigens were not detected except from one sample identified with the result of the sequence analysis confirming the need for the use of specific pathogen free (SPF) hosts for the isolation of IBDV and the difficulty in isolation of field IBDV in embryonated eggs and tissue culture. A 743bp fragment of the hvVP2 region was amplified from 143 (66.2%) out of 216 of the samples tested while a 722bp fragment of the VP1 gene was amplified from 86 (60.14%) of these by RT-PCR. The sequence results of the hvVP2 region revealed that the very virulent Infectious bursal disease virus (vvIBDV) and classic strain were present in the flocks sampled with vvIBDV being more prevalent. The amino acid sequences also showed mutations that were previously not present. The phylogenetic analysis revealed that the Nigerian IBDVs were closely related to vvIBDV from Europe and Asia but formed distinct clusters of their own. Results obtained from the sequence analysis of the VP1 region showed that reassorted IBDV strains were present in Nigerian poultry flocks. Sequence results also revealed motifs at the triplet amino acid positions especially at amino acid position 145 and showed that the Nigerian IBDV had vvIBDV-like and non-vvIBDV-like segment B with a segment A derived from vvIBDV. Phylogenetic analysis of the Nigerian IBDV revealed a novel lineage of reassorted IBDV segment B. These findings clearly show that the vvIBDV is the prevalent strain affecting the poultry population in Nigeria breaking through any protection conferred by the vaccine strains used in the field thereby causing post vaccination outbreaks. The presence of reassortant strains reported in this study has further compounded the problem of effective control of IBDV in Nigeria. Therefore, there is a need for continuous molecular and epidemiological study of infectious bursal disease virus in Nigeria to keep track of the changes (mutations) observed to avoid sudden emergence of new strains. There is also a need to review the current infectious bursal disease virus vaccines used in the field to prevent the high mortalities observed during outbreaks especially by the very virulent strain of infectious bursal disease virus.

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CHAPTER 1

INTRODUCTION

1.1 Background of the study

Agriculture is the most important sector of the Nigerian economy in terms of provision of employment (Labour Force Statistics, 2010). The poultry sub-sector is the most commercialized of all the sub-sectors of Nigeria's agriculture, holding the greatest promise in affording animal protein supply, food security and poverty alleviation in both rural and urban populace (Heise *et al.*, 2015). However, constraints such as diseases like infectious bursal disease (IBD) also known as Gumboro disease have affected the poultry industry in Nigeria and indeed the world negatively (van den Berg, 2000).

Infectious bursal disease (IBD) is an acute, highly contagious disease of young chicken caused by Infectious bursal disease virus (IBDV) that affects most commonly young chickens about 3-6 weeks of age. The target organ of IBDV is the bursa of fabricius where the virus causes a massive destruction of the B lymphocyte cells resulting in lymphopenia (immunosuppression) (Eterradossi and Saif, 2008). Ever since the disease was recognized by Cosgrove in 1962 in Gumboro, Delaware, U.S.A, IBD is now endemic in many countries of the world and have continued to pose a serious threat to the poultry industry worldwide (van den Berg, 2000). The economic impacts of the disease are manifold including losses due to morbidity and mortality, immunosuppression in the surviving chickens since IBDV infection exacerbates infections with other disease agents, reduction in the chicken's ability to respond to vaccination and risk of introduction to exotic places from importing infected poultry products. Sequelae that have been associated with immunosuppression induced by the virus include gangrenous dermatitis, inclusion body hepatitis-anemia syndrome, *E. coli* infections (Eterradossi and Saif, 2008). The economic impact of the disease is influenced by pathogenicity of the strain of virus, susceptibility and breed of flock, other prevalent pathogens and environmental and management practices.

IBDV is a non-enveloped bi-segmented dsRNA virus belonging to the *Avibirnavirus* genus within the *Birnaviridae* family (Delmas *et al*, 2011) with two distinct types designated as

serotypes 1 and 2 (Lukert and Saif, 1997). Serotype 1 is pathogenic to chickens and can be classified into very virulent (VV), classical (virulent, mild/intermediate or attenuated), antigenic variant, and Australian classic genotypes on the basis of their virulence and antigenicity (Sharma *et al*., 2000). Serotype 2 isolated from turkey is not pathogenic in chickens (Mahgoub, 2012). Strains of IBDV differ in their degree of pathogenicity in chickens. The classical IBDV strain induces 10-50% mortality with typical signs and lesions. The variant strain induces little if any clinical signs and mortality, but marked bursal lesion while the very virulent strain induces approximately 50-100% mortality with typical signs and lesions (OIE, 2008). vvIBDV can cause 70% mortality in layers, 25% mortality in broilers and 100% mortality in specific pathogen free (SPF) chickens and can also cause high mortalities in older birds (Eterradossi and Saif, 2008). IBDV is ubiquitous, extremely resistant to environmental conditions and can persist in the environment for a long time.

The viral genome of IBDV has two RNA segments; A is 3.2kb and B is 2.8kb. Segment A encodes viral proteins VP2, VP3, VP4, and VP5 while the segment B encodes VP1, the putative viral RNA polymerase. The VP2 protein is considered to be the major host-protective antigen and contains the major antigenic site responsible for eliciting neutralizing antibodies. The VP2 protein of the various IBDV strains displays high amino acid variability, with the hypervariable region (HVR) being located in amino acid residues 206-350, and responsible for antigenic variation, tissue-culture adaptation and viral virulence, thereby making the VP2 hypervariable region the prime target for the molecular techniques used for detection and characterization of IBDV. Initially, the VP2 was thought to be the primary determinant for virulence for vvIBDV phenotype, but the appearance of reassortant IBDV strains have proved otherwise. Reports suggest that both the major capsid protein (VP2) and the RNA-dependent RNA polymerase (VP1) located on genome segments A and B respectively, contribute to the virulence of IBDV thereby demonstrating that VP2 of vvIBDV is not the sole determinant for their pathogenicity (Escaffre *et al*., 2013). It has become imperative that in the molecular identification of IBDV, especially with regard to the vvIBDV phenotype, both the VP2 and VP1 regions need to be analyzed. So in order to ensure adequate protection of poultry flocks from IBDV, it is necessary to identify the strain(s) of IBDV circulating in the field because it will help in the development of a proper vaccination program to checkmate the losses farmers incur due to IBDV. Many

diagnostic techniques are available for the detection and characterization of IBDV strains in the field with the molecular technique (RT-PCR/RFLP, nucleotide and amino acid sequencing, phylogenetic analysis) being the most popular.

Vaccination is the only means for the prevention and control of Infectious bursal disease (IBD) in poultry worldwide but the rate of post vaccinal IBD outbreaks in Nigeria has reached a worrisome proportion. Numerous IBDV vaccines produced with different IBDV strains are used indiscriminately in the field during vaccination programs without full knowledge of the characteristics of the strain of IBDV present or prevalent in the field. This has led to many complications in IBD prevention and control with one of the obvious results being vaccination failure. Coupled with this problem is the indiscriminate importation and use of foreign IBD vaccine in the field with no effort made to know the strain of IBDV circulating in the country and if the foreign vaccines will confer adequate immunity in case of an outbreak . The factors that can lead to vaccination failure include; 1) presence of high level of maternally derived antibodies which can neutralize the vaccine, 2) improper storage, handling and administration of vaccine, and 3) emergence of new antigenic variants (Kabell *et al.*, 2005). The outbreaks being experienced in Nigerian poultry could be as a result of the circulation of a strain of IBD that is entirely different from the IBD vaccine strains used in the field that is able to break through any conferred immunity and cause disease and subsequent mortality or it could be that reassortant IBD virus strains are present in the poultry population in Nigeria. There is therefore the need to investigate and identify the strain(s) of IBDV circulating in the country poultry population that is responsible for the persistent post vaccinal outbreaks been experienced in the field and also ascertain if reassortant IBD viruses which have been implicated in IBD vaccination failure are present in Nigerian poultry.

1.2 Aim of the study

To carry out molecular characterization on the current Infectious bursal disease viruses responsible for post vaccinal outbreaks in poultry birds in Nigeria.

The objectives are to determine;

1) the distribution of IBD in chickens in some states of Nigeria with respect to host, place and time,

2) the molecular characteristics of IBDV responsible for wide spread mortality and morbidity in Nigeria and examine the sequences of the IBDV obtained for possible antigenic drifts or shifts,

3) the phylogenetic relationship/relatedness between the current Nigerian IBDV and IBDV strains from other parts of the world,

4) if reassortant IBDV strains are present in Nigeria poultry population, and

5) the phylogenetic relationship between the Nigeria reassortant IBDV and others in the GenBank.

CHAPTER 2

LITERATURE REVIEW

2.1 History of Infectious Bursal Disease Virus

Infectious bursal disease (IBD) was first recognized as a distinct clinical entity in 1957 (Cosgrove, 1962). IBD is a highly contagious viral disease of young chickens. The disease has a predilection for the bursa of Fabricius where it causes the destruction of lymphocyte cells in the bursa thereby leading to severe immunosuppression. Initially, Cosgrove (1962) described the malady as "avian nephrosis" on account of the tubular degenerative lesions found in the kidneys of infected broiler chickens. The syndrome adopted the name "Gumboro disease" since the first outbreaks occurred in and around the area of Gumboro, Delaware, USA. Predominant signs of illness included trembling, ruffled feathers, watery diarrhea, anorexia, depression, severe prostration, and death. In addition, hemorrhages in the thigh and leg muscles, increased mucus in the intestine, liver lobe infarction, renal damage, and enlargement of the bursa of Fabricius were lesions commonly observed at necropsy (Cosgrove, 1962).

Early studies suggested that the causative agent was a nephropathogenic strain of infectious bronchitis virus (IBV) due to similar gross changes observed in the kidney by Winterfield and Hitchner (1962). Hitchner (1970) proposed that the disease be termed "infectious bursal disease" due to its pathognomonic bursa lesions. Subsequent studies (Pejkovski *et al.*, 1979), however, revealed that IBV immunized birds could still be infected with the "infectious bursal agent" (IBA) and develop changes in their cloacal bursas specific for the disease. Following successful isolation of IBA in embryonated chicken eggs (Winterfield *et al.*, 1962), Allan *et al*, 1972 reported that IBD virus infections at an early age were immunosuppressive and the recognition of the immunosuppressive nature of the virus greatly increased the interest in the control of this infection (Eterradossi and Saif, 2008). In 1980, the existence of a second serotype was reported (McFerran *etal*., 1980). The control of the IBD was complicated by the recognition of "variant" strains of serotype 1 IBDV, which were first found in the Delmarva poultry producing area of the USA (Eterradossi and Saif, 2008). These strains were breaking through maternal immunity against "standard" strains, and they also differed from standard strains in their biological

properties (Rosenberger *et al.*, 1985; Rosenberger *et al.*, 1987). In the late 1980s, a very virulent strain of IBDV (vvIBDV) was isolated in the Netherlands, and quickly spread to Africa (Zierenberg *et al.*, 2000), Asia (Phong *et al.*, 2003; Sun *et al*., 2003) and lately to South America (van den Berg, 2000; Muller *et al.*, 2003; Eterradossi and Saif 2008) and more recently the United States(Jackwood *et al*., 2009; Stoute *etal.*, 2009). The overall characteristics of these new strains were: their appearance in vaccinated flocks, capability to induce high mortality (>60%) in specific-pathogen-free (SPF) chickens, and inability to grow in tissue culture. These viruses have been identified on almost every continent but have not been identified in Australia and New Zealand (Eterradossi and Saif 2008). Since its emergence, the virus has spread throughout most continents and by 1995, 80% of the member countries of the Office International Epizooties (OIE) reported the occurrence of vvIBDV (van den Berg, 2000). In West Africa severe acute IBD is no longer new. Before the emergence of the European vvIBDV strains, there have been several reports of unusually high IBD-induced mortalities in local or imported chickens (Gyening and Corkish, 1977; Okoye, 1987). However, the unusual severity of these African cases was attributed as much to concurrent aggravating factors such as climatic stress or coinfection with parasites, bacteria and other viruses than to any increased virulence of the local viruses (Eterradossi *et al.*, 1999). Very virulent (vv)IBDV has become an economically important pathogen in poultry industries worldwide (Hernandez *et al*., 2015).

2.2 History of IBDV in Nigeria

In Nigeria, IBD was first observed in 1969 in the West and Midwest regions and was first reported by Ojo in 1973 (Okoye and Uzoukwu, 2000., Abdu *etal*., 2001).Subsequent studies have shown that the disease has acquired endemic status among the Nigerian poultry populations (Abdu *et al*., 2001; El- Yuguda and Baba, 2004; Mai *etal.*, 2004; Oluwayelu *et al.*, 2007; Usman and Diarra, 2008). Outbreaks of IBD have been reported in all parts of the country in commercial and locally kept backyard chickens (Adene *et al.*, 1985; Haruna *et al.*, 2003), in crossbred chickens (Oluwayelu *et al*, 2002), in indigenous Nigerian ducks (*Anas platyrhynchos*) (Oluwayelu *et al.*, 2007), in unvaccinated Turkey (Oni *et al*, 2009), in vaccinated and unvaccinated chickens (El-Yuguda and Baba, 2004). Serological evidences of IBD have been reported in exotic birds (Abdu *et al.*, 2001; El- Yuguda and Baba, 2004; Usman and Diarra,

2008) and village indigenous chickens (Oni *et al.*, 2008; Nwankiti *et al.*, 2010; Nwankiti *et al.*, 2010; Anosa and Eze, 2010; Okwor *etal.*, 2012), in pearl guinea fowls (Adewuyi *et al.*, 1989; Nwagu *et al.*, 1997) and in double-spurred francolin , *Francolin bicalcaratus* (Oluwayelu *et al.*, 2014). Cases of IBD have also been reported in chickens 14-20 weeks of age in Nigeria over the years (Okoye and Uzoukwu, 1981; Owoade and Durojaiye, 1995; Igbokwe *et al.*, 1996; Dashe *et al.*, 2009; Musa *et al.*, 2010; Shekaro and Josiah, 2015). Previous studies carried out in Nigeria have shown that experimental IBD is more severe in village chickens than in pullets and broilers of exotic breeds (Okoye and Aba-Adulugba, 1996; Okoye *et al.*, 1998; M-El-Elahi *et al*., 2001). The presence of vvIBD has been reported in Nigeria (Zierenberg *et al.*, 2000; Owoade *et al.*, 2004; Adamu *et al.*, 2011b; Adamu *et al.*, 2013; Luka *et al.*, 2014; Owolodun *et al.*, 2015) with mortality rate of between 57 – 74.2% (Ezeibe *et al.*, 2012; Luka *et al.*, 2014).

2.3 The economic importance of IBDV field infection

The economic importance of IBD is evident in two ways; first, 20% or greater percentage mortality can be caused by some virus strains in chickens aged 3 weeks and older. The second and most important is that IBDV causes prolonged immunosuppression in chickens infected at an early age, which leads to higher susceptibility to secondary infections and impediment to host responses to vaccines against IBD and other diseases (Eterradossi and Saif, 2008). The disease has a predilection for the bursa of Fabricius where virulent IBDV strains target N-glycosylated membrane protein receptors on actively dividing and differentiating IgM-bearing B-lymphocytes (Mahgoub, 2012). The severity and economic impact of the disease is influenced by a combination of factors such as age and breed of flock, levels of maternally derived antibodies, virus strain, vaccination protocol utilized, presence of concurrent pathogens as well as environmental and management factors (Wu *et al*, 2009). IBD virus is not zoonotic and has no public health significance (Eterradossi and Saif, 2008).

2.4 Taxonomy and Classification

Infectious bursal disease is classified as a member of the Birnaviridae family. Viruses in this family possess bi-segmented, double-stranded RNA (dsRNA) genomes (Delmas, 2015), hence the name *birnaviruses*. This family of virus infects vertebrates, insects, mollusks and rotifers

(Delmas *et al.*, 2011; Delmas, 2015). On the phylogenetic tree, the birnaviruses form seven divergent genetic clusters with four genera: *Aquabirnavirus* whose type species is infectious pancreatic necrosis virus (IPNV), which infects fish, mollusks, and crustaceans; *Avibirnavirus* whose type species is infectious bursal disease virus (IBDV), which infects birds; *Entomobirnavirus* whose type species is *Drosophila X virus* (DXV), which infects insects, Esperito Santo virus (ESV) and *Blosnavirus* whose type species is blotched snakehead virus, BSNV (Delmas *et al.*, 2004, 2011; Delmas, 2015). The other birnaviruses have been isolated from bivalve mollusks such as Tellina virus 1 (TV-1), and Oyster Virus (Kitamura *et al.*, 2000; Delmas, 2015), and Japanese eels (Lee *et al.*, 1999), the Rotifer birnavirus (RBV) and the Drosophila B virus (DBV), chicken proventricular necrosis virus (CPNV). Another virus family with two or three segmented double-stranded RNA genome but smaller than birnaviruses are the *Picobirnaviridae* (Delmas, 2015).

2.5 IBDV Structure

Infectious bursal disease virus is a single-shelled, non-enveloped virion with icosahedral symmetry and a diameter varying from 55-65nm (Plate 1) (Coulibaly *et al*., 2005). Buoyant density of complete particles in cesium chloride gradient has been reported to range from 1.31-1.34g/mL (Eterradossi and Saif 2008). Detailed analysis of images of negatively stained and shadowed preparations has suggested that the architecture of the capsid of IBDV is based on the geometry of askew T=13 icosahedral lattice of the right-handed type with a diameter of about 700 A (T=13d) (Coulibaly *et al.*, 2005). IBDV have been shown in recent structural studies to have a typical *laevo* icosahedral geometry (Coulibaly *et al.*, 2005; Pous *et al.*, 2005). The VP2 forms the outer capsid which consists of 260 trimers (Plate1b) while the VP3 makes up 20-40% of the virion protein content and associates with the VP1 which is present in both free and a genome-linked form known as genome-linked protein (VPg) and also assists the VP1 during replication (Ferrero *et al.*, 2015). IBD virions contain two dsRNA genomic segments; A and B (Delmas, 2015).

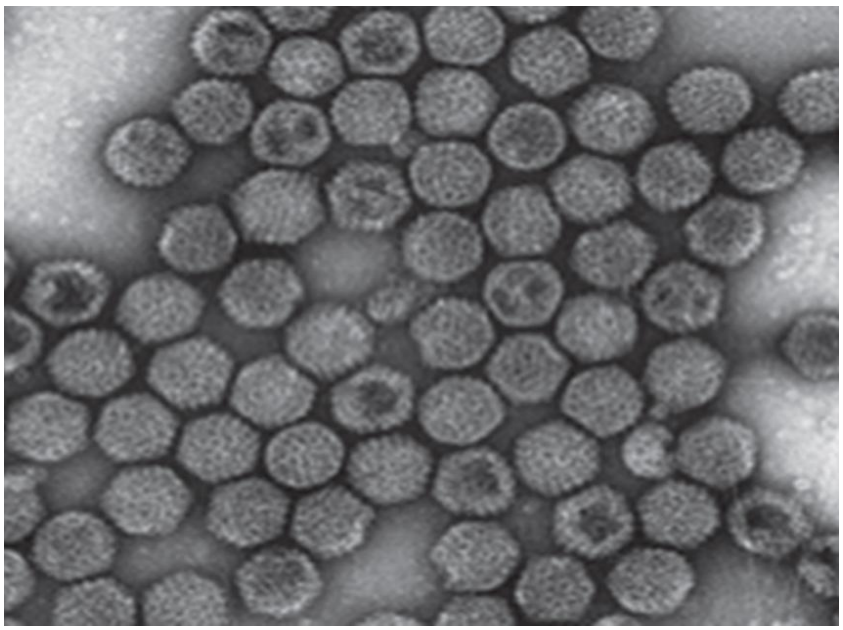

Plate1: Negative contrast electron micrograph of IBDV virions.Virions are about 60–70 nm in diameter. (Delmas, 2015)

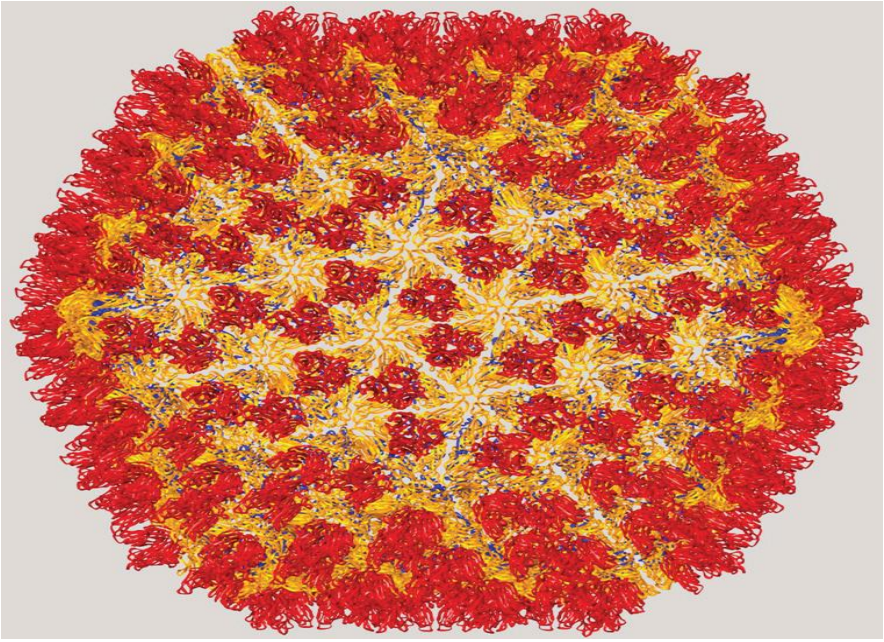

Plate 2: View of IBDV particle. The shell domains of VP2 is coloured in yellow and the projection in red. (Delmas, 2015)

2.6 IBDV Genomic Organization and Function

IBDV is a non-enveloped virus with a bisegmented dsRNA genome (Figure 1) (Delmas, 2015). Segment A (3.2kbp) contains two partially overlapping open reading frames (ORFs), the first of which codes for the nonstructural protein VP5 (145 residues with a M_r of 16,700) which is not essential for virus replication and with a virus-specific role in virulence (Delmas, 2015). The functions of VP5 during the virus cycle includes; targeting the plasma membrane and inducing cell lysis (Lombardo *et al.*, 2000), virus release (Wu *et al.*, 2009; Mendez *et al.*, 2015), binding to the regulatory subunit p85α of P13K preventing premature apoptosis (Wei *et al.*, 2011), being a major apoptosis inducer in DF-1 cells through interaction with the voltage-dependent anions channel 2 in the mitochondrion (Li *et al.*, 2012). The second ORF encodes polyprotein (91,012 residues; M_r of 109,700) that is trimmed by the viral Ser-Lys protease VP4 (Birghan *et al.*, 2000), providing three proteins: pVP2 (512 residues; M_r of 54,400; apparent M_r of ~48,000), VP3 (258 residues; M_r of 28,900; apparent M_r of 32,000 kDa), and VP4 (244 residues; M_r of 26,700). Most of the pVP2 C-terminal domain is further processed at three Ala-Ala bonds, which are secondary VP4 targets (positions 487, 494, and 501) (Sanchez and Rodriguez, 1999). The resulting intermediate pVP2 is again cleaved by an unknown mechanism between residues 441 and 442, giving the mature VP2 (441 residues; M_r of 47,100; apparent M_r of ~41,000). The pVP2 maturation process requires capsid assembly (Chevalier *et al.*, 2002), and the released C-terminal segment remains in its interior (Da Costa *et al.*, 2002). The VP3 plays some roles in the virus cycle by associating with the dsRNA genome, recruits VP1 into the capsid and interacts with the capsid protein precursor pVP2 to promote birnavirus morphogenesis (Delmas, 2015). Segment B (2.6kb) contains one large ORF encoding the VP1, the RNA-dependent RNA-polymerase (RdRp) responsible for the replication of the genome and synthesis of mRNAs (von Eniem *et al.*, 2004.; Pan *et al.*, 2007).

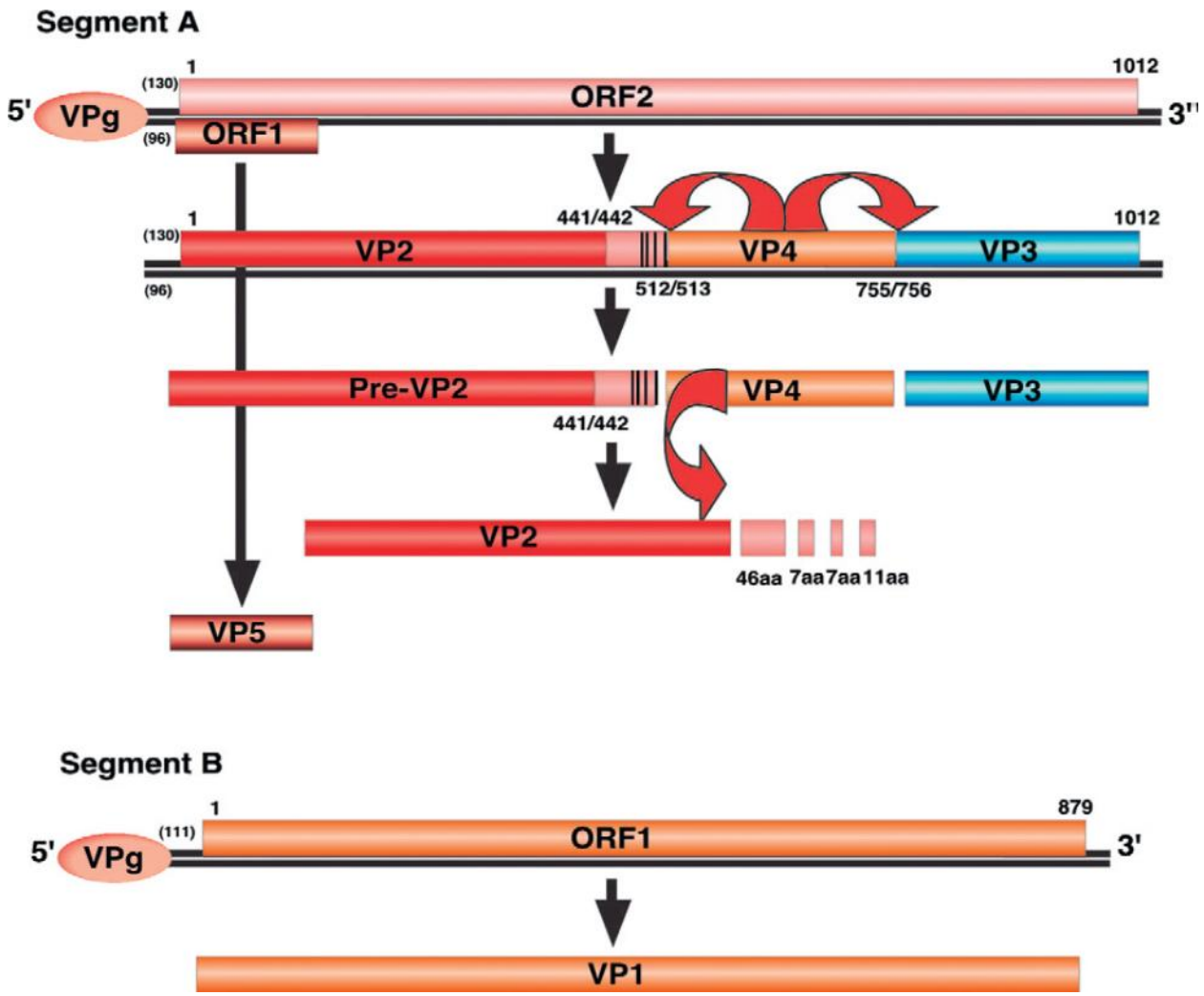

Figure 1: Schematic representation of the genome of infectious bursal disease virus (IBDV) and of the processing of the encoded proteins. ORF, opening reading frame; VP2 and VP3, capsid proteins; pre-VP2, precursor to VP2; VP1, RNA polymerase; VPg, genome linked VP1; VP4 protease; VP5 nonstructural protein; curved arrows indicate protease cleavage sites; numbers represent amino acid positions. Source: MacLachlan and Dubovi (2011).

2.6.1 VP2 protein

The VP2 protein encoded by the segment A of IBDV genome has been shown to be the major host-protective antigen (Lee *et al.*, 2003.; Yip *et al.*, 2007.; Villegas *et al*., 2008) and contains the major antigenic site responsible for eliciting neutralizing antibodies (Jackwood and Sommer-Wagner, 2011) and is located in the highly variable region (P domain) between amino acids 206-350 (Cui *et al.*, 2003.; Martinez-Torrecuadrada *et al.*, 2003.; Letzel *et al.*, 2007). The VP2 is folded into three domains: the base (B), shell (S), and projection (P) domains (Coulibaly *et al.*, 2005.; Pous *et al*., 2005.; Garriga *et al.*, 2006.; Lee *et al.*, 2006.; Letzel *et al.*, 2007.; Luque *et al*., 2007) The base and shell domains are formed by the conserved N- and C-terminal of VP2. The projection domain is the middle part containing the host cell receptor binding motifs and the hypervariable region of VP2 (hVP2) as well as being the antigenic site, virulence and cell culture adaptation determinants (amino acids 206 – 350) (Delmas, 2015). Within the hVP2 region, two major hydrophilic peaks known as A and B are recognized that are located at the outer most part of the P domain. Hydrophilic peak A spans amino acids 212 to 224 and peak B spans amino acids 314 to 325 (Schnitzler *et al.*, 1993). These regions form two loops: P_{BC} (aa 219 to 224) and P_{HI} (aa 316 to 324) (Coulibaly *et al.*, 2005), respectively which are the neutralizing antibody-binding domain. They also play an important role in cell culture adaptation and virulence. Two other loops known as P_{DE} (aa 249 to 254) and P_{FG} (aa 279 to 284) (Coulibaly *et al.*, 2005), have also been identified. The VP2 also has two minor hydrophilic peaks known as peak 1 that spans amino acids 248 to 254 and peak 2 that spans amino acids 279 to 290. The VP2 hypervariable region has the highest amino acid variation among serotype 1 strains (Jackwood and Sommer, 2005.; Kasanga *et al.*, 2007) and is the region mostly used for molecular diagnosis and genotyping of Infectious bursal disease viruses and it consists of sequence regions uniquely conserved to vvIBDV. Exchanges of amino acid residues in VP2 occur in the four hydrophilic loops of the viral capsid (Coulibaly *et al.*, 2005) and shows that the selective pressure for the evolution of IBDV is concentrated on the regions that are open and interacts with the immune system (Durairaj *et al.*, 2011). Amino acid changes in this region can affect the antigenicity of the virus (Jackwood and Sommer-Wagner, 2011).The putative amino acids responsible for virulence and cellular tropism were identified to be glutamine (Q) at amino acid position 253, aspartic acid (D) at amino acid position 279 and alanine (A) at amino acid position 284. Reverse

genetics studies have also revealed that amino acid at positions 249 and 256 are involved in IBDV replication efficiency and virulence. The very virulent (vv)IBDV displays a presumed genetic fingerprint in the VP2 hypervariable region at amino acid residues 222 (Ala), 242 (Ile), 256 (Ile), 294 (Ile) and 299 (Ser) (Hoque *et al.*, 2001.; Rudd *et al.*, 2002.,; Banda and Villegas, 2004.; Jackwood *et al.*, 2008.; Islam *et al.*, 2012.; He *et al.*, 2012.; Xu *et al*., 2015). Other amino acids conserved in vvIBDV are 249 (Glu), 253 (Glu), 254 (Gly), 279 (Asp), 284 (Ala), and 330 (Ser) (Xu et al., 2015). Avirulent IBDV have the following conserved amino acids; 222P, 256V, 279N, 284T, 294L and 299N while 249Q and 254G are seen in classical IBDV strains (He *et al.*, 2012), 222T, 249K and 254S are seen in variant strains while attenuated strains have 253H, 279N, 284T and 330R/K (Xia *et al.*, 2008). Located downstream after the second hydrophilic peak is a motif consisting of seven amino acid sequences SWSASGS also known as the serine-rich heptapeptide region. These sequences are conserved for vvIBDV, variant and vaccine strains. The region have been proposed by many workers as a confirmation of the highly virulent nature or virulence signature of vvIBDV (Mohamed *et al.*, 2014.; Xu *et al*., 2015).

2.6.2 VP1 protein

The VP1 protein is the only viral protein encoded by the segment B of IBDV. It is the RNA-dependent RNA polymerase (RdRp) that is responsible for viral genome replication and messenger RNA synthesis. It is found as a free protein in the viral capsid and is linked covalently to the 5´ end of the genomic RNA segment. The crystal structure of VP1 has been characterized (Pan *et al.*, 2007), and is made up of three domains: the N- terminal domain that spans amino acids 1 – 167 which possesses the putative self-guanylylation site and is responsible for protein priming. The central polymerase domain that spans amino acids 168 – 658 which folds to form a ‘fingers’, ‘palm’ and ‘thumb’ subdomains, contains all the structural motifs of RdRp which are involved in nucleotide recognition and binding. The C- terminal domain which spans amino acids 659 – 878, prevents back-primed RNA synthesis during protein priming (Garriga *et al.*, 2007.; Pan *et al.*, 2007). Phylogenetic studies of IBDV strains shows that the genome segment B of the vvIBDV is conserved and clearly different from non-vvIBDV (Islam *et al.*, 2001.; Jackwood *et al.*, 2012). Due to the bi-segmented nature of IBDV, homologous and non-homologous recombination and reassortment can take place in their genome. Hon *et al* (2006), showed that very virulent IBDV emerged after a reassorting of genome segment A with an

unidentified segment B. Reverse genetics studies have shown that VP1 also contributes to the virulence of IBDV (Boot *et al.*, 2000.; Le Nouen *et al.*, 2012.; Escaffe *et al.*, 2013.; Gao *et al.*, 2014). Multiple sequence analysis revealed that VP1 of vvIBDV strains i.e. D6948, UK661, HK46, OKYM contains 17 characteristic amino acid residues (4V, 13K, 61I, 145T,146D,147N, 242E, 287A, 390M, 508K, 511S, 546P, 562P, 646S, 687P, 695R) (Cui *et al.*, 2013). Yu *et al.*, 2010, reported similar sequences at positions 4, 61, 145, 287, 508, 511, 646 and 687 for seven vvIBDV isolated from China while the non-vvIBDV had 4I, 61V, 145N, 287T, 508R, 511R, 646G and 687S. High frequency of mutation is observed at the triplet amino acid position 145, 146 and 147 (Jackwood *et al.*, 2012). Phylogenetic analysis has revealed several lineages of segment B of IBDV, with the vvIBDV clustering separately from the non-vvIBDV (Wei *et al.*, 2006.; Gao *et al.*, 2007.; Jackwood *et al.*, 2012).

2.7 IBDV pathotypes

Based on virulence in susceptible chickens, IBDV serotype 1 strains were broadly classified into three pathotypes: classic, very virulent (vv), and variant strains. Classic IBDV strains induces approximately 10-50 % mortality and cause strong inflammatory response in the BF, and chickens infected with virulent classical IBDV show clinical signs (Cosgrove, 1962.; Kaufer and Weiss, 1980). In contrast, variant IBDV strains result in atrophy of the BF in the absence of any inflammatory changes (Rosenberger and Cloud, 1986.; Sharma *et al.*, 1989). The subclinical disease caused by variant IBDVs may almost not be noticed and at times only an increase in the incidence of respiratory diseases might be observed (Rosenberger and Cloud, 1986). The vvIBDV strain induces 50 – 100% mortality with typical signs and lesions (Eterradossi and Saif, 2008).

2.8 Pathogenesis and pathology of infectious bursal disease

Pathogenesis can be defined as the biological mechanism used by viruses to cause harm to the host with disease or death as a consequence (van den Berg, 2000). The most obvious feature of the pathogenesis and pathology of infectious bursal disease is the particular replication of virus in the cloacal bursa, which becomes grossly enlarged than its normal size 3-4 days after infection, with edema, hyperemia, and prominent longitudinal striations. Due to the massive

destruction of the lymphocytes by apoptosis and necrosis, the lymphoid follicles of the bursa of fabricius collapses with depleted lymphocytes observed in the bursa of birds that survived the infection. Very virulent virus strains also produce depletion of cells in the thymus, spleen, and bone marrow. Hemorrhages occur beneath the serosa, and there are necrotic foci throughout the bursal parenchyma. At the time of death, the bursa may be atrophic, and the kidneys enlarged from accumulation of urate secondary to dehydration. The outcome of IBDV infection is dependent on the strain and amount of the infecting virus, the age and breed of the birds, route of inoculation and presence or absence of neutralizing antibodies (Muller *et al*., 2003). The cecal tonsils and bone marrow may serve as non-bursal lymphoid tissues supporting virus replication at later time points (Elankumaran *et al*., 2002). Ma *et al*. (2013) recently described the effects of IBDV on Kupffer cells, macrophages found in the lining of the liver, suggesting that the liver is a site of IBDV infection and therefore possibly the innate immune response.

In the bursae of chickens infected with IBDV, productive viral replication is often associated with necrosis, apoptosis of lymphoid cells, inflammatory change, atrophy and hemorrhages (Van den Berg, 2000.; Kim *et al*., 2000.; Taylor *et al*., 2008).

The route of IBDV infection is usually oral (from contaminated food, water or excrements), but may be via the eye or respiratory tract, with an incubation period of 2-3 days. After oral infection, the virus replicates in gut-associated macrophages and lymphoid cells and enters the portal circulation, leading to primary viraemia (Mahgoub, 2012). Viral antigen can be detected in the macrophages and lymphoid cells of the caecum as early as 4 hours postinfection (hpi), reaching the liver by 5 hpi, andenters the blood stream where it causes primary viraemia. The virus is transported to the bursa of Fabricius and can be detected in the BF by 11 hpi (Eterradossi and Saif 2008). After replication of IBDV in the BF, the virus enters the blood stream causing secondary viraemia, which results in the spread of the virus to other tissues (Eterradossi and Saif 2008). The process of infection with vvIBDVs is the same except that replication at each stage is intensified (van den Berg, 2000). The cloacal bursa plays a central role in pathogenesis, because bursectomized birds survive otherwise lethal infection without developing clinical signs or disease (Kaufer and Weiss, 1980.; Hiraga *et al.*, 1994). However, the stage of differentiation of B lymphocytes in the cloacal bursa is crucial in supporting maximum virus replication, as only

non-immunoglobulin-bearing B lymphoblasts or IgM-bearing B lymphocytes support virus replication, whereas stem cells and peripheral B cells do not.

In the course of infection, surface immunoglobulin M-bearing B cells undergoing division are lysed, cells of the monocyte–macrophage lineage are infected and they play an important role in dissemination of the virus and in disease onset (van den Berg, 2000; Teshome *et al*., 2015). Apoptosis also known as programmed cell death is a mechanism used by cells in response to a particular stimulusdie in a controlled mode and it plays a crucial role in IBDV pathogenesis and immunosuppression(Mahgoub, 2012).In chickens infected with serotype I IBDV strain L, there was evidence of high level of apoptotic activities in the peripheral blood lymphocytes (Vasconcelos and Lam, 1994), and peripheral blood lymphocytes showed a high apoptotic index (nuclear fragmentation and cellular breakdown into apoptotic vesicles). Results of previous studies indicated a correlation between IBDV replication and the proportion of apoptosis in bursal cells (Nieper *et al*., 1999.; Jungmann *et al*., 2001). Remarkably, apoptotic cells were frequently located in the vicinity of antigen-expressing cells (Tanimura and Sharma, 1998.; Jungmann *et al*., 2001.; Taylor *et al*., 2008). This suggests the involvement of apoptotic processes in the pathogenesis of IBDV infection. The small, non-structural protein VP5 performs the function of inhibiting apoptosis initiated by viral replication and prevents infected cells from undergoing cell death before the virus finishes its life cycle (Liu and Vakharia, 2006.; Wei *et al.*, 2011). Studies of the genes expressed during acute IBD infection demonstrated the activation of spleen macrophages and bursal T cells (Kim *et al.*, 2000). Low level of expression of B-cell-specific genes in relation with B-cell depletion enhanced the expression of numerous genes related with the activation of macrophages, T cells and NK cells. Genes upregulated during the host response includes Th1 [interferon (IFN)-g, interleukins (IL)-2 and IL-12p40] and Th2 (IL-4, IL-5, IL-13 and IL-10), chIFN-c, *IL-6*, *IL-8*, *MX1*, *IFIT5*, *TLR3*, *NOS2A*, and Chondroitin Sulfate *N*-acetylgalactosaminyltransferase-2 (CSGalNAcT2) that had already been identified as being upregulated after IBDV infection (Liu *et al.*, 2010.; Rauf *et al.*, 2011.; Zhang *et al.*, 2015). Others are matrix metalloproteins *MMP9*, *MMP7*, *MMP1*, and *MMP3*, the chemokines *CCLi7*, CCL5 MIP family chemokine (RANTES) (*CCLi4*), *CCL5*, and CCL19 homeostatic chemokine (*CCL19*), the interferon (IFN)-induced transmembrane proteins *IFITM1*, *IFITM3*, and *IFITM5*, as well as *GBP*, *ART1*, *PDIP1*, *OASL*, *CSF3*, *IRF10*, and *TLR15*, heat shock proteins (HSP70

and 47). Genes downregulated during the early host response includes Biliverdin reductase A (*BLVRA*), Myeloblastosis oncogene-like 1 (*MYBL1*), Activation-induced cytidine deaminase (*AICDA*), Serum response factor binding protein 1 (*SFRP1*), *VPREB3*, *SFTPA2*, and Toll-like receptor 7 (*TLR7*) (Smith *et al.*, 2015). Studies carried out on genes expressed during IBDV infection in resistant and susceptible chickens lines by Smith *etal*, (2015) showed that (MHC class II antigen B-F minor heavy chain (*BLB1*) , Caspase recruitment domain family, member 9 (*CARD9*), *BLVRA*, *AICDA*, *MYBL1*, *SFRP1*, *B6.3*, *VPREB3* and *MMP13*, were highly expressed in the resistant chickens while *MMP9*, *IFNA*, *CCL5*, *MMP7*, *AVD*, *NOS2A*, *CXCLi2*, *IL-6*, *LYG2*, *GBP*, *CCL19*, *IFITM1*, *IFITM3*, and *IFITM5* were more highly expressed in the susceptible chickens. They also assumed that some of these genes may play an important role in the resistance and susceptibility of chickens to IBDV infection. Another gene of the cytokine family that is downregulated during IBDV infection is chCD132 (Wang *et al.*, 2014).

Macroscopically, birds that succumb to the infection are dehydrated, with dark hemorrhages on the thigh and pectoral muscles (Teshome *et al*., 2015). In the intestine, mucus is increased and renal change is prominent (Mahgoub, 2012). The target organ which is the bursa by 3 dpi begins to increase in size and weight due to edema and hyperemia and reaches double it normal weight by 4dpi before the size begins to recede and returns to its normal weight by 5 dpi but the size continues to atrophy and is one-third of its original weight or less from day 8 forward. A yellowish transudate starts to cover the bursa of Fabricius at 2 or 3 dpi and disappears as the bursa returns to its normal size. Some bursae show extensive hemorrhage and in such cases birds may void blood in their droppings. The spleens are enlarged with small gray foci uniformly dispersed on the surface (Teshome *et al*., 2015). At the proventiculus and gizzard mucosal junctions, hemorrhages are occasionally observed. vvIBDV strains cause greater decrease in thymic weight index with more severe lesions in the cecal tonsils, thymus, spleen and bone marrow but with similar bursal lesions.

Microscopically, lesions of IBD occur primarily in the lymphoid tissues with changes most obvious in the clocal bursa (Wang *et al.*, 2010.; Murum *et al.*, 2014). Degeneration and necrosis of lymphocytes start in the medullary area of the bursal follicles as early as 1 dpi (Lukert and Saif, 1997). Lymphocytes become replaced by heterophils, pyknotic debris, and hyperplastic

reticuloendothelial cells. By day 3 or 4 postinfection, all of the lymphoid follicles are affected. The increased bursa weight observed is due to severe oedema, hyperaemia, and marked accumulation of heterophils. Cystic cavities brought about by necrosis and phagocytosis develops in the follicular medulla as inflammation reaction reduces. Bursal tissue fibroplasia are prominent during atrophy of the bursa.(Eterradossi and Saif, 2008.; Mahgoub, 2012).

2.9 Immune response to IBDV

IBDV affects young birds, with the disease usually being diagnosed in 3- to 6-week-old birds. The disease peaks between 2 to 5 days dpi and clears out by day 7 (Ruby *et al.*, 2006). During this acute phase, bursal follicles are depleted of B cells, and the bursa becomes atrophic, which results in a diminished antibody response and increases the susceptibility of the surviving chicken to secondary infections. Abundant viral antigen can be detected in the bursal follicles and other lymphoid organs such as the cecal tonsils and spleen with CD4+ and CD8+ cells accumulating at the site of virus replication (Sharma *et al.*, 2000)

Following IBDV infection, depletion of B cells occurs in the bursal cortex and medulla, peripheral blood and thymic medulla (Mahgoub, 2012). Loss of surface antigen is also observed in the bursal epithelial cells and it has been reported that T cells are not susceptible to infection. Kim *et al*. (2000) showed that IBDV-infected bursa of Fabricius were infiltrated with $CD4^+$ and $CD8^+$ T cells starting from 4 dpi and kept on increasing until T cells represented 65 % of the bursal cell population at 7 dpi. In some recent reports, Withers *et al*. (2005), reported that at 1 dpi, the numbers of $CD4^+$ cells, $CD8^+$ cells, and macrophages increased in the BF following IBDV infection while Yu *et al*. (2015) in their investigation on the changes in CD4+CD25+ cells in lymph organs, e.g., the thymus, spleen, bursa of fabricius and peripheral blood during the first 1-5 days post infection of chickens with IBDV revealed a remarkable decrease in the percentage of CD4+CD25+ cells in the thymus from 1-5 dpi and in the spleen during early infection. Likewise they reported an increase in the percentage of CD4+CD25+ cells in the peripheral blood lymphocytes at 2dpi with CD4+CD25+ cells infiltrating the bursa along with CD4+ cell after infection, thereby causing a significant increase in interleukin (IL) 10 with the Harbin-1 strain of IBDV inducing higher IL-10 expression than the Ts strain thereby suggesting that CD4+CD25+ cells may play a role in IBDV pathogenicity by migrating from their sites of origin

and storage, the thymus and spleen, to the virally targeted bursa of Fabricius during IBDV infection. Birds that survive acute IBDV infection have the depleted bursal follicles repopulated with $IgM^{+}B$ cells.

2.10 Clinical signs

Young chickens aged 3 – 6 weeks are mostly susceptible to clinical infection of IBDV (van den Berg, 2000). IBDV is acute with short incubation period and clinical signs are seen within 2 – 3 days after infection. The disease lasts for 3 to 4 days after which the surviving birds recover rapidly (Mahgoub, 2012). An earliest sign of infection is the tendency of the birds to pick at their own vents. Other clinical signs involve whitish or watery diarrhea, anorexia, depression, ruffled feathers, trembling, severe prostration and death (Eterradossi and Saif 2008). Depending on the infecting strain virulence and the degree of protection in the flock infected, amount of infecting virus, age, breed of bird and route of inoculation, mortality rate can be as high as 100% (Müller, *et al*., 2003).. With the vvIBDV strain, mortality rates can be 100% in SPF flocks, 60% in layers and 30% in broilers (van den Berg, 2000).

2.11 Immunosuppression

Immunosuppression involves an act that reduces the activation or efficacy of the immune system. Infection of chicken at 1 to 2 weeks of age with IBDV causes immunosuppression thereby making the chicken fully susceptible to other viral, bacterial, protozoan and parasitic infections (Eterradossi and Saif, 2008). Immunosuppression induced by IBDV also causes vaccination failure to Newcastle disease vaccine and other vaccines (Eterradossi and Saif 2008, Nwagbo *et al.*, 2010). Other diseases that can cause immunosuppression in chicken apart from IBDV are Marek's disease, chicken anemia virus and reovirus. Other factors that are not disease agents that can cause immunosuppression in poultry includes food borne mycotoxins, exposure to ammonia, suboptimal nutrition and varying concentration of arginine supplementation (Tan *et al.*, 2015).

2.12 Isolation and detection of IBDV

For the isolation of IBDV, the organ of choice for the recovery of the virus is the bursa of Fabricius. Other organs such as the spleen may contain the virus but the concentration is usually

low when compared with bursa. Although IBDV isolation is not done routinely, when it becomes necessary to isolate the virus, host systems such as embryonated eggs especially from specific-pathogen-free (SPF) flocks (Eterradossi and Saif, 2008) and chickens (specific-pathogen-free (SPF)) are recommended. Of all the substrates used for the isolation of IBDV, the 9-11 day old embryonating egg is the most sensitive with the chorioallantoic membrane (CAM) the route for virus inoculation for all strains of IBDV (Eterradossi and Saif, 2008). VvIBDV strains induce embryo mortality within 3-5 days post inoculation with classic strain inducing erratic lower mortality (Eterradossi and Saif, 2008) while variant strains produce negligible mortality (Rosenberger *et al.*, 1985). The lesions observed in the embryo after infection include edema in the abdominal region, cutaneous congestion and petechial and ecchymotic hemorrhages (on cerebrum, toes and feather tracts, liver), pale "parboiled" appearance of the heart congestion in the kidney and lungs with mottled necrosis in the kidney, pale spleen with small necrotic foci. Small hemorrhagic areas are occasionally observed on the CAM but no plaques. The lesions seen on the embryo infected with the variant strain of IBDV includes; splenomegaly, liver necrosis, cerebral and abdominal edema and stunting.

Many strains of IBDV have been adapted to grow in tissue culture cell lines thereby facilitating quantitation of the virus by plaque assay or microtitre techniques. Cell lines used for IBDV isolation are; chicken embryo bursa, chicken embryo fibroblast (Eterradossi and Saif, 2008), turkey and duck embryo (van Loon *et al.*, 2002), mammalian cell lines derived from rabbit kidney (RK-13) (Rinaldi *et al.*, 1972), chicken embryo kidney, continuous fibroblast cell line of Japanese quail origin (QT35), baby hamster kidney (El-Ebriary *et al*., 1997), ovine kidney (Kibenge and MuKenna, 1992), normal chicken lymphocytes, B-cell lymphoblastoid, baby grivet monkey kidney (BGM-70) and M4-104 cells derived from foetus of rhesus monkey kidney (Jackwood *et al*.,1987), Vero cells (made from kidney of African green monkey (Jackwood *et al.*, 1987.; Peilin *et al*., 1997), DF-1 cell line (Rekha *et al.*, 2014). The BGM-70 cell line has been reported to be successful in supporting several strains of serotype 1 and 2 IBDV (Ley *et al.*, 1983.; Jackwood *et al.*, 1987). Very virulent strain of IBDV from the field is difficult to isolate and propagate in primary or secondary cell cultures of chicken embryo origin. The inability of vvIBDV to adapt in cell cultures may be due to the differences in field strains of the virus (Mannan *et al.*, 2009) and the process of trying to adapt vvIBDV to cell culture leads to

attenuation of the virus and a reduction in the ability of the virus to replicate in the bursa of Fabricius. Some cell lines have been used to isolate and propagate vvIBDV e.g. chicken mesenchymal cell (Mohammed *et al.*, 2012), lymphoid leukosis tumor cell lines (Hassan *et al.*, 2015).

For IBDV detection, and in order to differentiate between serotype 1 or 2 of IBDV, or between subtypes 1 or pathotypes, a number of techniques such as serology and molecular techniques can be used. The serological techniques used for IBD detection include; Agar gel immunodiffusion (AGID) test (Hirai *et al.*, 1972., Cullen and Wyeth, 1975) which can be used to detect IBDV group-specific antigen, virus neutralization (VN) test could be used to identify the virus in embryos or in tissue culture after adaptation to these host systems, serum neutralization in cell culture (Weisman and Hitchner, 1978), antigen and antibody capture enzyme linked immunosorbent assay (AC-ELISA) test for detecting and characterizing IBDV isolates (Marquardt *et al.*, 1980.; Meulemans *et al.*, 1987.; Snyder *et al.*, 1992). Polyclonal antibodies can also be utilized in the AC-ELISA and may be more effective for general screening of tissue samples for IBDV (Lombardo *et al.*, 2000), immunofluoresence and immunocytochemistry for detection of viral antigens in infected tissues. (Meuleman *et al.*, 1977). Strains of IBDV can be differentiated on the basis of pathotype and antigenic configuration. There are at least two distinct serotypes and several antigenic subtypes that can be identified by cross-neutralization and cross challenge tests. Isolates of IBDV may vary in pathogenicity from being nonpathogenic to virulent (Lombardo *et al.*, 2000). Molecular analysis and *in vivo* pathogenicity studies are also necessary in IBDV detection so as to monitor evolutionary changes and the emergence of quasispecies in the field viruses. Molecular techniques used for IBD detection include; reverse transcriptase-polymerase chain reaction (RT-PCR) including multiplex PCR and real-time PCR, restriction fragment length polymorphism (RFLP) used for molecular differentiation of IBDV strains (Jackwood and Sommer, 1999.; Zierenberg *et al.*, 2000.; Sapats and Ignjatovic, 2002), restriction endonuclease (RE) digestion, reverse transcription coupled with loop-mediated isothermal amplification (RT-LAMP) in one step (Xue *et al.*, 2009.; Xu *et al.*, 2009.; Kiatpathomchai *et al.*, 2007.; Chen *et al.*, 2008.; Peyrefitte *et al.*, 2008.; Li *et al.*, 2009.; Le Roux *et al.*, 2009) and nucleotide and deduced amino acid sequence analysis used to investigate

antigenic and genetic relationship between virus strains and/or isolates and also to explain the links between emergence, spreading and maintenance of the vvIBDV strains around the world.

2.13 IBDV reassortment

Reassortment is a form of genetic recombination that occurs in RNA viruses with segmented genomes, whether these are single- or double-stranded and whether these involve few or many segments. Reassortment has been documented in families with 2 (*Arenaviridae* and *Birnaviridae*), 3 (*Bunyaviridae*), 6, 7, or 8 (*Orthomyxoviridae*), or 10, 11, or 12 (family *Reoviridae*) genome segments. When two viruses that are related infect a cell, there is the likelihood of exchange of genetic materials which results in the production of viable and stable reassortants. Reassortment is a natural phenomenon and serves as a crucial means of genetic variability. High mutation rate, short generation time and high progeny yield are some of the characteristics of double-stranded RNA viruses (Domingo and Holland, 1997). Apart from the mutations introduced by their error-prone polymerases, genetic variations in RNA viruses is brought about by homologous and non-homologous recombination and reassortment in viruses with segmented genomes (Pita *et al.*, 2001.; Kew *et al.*, 2002.; Wei *et al.*, 2006). Reassortment of segmented viruses gives rise to new strains of viruses with the ability to evade immunological attacks (Diebold *et al.*, 2004.; Kobasa *et al.*, 2007.; Maines *et al.*, 2008.; Greenbaum *et al.*, 2009.; Jimenez_Baranda *et al.*, 2011). Genomic reassortment has been demonstrated for many animal RNA viruses such as influenza virus (Kilbourne, 2006.; Taubenberger and Morens, 2006.; Wallensten *et al.*, 2005), Rift valley fever virus (Bowen *et al.*, 2001), Jatobal virus (Saeed*et al.*, 2001), Cucumber mosaic virus (Roossinck, 2002), in plant viruses like Rice grassy stunt virus (Miranda *et al.*, 2000), Tobravirus (Robinson *et al.*, 1987), Cucumovirus (White*etal.*, 1995). Bluetongue virus strains are often reassortants, sometimes containing genes similar or identical to those of live-attenuated vaccine viruses.

Reassortment events have been reported in infectious bursal disease virus (IBDV) (Le Nouën *et al.*, 2006.; Hon *et al.*, 2006.; Wu *et al.*, 2007.; Gao *et al.*, 2007.; Jackwood *et al.*, 2011.; Jackwood *et al.*, 2012.; Chen *et al.*, 2012). The possible model/mechanism of genomic reassortment of IBDV in nature was from the co-infection of two viruses, vvIBDV, and the attenuated live vaccine strains (Wei *et al.*, 2006). Hon *et al*, (2006), showed that the emergence

of very virulent IBDV came about after reassortment activities between the genome A and undocumented segment B of IBDV. Further studies indicated that the genome segment B of vvIBDV was highly conserved and distinct from non-vvIBDV (Gao *et al.*, 2007.; Hon *et al.*, 2008.; Le Nouën *et al.*, 2006). Initially, the VP2 was thought to be the singular determinant of virulence in IBDV, but the reassortment events that occurred between the genome segment A and B of IBDV which gave rise to the very virulent IBDV strains (Hon *et al.*, 2006.; Le Nouen *et al.*, 2006) suggests that both the major capsid protein (VP2) and the RNA-dependent RNA polymerase (VP1) located on genome segment A and B respectively, contribute to the virulence of IBDV (Boot *et al.*, 2000.; van Loon *et al.*, 2002.; Liu and Vakharia, 2004.; Boot *et al.*, 2005), thereby signifying that the VP2 of vvIBDV is not the only determinant for their pathogenicity (Boot *et al.*, 2000.; Le Nouen *et al.*, 2012.; Escaffre *et al.*, 2013). Many natural reassortant IBDV isolates that occurred during field outbreaks have been characterized (Sun *et al.*, 2003.; Hon *et al.*, 2006.; Le Nouen *et al.*, 2006.; Wei *et al.*, 2006.; Wei *et al.*, 2008.; Jackwood *etal.*, 2011.; Kasanga *et al.*, 2012), and most of them have a segment A of vvIBDV strain and segment B from attenuated IBDV strains (Sun *et al.*, 2003.; Hon *et al.*, 2006.; Le Nouen *et al.*, 2006.; Wei *et al.*, 2006.; Gao *et al.*, 2007.; Wei *et al.*, 2008.; Jackwood *et al.*, 2011.; Kasanga *et al.*, 2012). Wei *et al*, (2006, 2008) reported a natural reassortant IBDV with a segment A derived from an attenuated strain with segment B derived from a very virulent strain. Attempts to isolate reassortant virus by experimentally co-infecting chickens with vvIBDV and attenuated IBDV was not successful therefore reassortment event may be more than just the co-infection of two types of IBDV.It is documented that vvIBDV cannot directly adapt to tissue culture (Brandt *et al*., 2001) so the possibility of vvIBDV to provide its segment A for reassortment in cell culture is not possible, so the event of reassortment likely occurs in the bursa tissues only. The process of reassortment may be more complex in the field than expected and factors such as vaccine pressure, environment, and time may contribute to the complexity of the process.

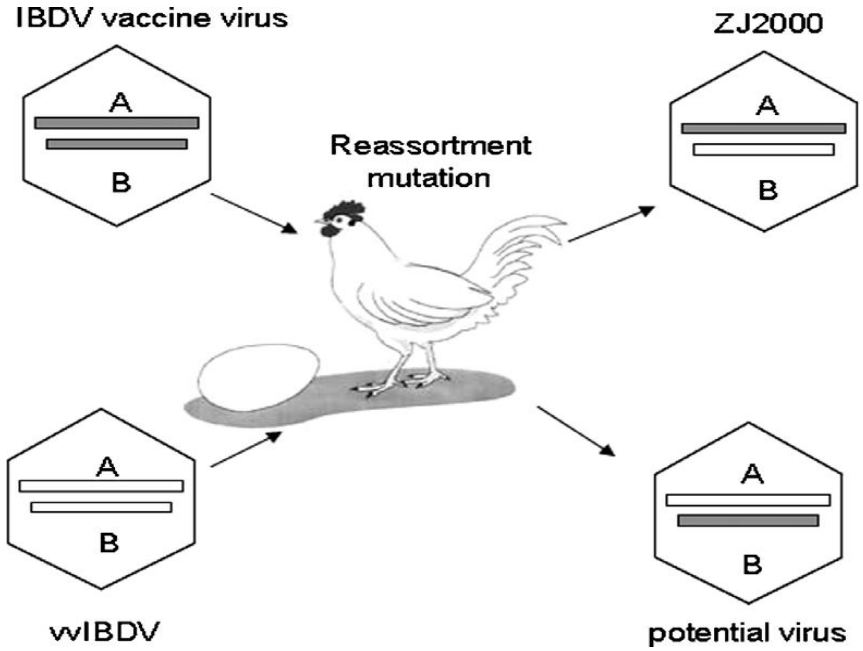

Figure 2: Model of genomic reassortment of IBDV in nature. (Wei *et al*, 2006.)

2.14 IBDV recombination

Recombination is very common with positive-sense RNA viruses (Chare and Holmes, 2006) but not with negative-sense RNA viruses. This is due to the fact that their negative-sense genome is not readily available for template-switching owing to encapsidation by ribonucleoprotein complex (Chare *et al*., 2003).Natural homologous intragenic recombination is described for many animal viruses (Lee *et al*., 2013) and homologous recombination can play an important role in the evolution of some RNA viruses. In studies on recombination in the Birnaviridae family, putative homologous recombinants were identified with recombination events detected in both genome segment A and B, with all the viruses with recombination events in genome segment A involving attenuated vaccine strains of IBDV in one of the studies. One of the recombination events occurred between a vaccine virus and a variant IBDV strain, whereas the other recombination events involved vvIBDV and attenuated vaccine strains of the virus (Hon *et al*, 2008.; He *et al.*, 2009). In IBDV, antigenic drift, reassorting of genome fragments, and homologous recombination have led to numerous pathogenic, antigenic variations and emergence of novel vvIBDV groups (Wei *et al.*, 2006.; Hon *et al.*, 2006.; Gao *et al.*, 2007.; Hon *et al.*, 2008.; He *et al.*, 2009.; Jackwood and Sommer-Wagner, 2011). Recombination has the potential to alter the interactions of IBDV proteins and the orientation of the capsid domains preventing neutralization by pre-existing Abs, which lead to vaccine failure.

2.15 Prevention and control

Infectious bursal disease virus is highly contagious, and spreads through the movement of poultry products, people, fomites (equipment, feedbags and vehicles), and, to a lesser extent, through aerosols of dust. IBDV is relatively stable and very resistant to inactivation, which accounts for its persistent survival on poultry farms, despite disinfection (van den Berg, 2000.; Eterradossi and Saif, 2008). Infectious bursal disease virus is resistant to ether and chloroform, pH extremes, high temperature, and phenol or quaternary ammonium disinfectants (Mandeville *et al.*, 2000).There is no treatment for IBD. Therefore in order to prevent and control IBD, a combination of sound biosecurity and effective vaccination of parents and progeny are recommended (van den Berg *et al.*, 2000.; Saif *et al*., 2008).Vaccination will be ineffective if major sanitary precautions are not taken and these include an ‘all-in/all-out’ farming methods,

cleaning and disinfection of premises between flocks especially using formaldehyde and iodophors is recommended (Pattison *et al*., 2008), and equipments and observance of a 'down time' (a period of rest between depopulation and restocking (Maris, 1986). It is also important to eliminate all vectors like insects e.g. lesser worms, mosquitoes (Eterradossi and Saif, 2008) and pests e.g. rats and mice and dogs. Old bedding and dung must be eliminated and composted (van den Berg, 2000) and that traffic (people, equipment and vehicles) onto the farm be controlled.

2.16 Vaccination

Vaccination of chickens is the major procedure used for the control of IBD in chickens. Before the introduction of attenuated vaccine strains, IBD was controlled by deliberately exposing chicks to infection at an early age and the chicks are normally protected due to the presence of maternal antibodies and at an early age (2 weeks) they do not normally exhibit signs of clinical IBD. Discovery of severe immunosuppression due to early exposure to IBD infection put a stop to the practice. In addition to strict compliance with rules of hygiene and disinfection, the success of vaccination depends on the choice of the vaccine strain and on the vaccination schedule. These must take account of the existence of certain pathotypes and the presence of antigenic variants in certain regions (van den Berg, 2000).

IBDV vaccines include live attenuated, inactivated/killed, immune complex vaccines (a mixture of hyper immune sera from specific-pathogen-infected chickens and embryo-adapted live pathogens) and DNA vaccines (Schijns *et al.*, 2008). Application route for IBDV vaccines varies from *inovo* administration to addition to drinking water or intramuscular injection. Breeder flocks are vaccinated by adding IBDV vaccine to drinking water to prevent infection of newly hatched chicks or by oral live virus vaccination of breeder stock at 18 weeks of age, together with injection of inactivated vaccine in oil adjuvant just before laying and is repeated a year later which ensures a well maintained high level of neutralizing antibody throughout the laying life of the bird (Mahgoub, 2012). Breeder flocks vaccinated with live and killed vaccines ensures the conferment of high titers of maternal antibodies to their progeny (van den Berg, 2000).This passive immunity protects chicks against early immunosuppressive infections for 1 to 3 weeks; however, boosting the immune response with killed, oil adjuvanted vaccines can provide extended protection for 4-5weeks of age (van den Berg, 2000., Eterradossi and Saif, 2008).

Vaccination of breeders at 8-12 weeks of age with live attenuated virus followed by a booster of inactivated oil-emulsion prior to the onset of lay (20-21) weeks resulted in greater transfer of maternal antibodies to the progeny (Eidson, 1980). Neutralizing antibodies can be induced by vaccinating chicks with live or inactivated vaccines (van den Berg, 2000). In this regard, classical live vaccines are recommended for a lifelong and broad immunity although they have residual pathogenicity and can revert to virulence. The major problem with active immunization of young maternally immune chicks is determining the proper time of vaccination that allows adequate replication of the vaccine virus at the same time protects young chicken from disease and this varies with levels of maternal antibody, route of vaccination, and virulence of the vaccine virus. For a successful vaccination program factors like environmental stresses, management and flock profiling for the presence of maternal antibodies should be taken into account (Eterradossi and Saif, 2008).Maternally-derived antibodies are known to interfere with vaccination of chicks, and the maternally-derived antibody titers may vary considerably due to differences in breeder vaccination programs, the age of the breeder flocks supplying progeny, and normal variation between hen titers in the same flock,thus serological monitoring is invaluable for deciding the best time for vaccination (Müller *et al*., 2003, Besseboua *et al.*, 2015) to prevent persistent maternal antibodies from potentially neutralizing the vaccine virus (Alam *et al.*, 2002.; Hair-Bejo *et al.*, 2004.; Moraes *et al.*, 2005.; Besseboua *et al.*, 2015). The optimal timing is often predicted based on serological data following detection of IBDV MDA by an enzyme-linked immunosorbent assay (ELISA) system during the first week post hatch (Besseboua *et al.*, 2015). A formula known as “the Deventer formula’’ was developed to estimate the optimal vaccination time point based on the half-life time of the MDA, the age of the chicken at sampling, the genetic background, break-through titre of the vaccine, and the requested percentage of the flock having antibody levels below the breakthrough titre of the vaccine at the time of administration (de Wit, 2001).

2.16.1 Types of IBD vaccines

2.16.2 Live and inactivated IBDV vaccines

For the production of live vaccines, classical and variant IBDV strains are passaged in tissue cultures or embryonated chicken eggs to attenuate them (Jackwood and Sommer-Wagner 2011).

Live IBDV vaccine mimic infection in the target host, replicates and induces both cellular and humoral immunity and does not require an adjuvant. They are suitable for mass vaccination but have the tendency of reversion to virulence (van den Berg, 2000.; van den Berg *et al.*, 2000). Live vaccines are classified according to virulence as mild, mild intermediate, intermediate and intermediate plus ('hot') strains depending on the degree of attenuation (Eterradossi and Saif, 2008). Mild vaccines are used mostly for breeder vaccination but exhibit only poor efficacy in the presence of certain levels of maternally derived antibodies and against vvIBDV. Intermediate and intermediate plus (hot) vaccines are more efficacious and have the ability to break through high levels of maternally derived antibodies as high as 1:500 but the disadvantage of these vaccines is their ability to induce moderate to severe bursal atrophy and immunosuppression (Kumar *et al*., 2000.; van den Berg, 2000.; Wu *et al.*, 2009.; Geerligs *et al*, 2015).

Inactivated IBD vaccines are mostly formulated as water-in-oil emulsions, usually combining several antigens. They are often used to boost levels of maternal antibodies and confer longer-lasting immunity in breeder hens. The duration and uniformity of this immunity may be influenced by the concentration and antigenic specificity of the vaccine strains (van den Berg, 2000). Inactivated vaccines are most efficiently used in a prime-boost regimen, using attenuated live IBDV as priming vaccine. Many oil-adjuvant vaccines contain both classic and variant IBDV strains and do not protect against vvIBDV strains (Eterradossi and Saif, 2008).

2.16.3 Subunit and DNA IBDV vaccines

Other vaccines used for the control of IBDV include subunit vaccines that make use of expression systems (vectors) e.g. Baculovirus (Vakharia *et al.*, 1993), fowlpox virus (Bayliss *et al*., 1991.; Shaw and Davidson, 2000), Semliki forest virus (Phenix *et al.*, 2001), yeast *Pichia pastoris* (Pitcovski *etal.*, 2003.; Wu *et al.*, 2005.; Taghavian *et al.*, 2013), *Kluyveromyces lactis* (Arnold *et al.*, 2012), *Escherichia coli* (Rong *et al.*, 2007), bacteriophage (Cao *et al*, 2005), avian herpes virus (Tsukamoto *et al*., 2002), fowl adenovirus (Francois *et al.*, 2004), Marek's disease virus (Tsukamoto *et al.*, 2000.; Tsukamoto *et al.*, 2002). DNA vaccine (Hsieh *et al*., 2010.; Brun *et al.*, 2011), plant made vaccine (Brun *et al*, 2011), plant edible vaccines (Aswathi *et al.*, 2014); *Arabidopsis thaliana* (Wu *et al.*, 2004), rice seeds *Oryzae sativa* (Wu *et al*, 2007), Tobacco plant produced via transgenesis mediated by *Cauliflower mosaic virus* (Taghavian 2013),

Chenopodium quinoa expressed in *Bamboo mosaic virus* (Chen *et al.*, 2012), *Nicotiana benthamiana* (Gomez *et al.*, 2013).

2.17 Factors responsible for vaccination failure

Several factors have been implicated in IBD vaccination failure. These factors are; Differences between the field and vaccine strains, emergence of new antigenic variants, high levels of maternally derived antibodies (Müller *et al.*, 2012), improper storage, handling and application of vaccine (Raue *et al*., 2004), immunosuppression (van den Berg, 2000), absence of certain antioxidant supplements in feeds (Shekaro *et al.*, 2012) and reassortment/recombination between field and vaccine IBDV strains (Le Nouën *et al.*, 2006.; Hon *et al.*, 2006.; Wu *et al.*, 2007.; Gao *et al.*, 2007.; Jackwood *et al.*, 2011.; Jackwood *et al.*, 2012.; Chen *et al.*, 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

Nigeria covering a total of 923,968 sq km is bordered to the north by Niger Republic and Chad, to the west by Benin Republic, to the East by the Republic of Cameroun and to the south by the Atlantic Ocean. It is situated between Latitudes 4° to 14° North and between Longitudes $2^{o}2'$ and 14° 30' East in West Africa (Aregheore, 2009). Currently, there are thirty-six states in Nigeria plus the Federal Capital Territory (FCT). Nigeria has two major seasons- the rainy or wet season that lasts from mid-March to November in the South and from May to October in the North, and the dry season for the rest of the year. Nigeria is classified into nine agro-ecological zones; (a) The mangrove forest and coastal vegetation, (b) the freshwater swamp communities, (c) the tropical high forest zone, (d) the derived Guinea savannah with relict forest, (e) the Southern Guinea savannah zone, (f) The northern Guinea savannah zone, (g) The Jos plateau, (h) The Sudan savannah, and (i) The Sahel savannah (Aregheore, 2009). The two major rivers in Nigeria are river Niger and river Benue. Temperatures in Nigeria are influenced by the rains and dry season. It can be as high as 44^{0}C and as low as 21^{0}C. The total amount of rainfall recorded in Nigeria varies decreasing from south to north e.g. Brass records rainfall of up to 379cm, Jos 143cm, Sokoto 71cm and Maiduguri 63cm. Humidity in the south is above 80% while in the north it is 60% (Aregheore, 2009).

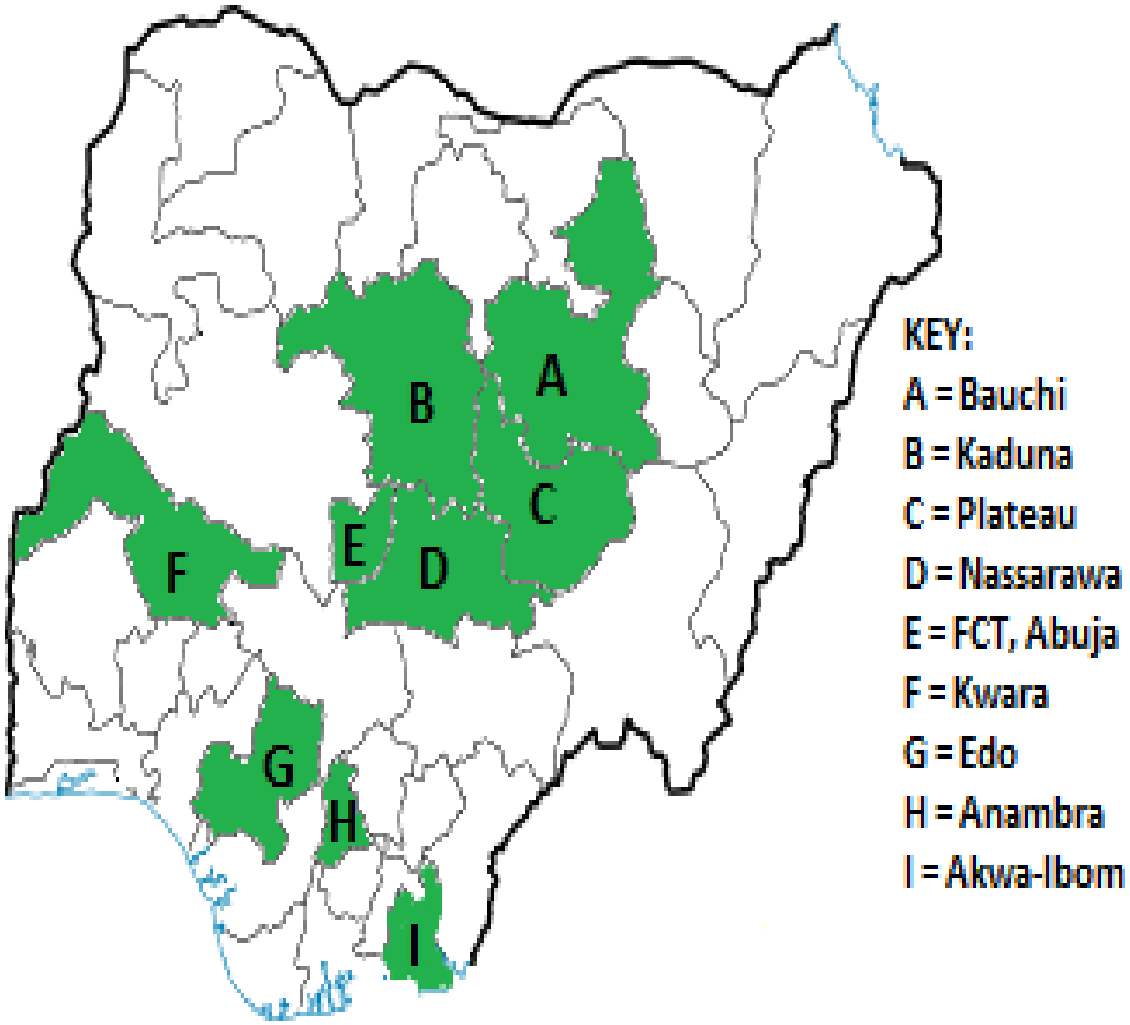

Figure 3: Map of Nigeria showing states of origin of isolated viruses/reported outbreaks.

3.2 Study population

The study population comprised poultry suspected to have died due to outbreak of infectious bursal disease virus in nine states. These birds included white leghorns, Isa brown and Black Harco (pullets and layers), New Hampshire (cockerel), Cobbs (broiler), *Numidia Meleagris* (Guinea fowl) and *Meleagris gallopavo* (turkey).

3.3 Sampling

Purposive sampling method was used for sample collection. Bursae were collected from poultry in nine states in Nigeria (Abuja FCT, Anambra, Akwa Ibom, Bauchi, Edo, Kaduna, Kwara, Nassarawa and Plateau) where outbreaks of IBDV were reported or suspected and carcasses of birds sent to the veterinary clinics in each of the states mentioned above. A total of three hundred and seventy seven (377) bursae were collected from two hundred and one (201) suspected IBD outbreaks. Clinical signs and postmortem lesions characteristic of IBD were also considered before bursae were collected.

3.4 Data collection

Information pertaining to the infected flock were collected and they were; Name of farm, Local government, State, species/breed of birds, age of birds, source of birds, number of birds, name of IBD vaccine used, vaccine manufacturer, vaccination dates and age at which birds were vaccinated and outbreak details.

3.5 Collection of virus infected samples

Collection of bursae from dead birds was by dissection of the bird and excision of the bursa located at the proctadael region of the cloaca with a pair of scissors. Bursae were placed in sterile universal bottles. The bottles were placed in ice box with ice and transported to Viral Research laboratory at the National Veterinary Research Institute, Vom where they were placed at -20^{0}C for two week before processing. Bursae were collected for a period of six (6) years from 2009 – 2014. Other sources of infectious bursal disease viruses used in this study were harvests from attempted isolation of five (5) bursae of fabricius cultivated in chicken embryonated eggs, two

(2) bursae cultivated in chicken embryo fibroblasts and two (2) uninfected chicken embryo controls.

3.6 Preparation of bursa homogenate

Twenty percent (w/v) of bursa homogenate in Phosphate buffer saline (PBS) containing 1mg/ml penicillin and 1mg/ml streptomycin (1000µg/ml) were prepared according to the procedure described in the Office International des Epizooties (2008) Manual of Standards for Diagnostic Tests and Vaccines. Samples were left at 4°C overnight for the antibiotic to eliminate bacterial contaminants. Samples were centrifuged at 3,000xg for 10mins and the supernatant collected and stored at-20°C until needed for analysis. When needed, 100 µl of supernatant from each homogenate was blotted on FTA® cards (Flinder Technology Associates, Australia). The cards were left to dry at room temperature for 1 hour and stored properly wrapped at -80^{0}C until they were shipped out to Prof Daral Jackwood's laboratory in The Ohio State University, Wooster campus for analysis.

3.7 Isolation of Infectious bursal disease virus in chicken eggs

Isolation of field IBD viruses from ten (10) homogenized bursa of fabricius was carried out. Eleven (11) day old chicken embryonated eggs were obtained from the National Veterinary Research Institute (NVRI), Vom hatchery. The eggs were candled with the aid of an egg candler and the chorioallantoic membranes were dropped after holes were bored at the centre of the air space and horizontally away from the embryo. According to the procedure described in the Office International des Epizooties (OIE) Manual of Standards for Diagnostic Tests and Vaccines (2008), five bursae homogenates used for inoculation were passed through 0.45µm Millipore filter to further ensure that the inoculums were sterile. While placed horizontally on the egg tray, with the aid of a sterile tuberculin syringe and needle, two hundred microlitres each of the bursae homogenates were inoculated into the embryonated chicken eggs through the chorioallantoic membrane (CAM) route. Six eggs per sample were used and 16 eggs (8 eggs per control) were used as positive and negative controls. Two hundred microlitres of a known positive IBDV sample was inoculated into the eggs used as positive control while two hundred microlitres of sterile Phosphate buffered saline (PBS) was inoculated into the eggs used as

negative controls. After inoculation, the eggs were incubated at 37°C for six days. The eggs were candled daily and dead embryos were removed. At the end of the incubation period, surviving embryos with their chorioallantoic membranes (CAMs) were harvested and checked for lesions. The harvested embryos only were homogenized and passaged into another set of eggs. The eggs were candled and at the end of 192h incubation, the living embryos with their CAMs were harvested and checked for lesions typical of IBD virus in embryos and on CAM. Isolation attempt was terminated at second passage. At the end of each incubation period, homogenized embryos were screened for IBD virus antigen using the agar gel immunodiffusion test.

3.8 Isolation of Infectious bursal disease virus using chicken embryo fibroblast (CEF) cells

Isolation of field IBD viruses from homogenized bursae samples was also carried out using chicken embryo fibroblast (CEF) cells in tissue culture plates using the method described in the Office International des Epizooties (OIE) Manual of Standards for Diagnostic Tests and Vaccines (2008). Ten (10) homogenized bursae of fabricius were used. Homogenates were passed through 0.45µm Millipore filters before use for inoculation. Twenty four hours confluent chicken embryo fibroblast cells in 24 well tissue culture Falcon plates and 25cm^{3} Falcon flasks were used. Two hundred microlitres (200µl) of filtrate was inoculated, each sample in duplicates into the CEF confluent wells and five hundred microlitres (500µl) in the 25cm^{3} Falcon flasks. The plates and flasks were incubated at 37°C for 1h for virus adsorption. At the end of an hour, maintenance medium (Eagle's minimum essential medium (EMEM) containing 2% newborn calf serum with penicillin and streptomycin) was added into the wells and flasks and returned back into the incubator. Cells were observed daily with the aid of an inverted light microscope to check for Cytopathic effects (CPE) and scored. Cells were frozen after 144h. Before harvesting, cells without CPE were freeze thawed three times, centrifuged at 3,000rpm and passaged again into fresh CEF. Isolation attempt was terminated after 2nd passage. Harvests were screened for IBDV antigen by agar gel immunodiffusion test after each passage using known positive IBDV serum.

3.9 Antigen detection by agar gel immunodiffusion (AGID) test

Infectious bursal disease virus antigen detection was first of all carried out on the samples after homogenization using agar gel immunodiffusion test as described in the Office International des Epizooties (OIE) (2008) Manual of Standards for Diagnostic Tests and Vaccines to determine positive and negative IBD bursa samples. Eight grams of sodium chloride and 1g of agarose were dissolved in 100ml of distilled water. After autoclaving at 115°C for 15mins, 15ml of the molten agar was poured into a 60mm plastic Petri dish and allowed to cool. Wells measuring 6mm in diameter and 3mm apart were made on the agar using a gel punch. Twenty-five microlitres of the homogenized bursa, embryo and tissue culture samples, plus known negative and positive IBDV samples were placed in the peripheral wells with the middle well containing a known positive IBDV serum. The plates were incubated overnight and results were read after 48hours with the aid of a light bulb. Positive results were determined by the presence of a white precipitin line at the boundary where the positive sample interacts with the known positive IBDV control serum. Negative results show no precipitation lines. Both AGID negative and positive bursae samples were put on FTA cards for RT-PCR, this was done in order to utilize the high sensitivity nature of RT-PCR against AGID test to further screen the samples.

3.10 IBDV GENOME SEGMENT A AND B: ISOLATION OF VIRAL RNA

3.10.1 Elution of viral RNA from FTA cards

This process involves using a hole puncher (2mm) to punch out between 10-15 punches from the FTA cards containing the sample. The punches were placed in a 1.7ml microcentrifuge tubes. Three hundred microlitres (300 µl) of TE buffer (10mM Tris-HCl, pH 8.0, 1mM ethylene diamine tetra acetic acid (EDTA)) was added to each tube. The tubes were vortexed and incubated for 10mins at room temperature to elute infectious bursal disease viral RNA from the cards. After incubation, tubes were vortexed again to obtain a uniform mix.

3.10.2 RNA Extraction

RNA in the tubes was extracted using the standard Trizol procedure described by Jackwood *et al* (2008). Nine hundred microlitres (900 µl) of Trizol® LS (Ambion, Life Technologies, Carlsbd,

CA)was added into each tube and mixed by vortexing the tubes for 20 seconds and incubating for 5mins at room temperature.

3.10.3 Phase Separation

After incubation, 240 µl of chloroform was added and the tubes vortexed for 15 sec and incubated at room temperature for 15 mins. Tubes were centrifuged at 12,000xg for 15 mins at 4^0C. At the end the centrifugation, three phases were observed and they included the upper aqueous phase containing the RNA, an inter phase containing proteins and a lower phase containing phenol/chloroform. The upper aqueous phase containing the RNA was transferred into new tubes and the interphase (containing protein) and lower phenol/chloroform phase were discarded (Jackwood *et al.,* 2008).

3.10.4 RNA Precipitation

Five hundred microlitres (500 µl) of cold isopropanol was added into each tube and incubated for 15mins at room temperature. After incubation, tubes were centrifuged at 12,000xg for 15mins at 4^oC. The isopropanol was poured off and tubes dried briefly upside down on a paper towel (Jackwood *et al.,* 2008).

3.10.5 RNA Washing

One mililitre (1ml) of 75% cold ethanol was added into each tube and centrifuged at 12,000xg for 15mins at 4^oC. The ethanol was poured off and excess ethanol in the tubes was pipetted off. The tubes were placed upside down for 10mins to dry. After drying, 25µl of 90% dimethyl sulfoxide (DMSO, Sigma Chemical Co)was added to each tube to redissolve the RNA. Tubes were then placed on ice for 1h before use for RT-PCR (Jackwood *et al.,* 2008).

3.10.6 Pre-denaturation of RNA

At the end of the 1h incubation on ice after redissolution with 90% Dimethyl sulfoxide (DMSO) (Sigma Chemical Co, USA), equal volumes of extracted RNA and nuclease free water (2.5 µl of RNA and 2.5 µl nuclease free water) were added into PCR reaction tube. RNA was denatured with the aid of a Thermal cycler 2720 (Applied Biosystems, Foster City, California) at 95^oC for 5mins before use for RT-PCR. This process uncoils the double stranded RNA into single strands.

3.11 RT-PCR (Reverse transcription-polymerase chain reaction) amplification

RT-PCR amplification was carried out with Thermal cycler 2720 (Applied Biosystems, Foster City, CA) and the AgPath-ID™ one-step RT-PCR kit (Ambion; Applied Biosystems, Foster City, CA) was used according to manufacturer's instruction. For genome segment A, a 743 base pair region of the hypervariable region of viral protein 2 (hvVP2) genes from nucleotides 737-1479 was amplified with primers 743-F: 5´-GCCCAGAGTCTACACCAT-3´ and 743-R: 5´-CCCGGATTATGTCTTTGA-3´ (Jackwood and Sommer-Wagner, 2005), while for the genome segment B, a primer pair B-168AF (5´-CAT AAA GCC TAC AGC TGG AC-3´) and B-889R (5´-GTC CAC TTG ATG ACT TGA GG-3´) (Jackwood *et al*, 2009) that amplifies a 722-base pair (bp) region starting from nucleotide 168 – 889 was used. Two microlitres (2.0µl) of denatured viral RNA was used for the assay. The extracted double stranded RNA was denatured at 95°C for 5mins before use for RT-PCR. RT was performed at 48°C for 30mins and 95°C for 10mins followed by 35 cycles of PCR at 95°C for 30 secs, 57°C for 1:30 mins (but 55^{0}C for 1:30 mins for segment B) and 72°C for 5 mins. After completion of the 35 cycles, a final extension at 72°C for 5mins was performed. Likewise, a 684bp of the genome segment B of six Nigerian IBD viruses were also amplified and procedure was same as above.

3.12 Restriction fragment length polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) was performed along with RT-PCR to detect and differentiate the strain of infectious bursal disease virus circulating in the field. Eleven Nigerian IBDV isolates were characterized by this method (RFLP). A primer pair designed to amplify a 743bp fragment of the VP2 gene was used and restriction fragment length polymorphism profiles (Jackwood *et al*., 2001) were determined for each isolate using three restriction enzymes, *BstN*I, *Mbo*I and *Ssp*I (Promega, Madison WI, USA) because of their ability to differentiate IBDV strains. Eight microlitres (8µl) of the RT-PCR products were digested with 1µl of each restriction enzyme (*BstN*I, 10U/µl; *Mbo*I, 4U/µl; *Ssp*I, 2U/µl), 2µl of the 10x buffer supplied by the manufacturer (Stratagene, California), and 9µl of 0.1% diethyl pyrocarbonate (Sigma chemical Co, USA), in water for 1h at 60°C for *BstN*I and 1h at 37°C for *Mbo*I and *Ssp*I. The digested cDNAs were separated on 2.0% Metaphor agarose gel (FMC Bio-Products, USA) stained with ethidium bromide for easy visualization.

3.13 Gel electrophoresis

RT-PCR products were electrophoresed on 0.8% Agarose gel with ethidium bromide for visualization to confirm the presence of IBDV in the amplified RNA (cDNA) product. This procedure involves the addition of 5 µl of cDNA mixed with 1 µl of loading dye into wells on Agarose slabs immersed in Tris borate EDTA buffer (TBE) solution in an electrophoresis tank. The first and second wells consisted of a DNA ladder (marker) and positive IBDV control followed by the test cDNAs. The tank was connected to an electrical source and electrical currents facilitated the migration and separation of the DNA according to their sizes. The gel results were visualized with the aid of ultra-violet light source. Amplicons of the appropriate size 743bp for genome segment A and 722bp, 684bp for genome segment B were identified by comparison with the DNA marker in the gel.

3.14 Nucleotide sequence analysis

After the gel electrophoresis results were read, RT-PCR products with moderately strong to strong bands on the gel were selected for sequencing. The RT-PCR products were purified and prepared for sequencing using a Wizard SV Gel and PCR Clean-Up System (Promega Corp., Madison, WI, USA). Sanger sequencing was conducted at the University of Wisconsin, Biotechnology Center, DNA Sequence Laboratory Madison, WI, USA). Nucleotide sequence results were downloaded using Chromas (Technelysium Pty. Ltd., Queensland, Australia). Sequence analysis, Clustal W alignments and sequence comparison were conducted using Accelrys Gene v2.5 software (Accelrys, San Diego, CA, USA).

3.15 Phylogenetic analysis

Phylogenetic and molecular analysis of IBDV segments A and B were conducted using MEGA version 6.0 software (Tamura *et al*, 2013) (http://www.megasoftware.net/.). Nucleotide and amino acid sequences were aligned using Clustal W and phylogenetic trees were prepared using Neighbor joining (NJ) and UPGMA methods with 1000 bootstrap replicates.

3.16 Genbank accession numbers

Nucleotide sequences of segments A and B after alignment were submitted to the Genbank and were allotted accession numbers. The Genbank accession numbers for the individual IBDV nucleotides sequences used in this study will be listed in the results.

3.17 Statistical analysis

Field data obtained from outbreaks were analyzed using Chi square.

CHAPTER 4

RESULTS

4. Distribution of sampled IBD outbreaks in Nigeria

Distribution of sampled IBD outbreaks with respect to age of birds

Apart from the population with unspecified age which recorded the highest number of outbreaks which will possibly have chickens within the age range where IBDV causes mortality, IBDV outbreak occurred more in chickens aged 1-6 weeks, with the rate of outbreak decreasing as age increases as shown in Table 1. The distribution of the IBD outbreaks with respect to age of birds was statistical significant at $p<0.01$.

Distribution of IBDV outbreaks with respect to States

The distribution of IBDV outbreak in the various states surveyed is shown in Table 2. Plateau state had the highest outbreak of IBDV (42.79%) between 2009 – 2014. This is followed by Kwara state (14.42%), Akwa Ibom (12.50%), Bauchi (8.65%), from undesignated state (7.21%), Edo state (5.77%), Kaduna state (2.88%), Nassarawa state (2.40%), Anambra state (1.92%) with Abuja FCT having the least outbreak of IBDV (1.44%). The distribution of IBDV outbreak with respect to States was statistical significant at $p<0.01$.

Distribution of IBDV outbreaks with respect to species and type of birds

The outbreak of IBDV was highest in pullets (52.66%) followed by broilers (25.60%) and cockerels (5.31%). The least outbreaks occurred in turkey, grower and guinea fowl (0.48%) as shown in Table 3. The distribution of IBDV outbreak with respect to species and type of birds was statistically significant at $p<0.01$.

Distribution of IBDV outbreaks with respect to breed of chicken

The breed of chickens mostly highly affected by IBDV was the Isa brown breed (24.15%) followed by the black harco (3.86%) as presented in Table 4. The distribution of IBDV outbreak with respect to breed of chicken was statistically significant at $p<0.01$.

Table 1: Distribution of sampled IBD outbreaks from 2009 – 2014 with respect to age of birds

S/no	Age Range (wks)	Number of outbreaks	Percent occurrence
1	1 – 6	66	31.9
2	7 – 12	21	10.1
3	13 – 18	3	1.45
4	19 – 24	3	1.45
5	25 – 30	1	0.5
6	Unspecified	113	54.6
	Total	**207**	**100**

Statistically, since the value of chi-square calculated is 41.4 and P-value is < 0.01, therefore IBDV outbreak is not independent or is dependent on age of birds.

Table 2: Distribution of IBDV outbreaks with respect to States

S/no	State	Number of outbreaks	Percent occurrence
1	Abuja (FCT)	3	1.44
2	Akwa Ibom	26	12.6
3	Anambra	4	1.93
4	Bauchi	18	8.69
5	Edo	12	5.79
6	Kaduna	6	2.89
7	Kwara	30	14.49
8	Nassarawa	5	2.41
9	Plateau	89	42.99
10	Undesignated	14	6.76
	Total	**207**	**100.00**

The statistical test shows that since the value of the Chi-square calculated is 20.7 and p-value is < 0.01, therefore IBDV outbreak is dependent on state.

Table 3: Distribution of IBDV outbreaks with respect to species and type of birds

S/No	Specie	Type	Number of outbreaks	Percent occurrence
1	*Gallus gallus*	Broilers	52	25.60
		Layers	4	1.93
		Pullet chicks	109	52.66
		Pullet Grower	1	0.48
		Cockerels	11	5.31
2	*Melleagris gallopavo*	Turkey	1	0.48
3	*Guinea numida meleagris*	Guinea fowl	1	0.48
4	Unspecified	Unspecified	28	13.53
	Total		**207**	**100.00**

Statistically, since the value of the Chi-square calculated is 35.8 and p-value is < 0.01, therefore the statistical analysis shows that IBDV outbreak is dependent on specie and type of birds.

Table 4: Distribution of IBDV outbreaks with respect to breed of chickens

S/No	Breed	Number of outbreaks	Percent occurrence
1	Black harco	8	3.90
2	Isa brown	48	23.41
3	White leghorn	1	0.49
4	Unspecified	148	72.20
	Total	**205**	**100.00**

Statistics test shows that since the value of the Chi-square calculated is 51.3 and p-value is < 0.01, therefore IBDV outbreak is dependent on breed of chickens.

Yearly distribution of IBDV outbreaks from 2009 – 2014

The highest outbreak of IBDV occurred in 2014 (41.55%) than in other years sampled. The least outbreak of IBDV occurred in 2010 (4.35%) as shown in Table 5. The distribution of IBDV outbreak with respect to year was statistics significant at $p<0.01$.

Monthly distribution of IBDV outbreaks from year 2009-2014

In the month of May, the highest number of outbreaks were recorded (25.60%) closely followed by the month of October (14.01%) and the month of June (12.56%). The least outbreak of IBDV occurred in the month of December (0.48%) as shown in Table 6. The distribution of IBDV outbreak with respect to month was statistical significant at $p<0.01$.

Distribution of IBDV outbreaks with respect to season from year 2009-2014

The seasonal distribution of IBDV outbreak show that between May – November which are classified as rainy season, outbreak of IBDV was highest during this season (68.60%), followed by February – April, dry hot season (14.50%), with the least outbreak occurring between December – January , dry harmattan season (3.86%) as shown in Table 7. The distribution of IBDV outbreak with respect to season was statistically significant at $p<0.01$.

Distribution of IBDV outbreaks with respect to type of vaccine used

Specifically, 4.35% of the sampled populations were not vaccinated. For 37.20% of the sampled population, no information of the vaccination status was available. Approximately, 23.19% of the population sampled confirmed that their flocks were vaccinated but did not know the name of the IBD vaccine used, while 20.77% of the population vaccinated their flock using foreign IBD vaccine but could not recall the name of the vaccine used. From the data, foreign IBD vaccines were used more for vaccination than the locally manufactured vaccine produced in the country (NVRI Vom IBD Vaccine). Highest post vaccinal outbreak from available data was from the group where it was not known whether they were vaccinated or not as shown in Table 8. The distribution of IBDV outbreak with respect to type of IBD vaccine used was statistically significant at $p<0.01$.

Table 5: Yearly distribution of IBDV outbreaks from 2009 – 2014

S/No	Year of outbreak	Numbers of outbreak	Percent occurrence
1	2009	41	19.81
2	2010	9	4.35
3	2011	16	7.73
4	2012	33	15.94
5	2013	22	10.62
6	2014	86	41.55
	Total	**207**	**100.00**

The statistical analysis shows that since the value of the Chi-square calculated is 34.5 and p-value is < 0.01, therefore IBDV outbreak is dependent on time (year).

Table 6: Monthly distribution of IBDV outbreaks from year 2009-2014

S/No	Month	Number of outbreaks	Percent occurrence
1	January	3	1.45
2	February	8	3.86
3	March	9	4.35
4	April	11	5.31
5	May	53	25.60
6	June	26	12.56
7	July	19	9.18
8	August	5	2.42
9	September	5	2.42
10	October	29	14.01
11	November	7	3.38
12	December	1	0.48
13	Unspecified	31	14.97
	Total	**207**	**100.00**

Statistically, since the value of the Chi-square calculated is 17.3 and p-value is < 0.01, therefore IBDV outbreak is not independent or is dependent on the month of the year.

Table 7: Distribution of IBDV outbreaks with respect to seasons from year 2009-2014

S/No	Season	Number of outbreaks	Percent occurrence
1	Dry Harmattan- December - January	4	1.93
2	Dry hot season - February - April	28	13.52
3	Rainy season - May - November	144	69.6
4	Unspecified	31	14.97
	Total	**207**	**100**

Statistically, since the value of the Chi-square calculated is 51.8 and p-value is < 0.01, therefore IBDV outbreak is dependent on the season of the year.

Table 8: Distribution of IBDV outbreaks with respect to type of vaccine used

S/No	Name of vaccine	Number	Percent occurrence
1	Abic	3	1.44
2	Biovac	2	0.96
3	Bur706	1	0.48
4	Georgia strain	1	0.48
5	Indovac	1	0.48
6	Izovac	3	1.44
7	NVRI Vom IBD	9	4.35
8	Ornibur	10	4.83
9	Foreign vaccine (name unknown)	43	20.77
10	Vaccinated (name of vaccine unknown)	48	23.19
11	Not vaccinated	9	4.35
12	Unspecified (whether vaccinated or not)	77	37.20
	Total	**207**	**100.00**

Statistically, since the value of the Chi-square calculated is 25.9 and p-value is < 0.01, therefore IBDV outbreak is not independent or is dependent on the vaccine used.

Distribution of IBDV outbreaks with respect to husbandry type and scale

Apart from the unknown husbandry type, the large scale intensive type has the highest percentage of IBDV outbreaks (22.71%) followed by the small scale type (21.74%) and the medium scale intensive type (14.50%) as presented in Table 9. The distribution of IBDV outbreak with respect to husbandry type and scale was statistically significant at $p<0.01$.

4.2 Isolation of Infectious bursal disease virus in embryonated chicken eggs

At the end of incubation, control and IBDV infected embryos were dissected and organs checked for typical lesions associated with IBDV serotype 1. Livers of control embryos were red and normal while livers of infected embryos were swollen, greenish and congested. Plate 3 shows the livers of control and IBDV infected embryos.

4.3 Agar gel immunodiffusion test

The validity of the screening test was verified using agar gel immunodiffusion test and reverse transcription-polymerase chain reaction. Out of the one hundred and thirty nine (139) samples comprising 129 bursa of Fabricius tissues, 1 IBDV vaccine, 2 chicken embryonated eggs used as control, 2 harvests from IBDV infected chicken embryo fibroblast cells, and 5 harvested IBDV infected embryos screened for infectious bursal disease antigen subjected to agar gel immunodiffusion test (Appendix 2), 93 (66.9%) were positive while 46 (33.1%) were negative. When the same number of samples were subjected to RT-PCR, 78 (56.1%) were positive while 61 (43.8%) were negative. Calculation of the validity of the test used showed a specificity of 67.2% and sensitivity of 93.5%. The predictive value of the positive test was 78.5%, predictive value of the negative test 89.1%, percentage false positive 32.8% and percentage false negative 6.4% as shown in Table10.

4.4 RT-PCR for segment A and gel electrophoresis

Out of the two hundred and sixteen (216) viral RNA tested for the presence of infectious bursal disease VP2 gene using RT-PCR and primer pair that amplifies a 743bp of the viral protein (VP2) hypervariable region in the segment A of IBDV (Appendix 2), one hundred and forty-three (143) (66.2%) showed corresponding bands of 743bp comparable with the ladder and

known IBDV positive control indicating a positive result and presence of IBD VP2 in the samples while seventy-three (73) out of 216 (33.8%) showed no corresponding bands indicating negative results. Plates 4a and 4b show examples of amplicons of the expected 743bp that were obtained by RT-PCR. It was observed that 19 samples that were originally positive for IBDV by AGID were negative by RT-PCR while 5 samples that were originally negative by AGID were positive by RT-PCR. IBDV antigen was detected within the age range of 3 – 19 weeks.

Table 9: Distribution of IBDV outbreaks with respect to type of husbandry

S/No	Husbandry type	Number sampled	Percentage
1	Small scale (1-200 birds)	45	21.74
2	Medium scale intensive (<1000 birds)	30	14.50
3	Large scale intensive (>1000 birds)	47	22.71
4	Unspecified	85	41.06
	Total	**207**	**100.00**

Statistically, since the value of the Chi-square calculated is 51.8 and p-value is < 0.01, therefore IBDV outbreak is dependent on the type of husbandry.

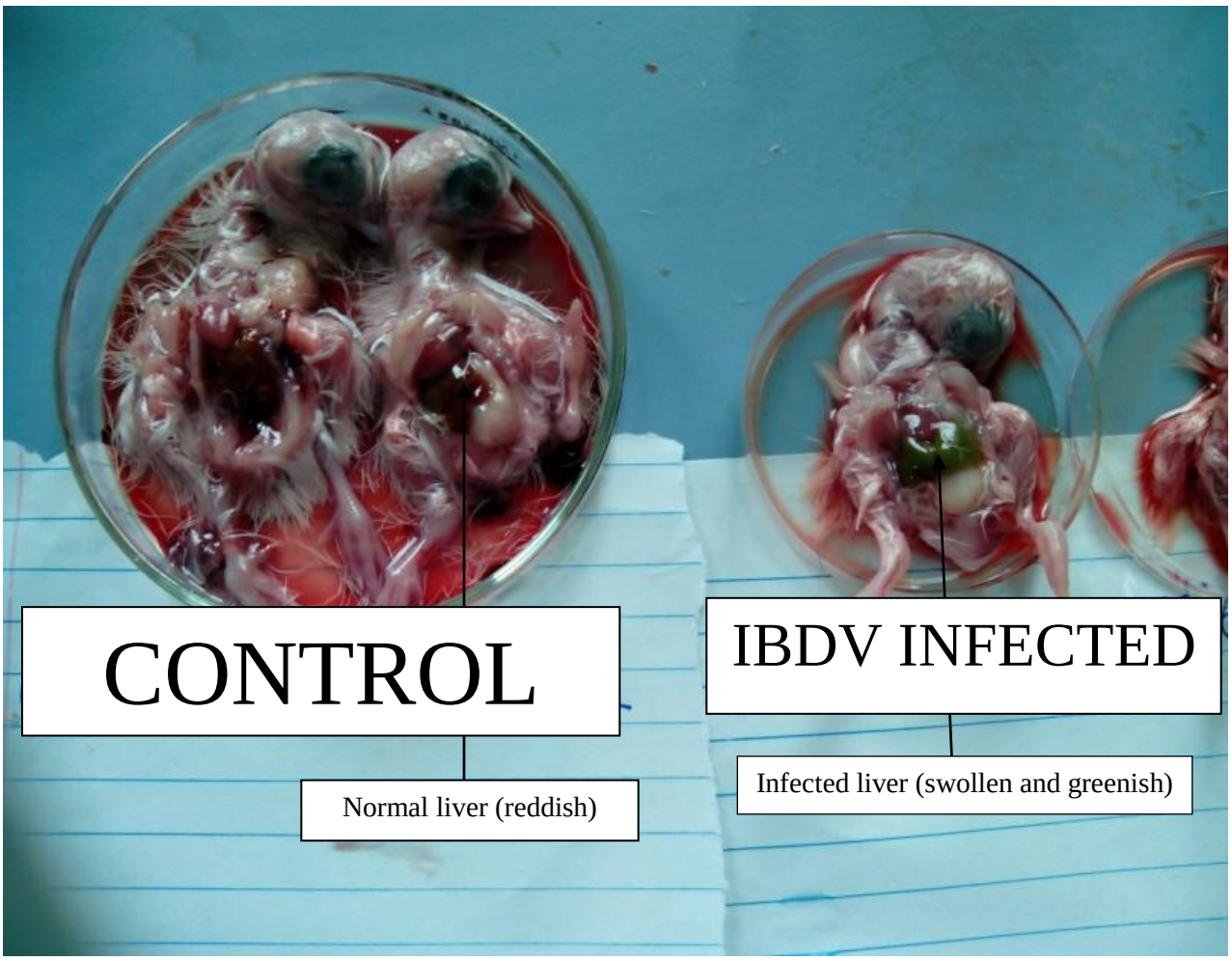

Plate 3: Uninfected and IBDV infected embryos. Arrows shows livers of control embryos that are normal while the liver of IBDV infected embryo is swollen and greenish.

Table 10: Computation of validity of screening test using AGID against diagnostic test RT-PCR

	DIAGNOSTIC TEST RT-PCR		
SCREENING TEST AGID	**Positive**	**Negative**	**Total**
Positive	73	20	93
Negative	5	41	46
Total	**78**	**61**	**139**

- AGID= Agar gel immunodiffusion
- RT-PCR= Reverse transcriptase-polymerase chain reaction

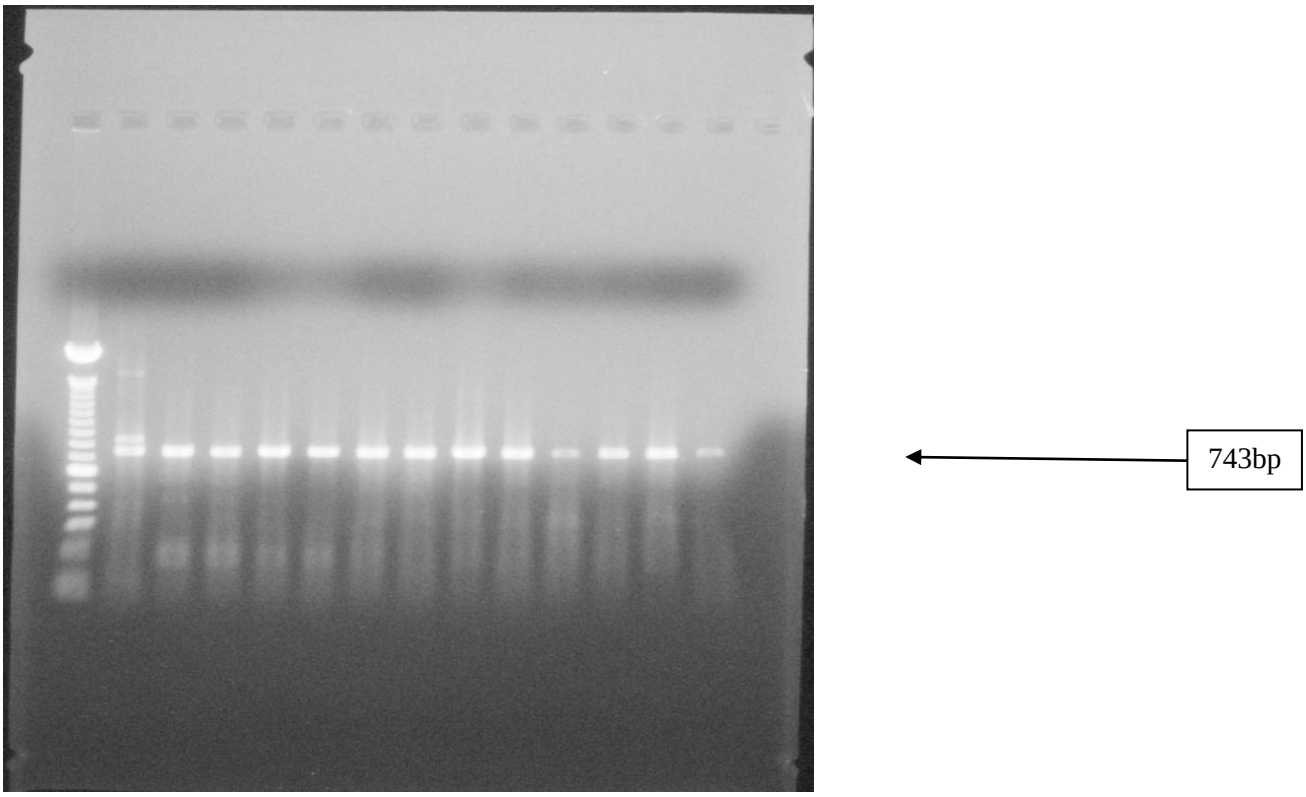

Plate 4a. Positive RT-PCR of the IBDV VP2 gene of bursa/ embryo homogenate samples indicated by a 743bp band (arrowed). Lane 1 – Molecular marker (100bp), lane 2 – known positive IBDV sample, lane 3-13 – bursa samples.

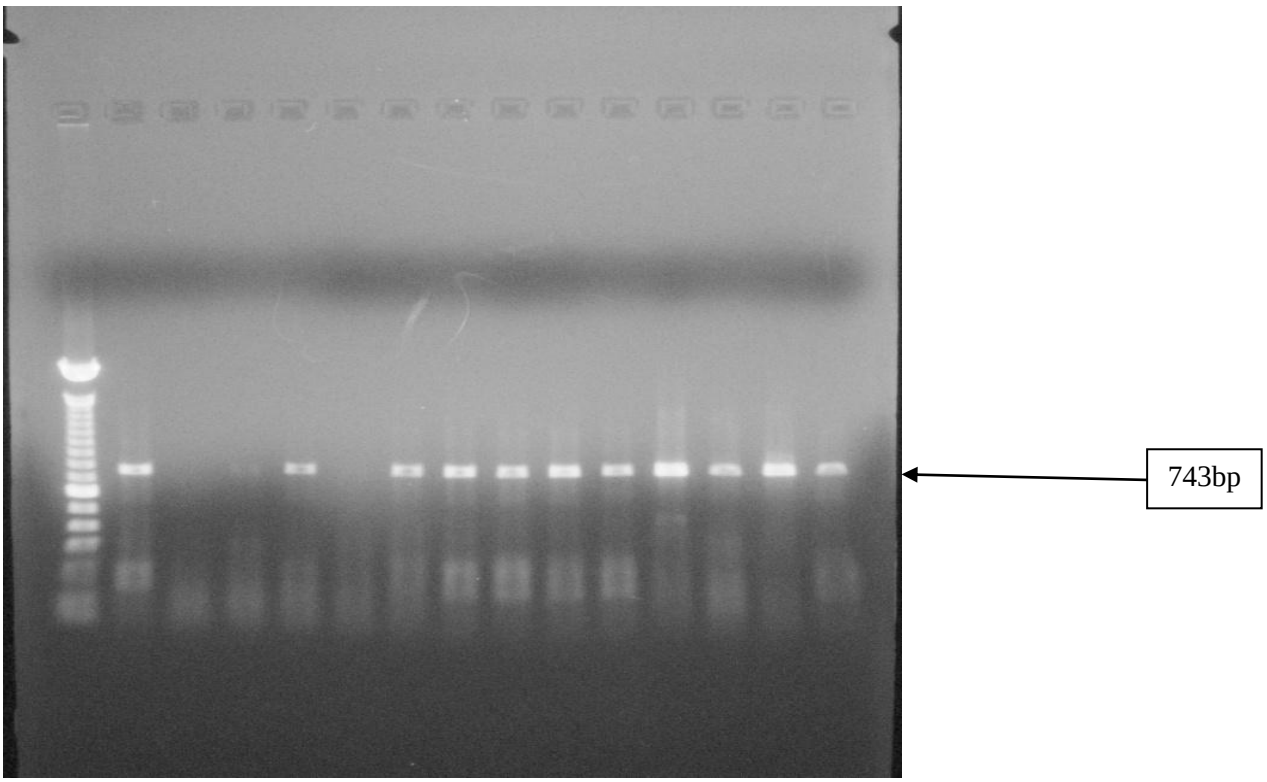

Plate 4b. Positive and negative RT-PCR of the IBDV VP2 gene of bursa/ embryo homogenate samples indicated by a 743bp band (arrowed). Lane 1 – Molecular marker (100bp), lane 2 – known positive IBDV sample, lane 3-13 – bursa samples.

4.5 Restriction fragment length polymorphism analysis

From the results obtained after digestion of the RT-PCR product with three restriction enzymes *BstN*I, *Mbo*I , *Ssp*I, 9 out of the 11 IBDV samples belong to molecular group 6 (Table11). This molecular group 6 contains very virulent (vv) IBDV strains that correlated with the presence of an *Ssp*I site. Interestingly, new RFLP patterns were observed in 6 out of the 11 isolates studied and all were *Ssp*I positive except for 2. When the segment A was digested using *BstN*I, two different molecular patterns were observed, Kaduna61/NG/2012, and Plateau53/NG/2010 yielded 3 bands of 367, 172 and 119bp while a previously unreported RFLP pattern designated 424 was observed in three (3) isolates Bauchi58/NG/2012, Anambra54/NG/2012, Nassarawa77/NG/2013 and Nassarawa77/NG/2013/1 which yielded 3 bands of 424, 172, and 119bp. With *Mbo*I two patterns were also observed with Kaduna61/NG/2012, and Plateau53/NG/2010 yielding 2 bands of 362 and 229bp while Bauchi58/NG/2012, Anambra54/NG/2012 and Nassarawa77/NG/2013/1 yielded 2 bands of 289 and 229bp. Although Anambra54/NG/2012 had a similar *BstN*I and *Mbo*I pattern with Bauchi58/NG/2012, it was *Ssp*I negative. The same observation was made with Nassarawa77/NG/2013/2 which had the same RFLP pattern with Nassarawa77/NG/2013/1 but was *Ssp*I negative.

4.6 Sequence Analysis

4.6.1 Nucleotide and the deduced amino acid sequence analysis of segment A of the Nigerian IBD viruses

The hvVP2 of IBDV genome segment A of the Nigerian isolates were amplified in the RT-PCR assay, and the nucleotide sequences were determined for 105 out of the 143 positive samples. Their Genbank accession numbers are KP152231-KP152322 and KP266322-KP266334 (Appendix 3). The percentage nucleotide identities (starting from nucleotide position 201 – 1133) of the 105 Nigerian viruses were calculated and compared with some reference IBDV strains from Genbank. The reference IBDV strains used for comparison include two very virulent (vv) IBDV strains UK 661and OKYM and a variant IBDV strain Del E. The nucleotide percentage identities of the Nigerian viruses when compared with vv UK 661 and OKYM ranged between 95.0 – 98.9%, with Del E it was 91.1 – 94.9% with one IBD sample Plateau7/NG/2012 having the highest 97.6% identity with Del E. The nucleotide sequences of the Nigerian viruses

with the reference IBDV strains are shown in Appendix 8. The percentage identities of the amino acid of the Nigerian viruses (starting from amino acid position 210 – 443) when compared with vv UK 661 and OKYM ranged from 97.4 – 99.6% while with Del E it was 93.6 – 98.3%. Appendix 4 shows a general summary of the comparison of amino acid substitutions at different positions between Nigerian field viruses used in this study and other published strains of IBDV for segment A. The deduced amino acid sequences of the hypervariable sequences in the VP2 gene (Appendix 9) of the one hundred and five (105) Nigerian viruses were determined and compared with the variant strains Del E and T1, classical strain STC and the very virulent strains OKYM and UK 661. The amino acid residue A222 which is known to be conserved among most vvIBDV was observed in all the Nigerian viruses except in Bauchi37/NG/2012 and Bauchi38/NG/2012 that had an A222T. The codon used for A222 was exclusively GCA, while it was ACA for 222T. Amino acid I242 was conserved in all the viruses but a mutation that was non-synonymous was observed in Nasarawa77/NG/no2/2013 and Plateau78/NG/2011/2 that had I242V, these viruses exhibited dual amino acid sequences that matched both the very virulent IBD strain and the Del E strain. Amino acid I256, I294 and S299 were conserved in all except for the amino acid mutation (S→N) observed in Nasarrawa77/NG/no2/2013. The amino acid mutations Q253H, A284T and D279N was observed in one of the Nigerian viruses (Nasarawa77/NG/no2/2013). Interestingly two other isolates (Nasarawa77/NG/2013 and Plateau78/NG/2011/1) had Q253L in this position with the nucleotide substitution occurring in the 2nd position of the codon used and it was non-synonymous but maintained the D279 and A284 seen in the vvIBDVs. Four (4) Nigerian viruses (Kwara148/NG/2014, Benin142E/NG/2013 (an embryo derived IBDV isolate), Plateau40/NG/2012 and Kwara141/NG/2014) showed an amino acid substitution at position I272T. This position was conserved in the other Nigerian viruses and in the reference IBDV strains used for comparism. It was also observed that these four viruses had a mutation D279N with variant T1 and classical strain STC. Sixteen (16) out of the one hundred and five (105) Nigerian viruses had a Q219T with one virus (Plateau78/NG/2011/2) having a Q219P at this position. One hundred and one (101) out of 105 had a S254, instead of the G254 seen in the vvIBDV as well as strains belonging to the antigenic variants. Nineteen (19) Nigerian viruses showed amino acid changes at position 269 (T→S). Similarly, 81 of 105 Nigerian viruses have an A300 instead of the E300

conserved in 5 Nigerian viruses and all other IBDV strains including the very virulent strains, while the remaining nineteen (19) viruses have a unique Q300 and were non-synonymous. The conserved serine-rich heptapeptide sequence SWSASGS adjacent to the second hydrophilic region was found in all of the viruses used in this study except in two that had R330 instead of S330, this amino acid R330 is conserved in all attenuated IBDV strains.Plateau92/NG/2013 had an amino acid substitution at position 261 (I→T) that was not seen in the other Nigerian viruses, variant and vvIBDV strains used for comparism.One isolate Benin142E/NG/2013 (this isolate has its origin from bursa homogenate Benin87/NG/2013), the bursa homogenate was propagated in eggs and at second passage had similar sequences with three other Nigerian viruses (100%) and the vvIBDV strains OKYM and UK 661 except that at position 272 the three had Lysine substituted with Threonine. The nucleotide and amino acid percentage identity between Benin142E/NG/2013, UK661and OKYM was 98.9 and 99.1%, respectively. When compared with the sequence of its original source (Benin87/NG/2013) they had similar amino acid sequence except for changes observed at positions 254 (Q→G), 272 (I→T), 279 (D→N) and 300 (A→E). The nucleotide and amino acid percentage similarity between the two viruses were 97.5% and 98.3%, respectively. It was observed that majority of the mutations that occurred in the amino acid sequences were synonymous and therefore silent while others were non-synonymous. Comparison of amino acid substitutions at different positions grouped according to states are shown in Tables12a-j. The total numbers of amino acid substitutions observed in the Nigerian viruses per state and the positions where the substitutions occurred in the VP2 region when compared with vvIBDV UK661 and Del E strains are shown in Table13 and 14, respectively. Tables 15 and 16 show the temporal distribution of amino acid substitutions and their positions between the Nigerian viruses with reference very virulent (vv) UK661 and Del E IBDV strains.

4.6.2 Comparism of the nucleotide and deduced amino acid sequences of segment A of IBDV viruses used in this study and previously published Nigerian IBDV strains

The nucleotide sequences of the Nigerian viruses and previously published Nigerian IBDV strains obtained from Genbank were aligned and nucleotide percentage identities when calculated ranged from 92.7 – 100%. It was observed that the 100% nucleotide identity was observed with two previously published Nigeria IBDV strains (Accession nos JX424071.1 and

JX424073.1) (Adamu *et al*, 2013), isolated from Kaduna State and eleven viruses used in this study from 3 states (Akwa Ibom, Edo and Plateau). The amino acid percentage identities of the IBD viruses from 2 Northwestern states Kano and Kaduna, 3 North central states Abuja, Nassarawa and Plateau, and 1 North-Eastern state Bauchi in Nigeria used in this study when compared with previously published IBDV strains from 3 Northwestern states Kano, Kaduna and Katsina and 1 North central state Abuja (FCT) in Nigeria were 98 – 100%. The amino acid percentage identities between IBD viruses used in this study and other previously published Nigeria IBDV strains used for comparison were 98.6 – 100%. The deduced amino acid sequences (211 – 364) of the one hundred and five (105) Nigerian viruses used in this study (Appendix 10) were aligned with the sequences of the VP2 hypervariable region of 33 strains of IBDV from southwestern Nigeria (Owoade *et al.*, 2004), 3 from an undesignated region of Nigeria (Zierenberg *et al.*, 2000), 2 (submitted by Fagbohun and Giambrone, 2007), 15 from northwestern Nigeria (Adamu *et al.*, 2013), variant IBDV strains Del E, T1, classical strain STC and a locally produced IBD vaccine (Vom vaccine) obtained from the Genbank. The amino acids of the viruses used in this study and the strains obtained from the Genbank were truncated to obtain a uniform alignment. Amino acid substitutions were seen at position 219, Q→T in 16 of 105 and Q→P in 1 of 105 of the Nigerian viruses studied but this position Q219 was conserved in the previous Nigerian strains, variant and very virulent IBDV used for comparison. These mutations Q→T were observed in IBD viruses from the following states; Plateau, Anambra, Bauchi, Kaduna and Akwa Ibom. Interestingly, none of the previous published IBDV strains from Kaduna state had this mutation. A222 was conserved in all except in one previously reported Nigerian strain from southwest and in Bauchi38/NG/2009 and Bauchi37/NG/2102 that were similar to the variant IBDV strains (A→T). I242 was conserved in all others except in Nasarawa77/NG/no2/2013 and Plateau78/NG/2011/2 that had a (I→V) that was also seen in the variant strains. S254 that was observed in 101 of 105 Nigerian isolates was also seen in some of the previously published Nigerian strains and Del E, while the others have G254 as seen in vvIBDV. It was observed that S254 were seen more in IBD viruses from the North central, North west, North east, South east and South south states of Nigeria than in the South west states. Nineteen (19) of 105 of the viruses used in this study had an S at amino acid position 269. This region was conserved in all other Nigerian viruses studied (T269) including other previously

published Nigerian strains, variant and very virulent IBDV used for comparison. Four (4) of 105 viruses including one previous Nigerian strain were observed to have a T instead of I at position 272 which was conserved in others. Plateau92/NG/2013 when compared with the others was the only virus that had an amino acid substitution at position 261 (I261T). Q253, D279 and A284 was conserved except in Nasarawa77/NG/no2/2013 that had Q253H, D279N, and A284T, however 4 other viruses used in this study and one previously published Nigerian IBDV had D279N. Viruses containing Q253 and A284 are pathogenic while viruses with H253 and T284 are attenuated and can replicate *in vitro*. I272 was conserved in all the viruses used for this study and those used for comparison except in 4 of 105 viruses used in this study and one previously published Nigerian strain. Comparison of amino acid at positions 299 and 300 between the isolates used for this study and other Nigerian isolates showed that S299 was conserved in all the isolates except for one isolate Nasarawa77/NG/no2/2013 that had S299N that was also seen in the classic, variant and vaccine (Vom vaccine) strains. E300Q was seen in 19 of 105 viruses used in this study and one from previously published Nigerian IBDV strain used for comparism, while E300A was seen in 81 of 105. E300 was seen in 5 of 105 viruses used in this study and in 5 previously published Nigerian IBDV strains used for comparison. Interestingly, Q300 was not seen in any of the isolates from northwestern Nigeria and some of the isolates used in this study are from northwestern Nigeria. The serine-rich heptapeptide sequence SWSASGS was conserved in all the IBDV isolates used for this study and others used for comparison except in Nasarawa77/NG/2013, Plateau78/NG/2011/1 and Vom vaccine. The three had R330 instead of S330.

Table 11: RFLP band sizes for 11 IBDV viruses from Nigeria

		Restriction enzyme (product bp)									
S/no	**Sample ID**	***BstN1***				***Mbo1***			***Ssp1***	**Molecular group**	**New pattern**
		424	367	172	119	362	289	229			
1	Plateau19/NG/2009	✓		✓	✓	✓		✓	+	6	
2	Kaduna61/NG/2012		✓	✓	✓	✓		✓	+		✓
3	Bauchi58/NG/2012	✓		✓	✓		✓	✓	+		✓
4	Plateau67/NG/2011	✓		✓	✓	✓		✓	+	6	
5	Plateau53/NG/2010		✓	✓	✓	✓		✓	+		✓
6	Anambra54/NG/2012	✓		✓	✓		✓	✓	-		✓
7	Plateau7/NG/2009	✓		✓	✓	✓		✓	+	6	
8	Benin35/NG/2012	✓		✓	✓	✓		✓	+	6	
9	Bauchi37/NG/2012	✓		✓	✓	✓		✓	+	6	
10	Nassarawa77/NG/2013/1	✓		✓	✓		✓	✓	+		✓
11	Nassarawa77/NG/2013/2	✓		✓	✓		✓	✓	-		✓

Table 12a: Comparison of amino acid substitutions at different positions in IBDV genome segment A between viruses from Plateau State and very virulent (vv) UK661 strain

STRAIN/ISOLATE	AMINO ACID SUBSTITUTION AT POSITIONS															
	219	222	242	253	254	256	261	269	272	279	284	294	299	300	315	330
vvIBDV_UK_661	Q	A	I	Q	G	I	I	T	I	D	A	I	S	E	S	S
PLATEAU43/NG/2012	.	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
PLATEAU164/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU163/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU162/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU161/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU160/NG/2014	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
PLATEAU159/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU158/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU157/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU156/NG/2014	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
PLATEAU155/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU154/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU16/NG/2009	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU4/NG/2009	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU19/NG/2009	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU39/NG/2012	.	.	.	.	S	.	.		.	.	.	.	.	A	.	.
PLATEAU67/NG/2011	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU53/NG/2010	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU7/NG/2009	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU133/NG/2014	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
PLATEAU135/NG/2014	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
PLATEAU78/NG/2011/2	P	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	R
PLATEAU78/ NG/2011/1	T	.	.	L	S	.	.	S	.	.	.	.	.	Q	.	.
PLATEAU99/NG/2011	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU92/NG/2013	.	.	.	.	S	.	T	.	.	.	.	.	.	A	.	.
PLATEAU83/NG/2013	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU90/NG/2013	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU95/NG/2013	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU96/NG/2013	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU131/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU134/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU110/NG/2010	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU104/NG/2010	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU40/NG/2012	.	.	.	.	.	.	.	.	T	N	.	.	.	.	.	.
PLATEAU119/NG/2013	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
PLATEAU120/NG/2013	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
PLATEAU132/NG/2014	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
PLATEAU82/NG/2013	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
PLATEAU75/NG/2011	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.

- **NOTE**:A- Alanine,R- Arginine, N- Asparagine, D- Aspartic acid, E- Glutamic acid, Q- Glutamine, G- Glycine, I- Isoleucine L- Leucine, P- Proline, S- Serine, T- Threonine.

Table 12b:Comparison of amino acid substitutions at different positions in IBDV genome segment A between viruses from Akwa Ibom (Uyo) State and very virulent (vv) UK661 strain

STRAIN/ISOLATE	AMINO ACID SUBSTITUTION AT POSITIONS															
	219	222	242	253	254	256	261	269	272	279	284	294	299	300	315	330
vvIBDV_UK_661	Q	A	I	Q	G	I	I	T	I	D	A	I	S	E	S	S
UYO167/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO166/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO185/NG/2014	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
UYO187/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO188/NG/2014	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
UYO189/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO190/NG/2014	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
UYO191/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO192/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO193/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO194/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO195/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO196/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO197/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO198/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO199/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO200/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO201/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO202/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO203/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO204/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO205/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO206/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.

- **NOTE**:A- Alanine,D- Aspartic acid, E- Glutamic acid, Q- Glutamine, G- Glycine, I- Isoleucine, S- Serine, T- Threonine.
  S- Serine, T- Threonine.

Table 12c: Comparison of amino acid substitutions at different positions in IBDV genome segment A between viruses from Kwara State and very virulent (vv) UK661 strain

STRAIN/ISOLATE	AMINO ACID SUBSTITUTION AT POSITIONS															
	219	222	242	253	254	256	261	269	272	279	284	294	299	300	315	330
vvIBDV_UK_661	Q	A	I	Q	G	I	I	T	I	D	A	I	S	E	S	S
KWARA174/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA152/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA151NG/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA150/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA149/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA148/NG/2014	.	.	.	.	.	.	.	.	T	N	.	.	.	.	.	.
KWARA147/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA145/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA139/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA141/NG/2014	.	.	.	.	.	.	.	.	T	N	.	.	.	.	.	.
KWARA175/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA176/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	T	.
KWARA177/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA178/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	T	.
KWARA179/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA180/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA181/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA182/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA184/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA171/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.

- **NOTE**:A- Alanine,N- Asparagine, D- Aspartic acid, E- Glutamic acid, Q- Glutamine, G- Glycine, I- Isoleucine, S- Serine, T- Threonine.

Table 12d: Comparison of amino acid substitutions at different positions in IBDV genome segment A between viruses from Bauchi State and very virulent (vv) UK661 strain

STRAIN/ISOLATE	AMINO ACID SUBSTITUTION AT POSITIONS															
	219	222	242	253	254	256	261	269	272	279	284	294	299	300	315	330
vvIBDV_UK_661	Q	A	I	Q	G	I	I	T	I	D	A	I	S	E	S	S
BAUCHI37/NG/2012	.	T	.	.	S	.	.	.	.	.	.	.	.	A	.	.
BAUCHI36/NG/2009	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
BAUCHI58/NG/2012	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
BAUCHI37/NG/2009	.	T	.	.	S	.	.	.	.	.	.	.	.	A	.	.
BAUCHI124/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
BAUCHI126/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
BAUCHI127/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
BAUCHI128/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
BAUCHI121/NG/2013	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.

- **NOTE**:A- Alanine,D- Aspartic acid, E- Glutamic acid, Q- Glutamine, G- Glycine, I- Isoleucine, S- Serine, T- Threonine.

Table 12e: Comparison of amino acid substitutions at different positions in IBDV genome segment A between viruses from Anambra State and very virulent (vv) UK661 strain

STRAIN/ISOLATE	AMINO ACID SUBSTITUTION AT POSITIONS															
	219	222	242	253	254	256	261	269	272	279	284	294	299	300	315	330
vvIBDV_UK_661	Q	A	I	Q	G	I	I	T	I	D	A	I	S	E	S	S
ANAMBRA54/NG/2012	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
ANAMBRA50/NG/2012	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.

- **NOTE**:A- Alanine,D- Aspartic acid, E- Glutamic acid, Q- Glutamine, G- Glycine, I- Isoleucine, S- Serine, T- Threonine.

Table 12f: Comparison of amino acid substitutions at different positions in IBDV genome segment A between viruses from Abuja (FCT) and very virulent (vv) UK661 strain

STRAIN/ISOLATE	AMINO ACID SUBSTITUTION AT POSITIONS															
	219	222	242	253	254	256	261	269	272	279	284	294	299	300	315	330
vvIBDV_UK_661	Q	A	I	Q	G	I	I	T	I	D	A	I	S	E	S	S
ABUJA93/NG/2013	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.

- **NOTE**:A- Alanine,D- Aspartic acid, E- Glutamic acid, Q- Glutamine, G- Glycine, I- Isoleucine, S- Serine, T- Threonine.

Table 12g: Comparison of amino acid substitutions at different positions in IBDV genome segment A between viruses from Edo (Benin) State and very virulent (vv) UK661 strain

STRAIN/ISOLATE	AMINO ACID SUBSTITUTION AT POSITIONS															
	219	222	242	253	254	256	261	269	272	279	284	294	299	300	315	330
vvIBDV_UK_661	Q	A	I	Q	G	I	I	T	I	D	A	I	S	E	S	S
BENIN47/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
BENIN35/NG/2012	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
BENIN142E/NG/2013	.	.	.	.	.	.	.	.	T	N	.	.	.	.	.	.
BENIN87/NG/2013	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
BENIN34/NG/2012	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.

- **NOTE**:A- Alanine, D- Aspartic acid, E- Glutamic acid, Q- Glutamine, G- Glycine, I- Isoleucine, S- Serine, T- Threonine, N- Asparagine

Table 12h: Comparison of amino acid substitutions at different positions in IBDV genome segment A between viruses from Kaduna State and very virulent (vv) UK661 strain

STRAIN/ISOLATE	AMINO ACID SUBSTITUTION AT POSITIONS															
	219	222	242	253	254	256	261	269	272	279	284	294	299	300	315	330
vvIBDV_UK_661	Q	A	I	Q	G	I	I	T	I	D	A	I	S	E	S	S
KADUNA61/NG/2009	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KADUNA136/NG/2014	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.

- **NOTE**: A- Alanine, D- Aspartic acid, E- Glutamic acid, Q- Glutamine, G- Glycine, I- Isoleucine, S- Serine, T- Threonine.

Table 12i: Comparison of amino acid substitutions at different positions in IBDV genome segment A between viruses from Nassarawa State and very virulent (vv) UK661 strain

STRAIN/ISOLATE	AMINO ACID SUBSTITUTION AT POSITIONS															
	219	222	242	253	254	256	261	269	272	279	284	294	299	300	315	330
vvIBDV_UK_661	Q	A	I	Q	G	I	I	T	I	D	A	I	S	E	S	S
NASSARAWA77/NG/no2/_2013	.	.	V	H	S	V	.	.	.	N	T	L	N	.	.	.
NASSARAWA77/NG/2013	.	.	.	L	S	.	.	S	.	.	.	.	.	Q	.	R

- **NOTE**:A- Alanine, D- Aspartic acid, E- Glutamic acid, Q- Glutamine, G- Glycine, I- Isoleucine, S- Serine, T- Threonine, V-Valine, H- Histidine, R- Arginine, L- Leucine.

Table 12j: Comparison of amino acid substitutions at different positions in IBDV genome segment A between viruses from undesignated State and very virulent (vv) UK661 strain

STRAIN/ISOLATE	AMINO ACID SUBSTITUTION AT POSITIONS															
	219	222	242	253	254	256	261	269	272	279	284	294	299	300	315	330
vvIBDV_UK_661	Q	A	I	Q	G	I	I	T	I	D	A	I	S	E	S	S
NIG20/NG/2009	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
NIG79/NG/2009	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.

- **NOTE**:A- Alanine, D- Aspartic acid, E- Glutamic acid, Q- Glutamine, G- Glycine, I- Isoleucine, S- Serine, T- Threonine.

Table 13: Comparative spatial distribution of IBDV genome segment A amino acid substitutions and their positions between the Nigerian viruses and a reference very virulent (vv) UK661 strain

S/no	States vs UK661	No of Isolates	No of Amino Acid Substitutions	Amino Acid Positions
1	Plateau	39	11	219, 253, 254, 261, 269, 272, 279, 300, 330
2	Akwa Ibom (Uyo)	23	4	219, 254, 269, 300
3	Kwara	20	5	254, 272, 279, 300, 315
4	Bauchi	9	6	219, 222, 254, 269, 300
5	Anambra	2	4	219, 254, 269, 300
6	Abuja	1	2	254, 300
7	Edo (Benin)	5	4	254, 272, 279, 300
8	Kaduna	2	4	219, 254, 269, 300
9	Nassarawa	2	11	242, 253, 254, 256, 269, 279, 284, 294, 299, 300, 330
10	Undesignated	2	2	254, 300

Table 14: Comparative spatial distribution of amino acid substitutions and their positions between the Nigerian viruses and a reference variant Del E IBDV strain

S/no	States vs Del E	No of Isolates	No of Amino Acid Substitutions	Amino Acid Positions
1	Plateau	39	23	213, 219, 222, 242, 249, 253, 254, 256, 261, 269, 272, 279, 286, 294, 299, 300, 318, 323, 330, 416, 422, 427
2	Akwa Ibom (Uyo)	23	15	213, 219, 222, 242, 249, 256, 269, 279, 286, 294, 299, 300, 318, 323
3	Kwara	20	16	213, 222, 242, 249, 254, 256, 272, 279, 286, 294, 299, 300, 315, 318, 323
4	Bauchi	9	14	213, 219, 222, 242, 249, 256, 269, 286, 294, 299, 300, 318, 323
5	Anambra	2	15	213, 219, 222, 242, 249, 256, 269, 286, 294, 299, 300, 318, 323, 410
6	Abuja	1	12	213, 222, 242, 249, 256, 279, 286, 294, 299, 300, 318, 323
7	Edo (Benin)	5	14	213, 222, 242, 249, 254, 256, 272, 279, 286, 294, 299, 300, 318, 323
8	Kaduna	2	14	213, 219, 222, 242, 249, 256, 269, 286, 294, 299, 300, 318, 323
9	Nassarawa	2	17	213, 222, 242, 249, 253, 256, 269, 284, 286, 294, 299, 300, 318, 323, 330
10	Undesignated	2	13	213, 222, 242, 249, 256, 279, 286, 294, 299, 300, 318, 323, 395

Table 15: Comparative temporal distribution of amino acid substitutions and their positions between the Nigerian viruses and reference very virulent (vv) UK661 IBDV strain

S/no	Year	No of Isolates	No of Amino Acid Substitutions	Amino Acid Positions
1	2009	9	3	219, 222, 254, 300
2	2010	3	2	254, 300
3	2011	5	6	219, 253, 254, 269, 300, 330
4	2012	9	7	219, 222, 254, 269, 272, 279, 300
5	2013	14	14	242, 253, 254, 256, 261, 269, 272, 279, 284, 294, 299, 300, 330
6	2014	65	7	219, 254, 269, 272, 279, 300, 315

Table 16: Comparative temporal distribution of amino acid substitutions and their positions between the Nigerian viruses and reference Del E IBDV strain

S/no	Year	No of Isolates	No of Amino Acid Substitutions	Amino Acid Positions
1	2009	9	12	213, 222, 242, 249, 256, 279, 286, 294, 299, 300, 318, 323
2	2010	3	12	213, 222, 242, 249, 256, 279, 286, 294, 299, 300, 318, 323
3	2011	5	17	213, 219, 222, 242, 249, 253, 256, 269, 279, 286, 294, 299, 300, 318, 323, 330
4	2012	9	18	213, 219, 222, 242, 249, 254, 256, 269, 272, 279, 286, 294, 299, 300, 318, 323, 410, 416
5	2013	14	21	213, 219, 222, 242, 249, 253, 254, 256, 261, 269, 272, 279, 284, 286, 294, 299, 300, 318, 323, 330
6	2014	65	20	213, 219, 222, 242, 249, 254, 256, 269, 272, 279, 284, 294, 299, 300, 315, 318, 323, 422, 427, 441

4.6.3 Comparison of the nucleotide and deduced amino acid sequences of segment A of IBDV viruses used in this study and some previously published IBDV strains from other African countries

The resulting nucleotide and amino acid sequences of the Nigerian IBD viruses used in this study were truncated to achieve uniform alignment. Nucleotide and amino acid sequences of some IBDV strains from Africa in Genbank published by Le Nouen *et al* (2006) and other workers were retrieved and used for comparison. Thus, the amino acid sequences start from position 220 to 344. The nucleotide sequence percentage identities of the Nigerian viruses when compared with the nucleotide sequences of some of the African IBDV strains were as follows; Egypt (93.4 – 95.9%), Ethiopia (93.4 – 97.7%), Ivory Coast (90.6 – 93.7%), Tanzania (89.7 – 95.9%), South Africa (92.9 – 98.8%) and Senegal (89.8 – 94%). The amino acid percentage identities of the Nigerian viruses when compared with 3 vvIBDV strains from Ivory Coast; AJ878888_88035.628, AJ878889_88035.693 and AJ878890_88035.737 ranged from 93.6 – 95.7. Their percentage identity with AJ58500_99323 a vvIBDV strain from Egypt ranged from 93.6 – 97.9%. With AJ878887_99006/1552 a vvIBDV from Senegal, their percentage identity ranged from 90.7 – 94.3%. When the Nigerian IBDV sequences were compared with two IBDV strains from Tanzania; BAG31964.1 and BAG31963.1, percentage identities ranged from 92.4 – 96.9%, with IBDV strains from South Africa, the percentage identities ranged from 88.5 – 99.2% and with IBDV strains from Ethiopia the percentage identities ranged from 93.6 – 99.2%. The deduced amino acid sequences (220-344) of the one hundred and five (105) Nigerian viruses used in this study were aligned with the sequences of the VP2 hypervariable region of some African IBDV strains from Genbank plus a vvIBDV and variant IBDV reference strains (Appendix 11). Position 219 was conserved in all the African strains used for comparison and the vvIBDV (UK661) and in the Nigerian viruses except for the substitution Q→T in this position seen in 16 of 105 and Q→P seen in 1 of 105 of the Nigerian viruses. A222 was conserved in all the Nigerian viruses except in 2 out of the 105 used in this study that had mutation A→T at this position. It was also observed that IBDV strain from Tanzania (BAG31963.1) and Senegal (AJ878887_99006/1552) had a P in this position and all the three strains from Ivory Coast had a Q in this position. I242 was conserved in all the Nigerian viruses and other African strains used for comparism except in two Nigerian viruses, one from Tanzania and one from Senegal that has a V instead of an I at this position. S254 was conserved in all the Nigerian viruses and other

African IBDV strains used for comparism except in four Nigerian viruses, one Tanzania, Senegal and three Ivory Coast strains that had a G in this position like the vvIBDV (UK661) but one South African strain had an N at this position. I256 was conserved in all the Nigerian viruses and African strains except in one virus from Nigeria, Tanzania, Senegal and seven from South Africa that had a V→I in this position like the variant Del E. I272 was conserved in all except in 4 Nigeria viruses (Kwara148/NG/2014, Benin142E/NG/2013 (an embryo derived IBDV isolate), Plateau40/NG/2012 and Kwara141/NG/2014) showed an amino acid substitution at this position I272T. D279 was conserved in all except in 5 Nigerian viruses and 7 South African strains that had a mutation D279N at this position. I294 and S299 were conserved in all the strains except in 1 out of 105 Nigerian viruses and 12 out of 19 African strains that had an I294L at this position and 1 out of 105 Nigerian viruses and 12 out of 19 African strains that had a S299N at this position as seen in the variant Del E. E300 seen in the vvIBDV was observed in 8 of the African strains and 5 of the Nigerian viruses while E300A was seen in 81 out of 105 Nigerian viruses and E300D seen in 5 African strains. The unique Q300 seen in 19 out of 105 Nigerian viruses was observed in 8 African strains. None of the African strains and the others used for comparison had an A at position 300 as seen in some of the Nigerian viruses. The conserved serine-rich heptapeptide sequence SWSASGS adjacent to the second hydrophilic region was found in all except in two Nigerian viruses that had R330 instead of S330.

4.6.4 Comparison of the nucleotide and deduced amino acid sequences of Nigerian IBDV viruses used in this study and previously published IBDV strains from other countries of the world

Nucleotide and amino acid sequences obtained from the Nigerian IBDV viruses used in this study were compared with IBDV strains retrieved from Genbank from other countries submitted by Le Nouen *et al* (2006). The Nigerian IBDV sequences were truncated so as to align with the sequences from Genbank with the amino acids starting from position 211-350 (Appendix 12). The nucleotide sequence percentage identity ranges of the Nigerian viruses when compared with other IBDV strain from other countries are as follows; Australia 82.1 – 85.8%, United Kingdom 90.8 – 94.5%, Germany 90.5 – 94.9, Poland 88 – 98.6%, France 92.9 – 98.6%, Belgium 93.7 – 98.8%, The Netherlands 93.7 – 98.8%, Hong Kong 92.7 – 98.4%, China 92.9 – 98.1%, Brazil 93.7 – 98.8, Bangladesh 93.7 – 97.9%, Dominican Republic 92.9 – 96.7%, USA 89.4 – 95.5 and Venezuela 93.7 – 98.6%. The amino acid percentage identities of the Nigerian IBDV strains

when compared with IBDV strains from Australia ranged from 86.4 – 91.4%. With IBDV strains from United Kingdom the percentage identities ranged from 95 – 95.7%. When compared with IBDV strains from France the percentage identities ranged from 93.6 – 98.6%. With IBDV strains from Venezuela the percentage identities ranged from 97.1 – 98.6%. With strains from Poland the percentage identities ranged from 87.9 – 98.6%. When compared with IBDV strains from Germany, the percentage identities ranged from 93.6 – 95.7%. When compared with IBDV strain from Belgium the percentage identities ranged from 93.6 – 95.7%. With IBDV strains from China, percentage identities ranged from 94.3 – 98.6%. With strain from Bangladesh percentage identities ranged from 93.6 – 99.3%. With strain from Dominican Republic the percentage identities ranged from 92.9 – 98.6%. With strains from Brazil percentage identities ranged from 94.3 – 98.6%. When compared with strains from the United States of America, the percentage identities ranged from 90 – 97.1%. With IBDV strain from The Netherlands, percentage identities ranged from 90 – 97.1%. When the deduced amino acid sequences of the 105 Nigerian IBDV viruses were compared with amino acid sequences of IBDV strains from other countries, position 219 was conserved in all the IBDV strains from other countries and in the Nigerian viruses except for the substitution seen in 16 out of 105 (Q→T) and 1out of 105 (Q→P) in the Nigerian viruses. It was observed that only the IBDV viruses from Nigeria had this substitution. Position 222 was conserved except in 2 Nigerian viruses (A→T), in the variant strains from the USA (A→T), another variant strain from USA (A→Q), classic STC (A→P), 2 strains from Poland (A→S). A strain F50/70 from UK had A→P at position 222, 002-73, CU-1WT from Germany, 2 Australian strains, CT4/4 and USA Georgia also had this change. Positions 242 was conserved except in the variant, classic, 002-73, 3 Australia, 2 Poland, CT4/4 and 2 Nigerian strains that had amino acid mutation I→V. I256 was conserved in all the strains except in the variant, classic, CU-1WT from Germany, 2 Australian, 2 Poland, CT4/4, F50/70, Belgium and 1 Nigerian virus. 002-73 was observed to have an I→L and 05/5 had an I→A at position 256. Likewise, I294 was conserved in all the Nigerian viruses except in one (Nasarawa77/NG/no2/2013), the variant, classic, 022-73, 3 Australia, 2 Poland, CT4/4 and F52/70 strains where substitution of amino acid I→L was observed. Position S299 was also conserved except in all the strains mentioned for position 294 and they have an S299N substitution. It should be noted that positions 222, 242, 256, 294 and 299 are considered as the

virulence markers on the VP2 gene of IBDV. Positions Q253, D279 and A284 that are known as sites for cell culture adaptation were conserved except for the substitutions seen at position 253 in CT4/4, Nasarawa77/NG/no2/2013, Variant A and Georgia that had Q→H, while 2 Nigerian viruses had Q→L, substitution in position 279 D→N seen in 2 variants, 2 Poland, CT4/4, 5 Nigerian viruses with 2 Australian and 022-73 having a D→G, substitution in position 284 A→T seen in CT4/4, 1 Nigerian virus and 2 variant strains. G254 was conserved in some of the IBDV strains from other countries used for comparison except in variants E and GLS5, 05/5 and 101 Nigerian viruses have amino acid mutation G→S, variant T1 had a G→N and 80GA from Poland had a G→D. Position E300 was conserved in all the strains from other countries used for comparison and in 5 out of 105 Nigerian viruses except in strains from Bangladesh, Dominican Republic and in 81 out of 105 Nigerian viruses that had a E→A, while 19 out of 105 Nigerian viruses had a E→Q. The conserved serine-rich heptapeptide sequence SWSASGS (amino acid position 326-332) adjacent to the second hydrophilic region was found in all of the strains except for mutations seen in 06/95 and N1/99 that had a S→L, S→N at positions 328 and 332 respectively, N1/99 (S→T), 05/5 (S→V) in position 329, CT4/4, Nasarawa77/NG/2013, Plateau78/NG/2011/1, USA Georgia had a S→R in position 330 while 002-73, 06/95, N1/99 and 05/5 had a S→N at position 332.

4.6.5 Comparison of the nucleotide and deduced amino acid sequences of Nigerian IBDV viruses with some IBDV vaccines commonly used in Nigeria and others

Amino acid sequences of some IBDV vaccines commonly used in Nigeria for vaccination of chickens and some others were retrieved from Genbank and used to compare the Nigerian IBDV viruses used in this study. The percentage identities ranged from 86.9 – 96.9%. It was observed that two vaccines MB and ABIC both from Israel had a 100% identity with 4 Nigerian viruses namely; Kwara141/NG/2014, Kwara148/NG/2014, Plateau40/NG/2012 and Benin142E/NG/2013, while their percentage identities ranged from 93.8 – 96.9%. Unfortunately, when the vaccination records of the origin of these strains were checked, the vaccine used on the flock was unavailable except for Benin142E/NG/2013 which originated from an IBDV sample that was propagated in eggs. When the deduced amino acid sequences of the Nigerian strains were compared with the amino acid sequences of the vaccine strains retrieved from Genbank (appendix 13), the amino acid mutation observed at position 219 (T) was not seen in any of the

vaccine strains. Position A222 was conserved in the Nigerian viruses, MB and ABIC vaccine strains only. Other vaccine strains had a A222P, A222S and A222L at these positions. V242 was conserved in some of the vaccines and 2 Nigerian viruses except for some strains that had a V242I at this position with the Nigerian viruses. 253Q was conserved in the Nigerian viruses and in some of the vaccine strains except for 2 Nigerian viruses that had an L at this position and other vaccine strains and 1 Nigerian virus that had a H. Likewise G254 was conserved in all the vaccine strains and in 4 Nigerian viruses but the other Nigerian viruses had a G254S in this position. V256I amino acid substitution was seen in all the Nigerian viruses except in one but was conserved in the vaccine strains. One Nigerian virus had an amino acid substitution at position 261(I→T). T268 was conserved in all the vaccine strains and Nigerian viruses except for 19 out of 105 Nigerian viruses that had a T268S while position T269 was conserved in the vaccine strains except for 2 vaccine strains that had T269A with all of the Nigerian viruses. Position 272 was conserved in all the Nigerian viruses except in 4 out of 105 of the Nigerian viruses and in 4 of the vaccine strains that had a substitution I272T. N279 was conserved in some of the vaccines and 4 Nigerian viruses except in 2 vaccine strains that had a G at this position while the rest had a D at this position with the other Nigerian viruses. Amino acid substitution T284A was seen in all but one of the Nigerian virus and vaccine strains but the other vaccine strain maintained T284. I294L was seen in all the Nigerian viruses and 3 vaccine strains. Three combinations of amino acids substitutions N299S, E300A and E300Q were seen at these positions. All the vaccine strains and one Nigerian virus has an N299 and E300. The serine-rich heptapeptide region was conserved in all the strain except for substitutions seen at position R328L in two vaccine strains and R330S seen in all the Nigerian viruses except in two.

4.7 Phylogenetic analysis

4.7.1 Phylogenetic analysis of the segment A of the Nigerian viruses with reference IBDV strains from GenBank

To gain insight into the genetic relatedness among the Nigerian viruses and other IBDV strains, a phylogenetic tree based on the IBDV-vVP2 (701-1133) nucleotide sequences was constructed (Figure 4) using the neighbor joining (NJ) method with 1000 bootstrap replication with seven reference vvIBDV strains (UK661, AJ583500.1_Egypt, OKYM (Japan), Harbin, Henan and Gx

(China), HK46 (Hong Kong)), variant IBDV strains (Del E and T1), classic IBDV strain (STC) and serotype 2 IBDV strains (OH (USA), TY89 and 23/82) obtained from Genbank. The resulting tree showed that the Nigerian IBD viruses belong to the serotype 1 IBDV. The tree showed three major branches with bootstrap value of 51 with the Nigerian viruses falling into 3 distinct groups. The largest group with bootstrap value of 96 has three major clusters, with the first cluster consisting of Nigerian IBDV viruses exclusively (81 out of 105) with bootstrap value 89, the second cluster has all the reference vvIBDV strains with 4 Nigerian viruses on the same branch, with the Nigerian viruses forming a separate cluster within this branch (bootstrap value 67) and the third cluster consists of 19 Nigerian IBDV viruses exclusively (bootstrap value 82). The second major branch consists of one Nigerian IBDV virus with the variant and classic IBDV strains. The third branch contains the serotype 2 IBDV strains with none of the Nigerian viruses or reference IBDV strains found within this group.

4.7.2 Phylogenetic analysis of the segment A of the Nigerian viruses with previously published Nigerian IBDV strains from GenBank

Phylogenetic tree constructed using the nucleotide sequences from the VP2 hypervariable region of IBDV used in this study and some previously published Nigerian, (Figure 5) very virulent and variant IBDV strain sequences from GenBank using the neighbor joining (NJ) method with 1000 bootstrap replicates produced three major branches. The first branch which is the largest comprised the IBDV viruses used in this study, the previously published Nigerian strains and the vvIBDV reference strains but it was observed that the IBDV viruses used in this study formed a cluster of their own with sub clusters within their clusters. Four IBDV viruses used in this study were seen on the same branch with reference vvIBDV and some previously published Nigerian IBDV strains mostly from Southwestern Nigeria (88% bootstrap value) but they formed their own unique cluster on this branch. The second branch consist exclusively the IBDV viruses used in this study (89% bootstrap value). None of the previously published Nigeria and reference vvIBDV strains were seen on this branch. The third branch consists of a variant reference IBDV Del E with one IBDV virus used in this study (98% bootstrap value).

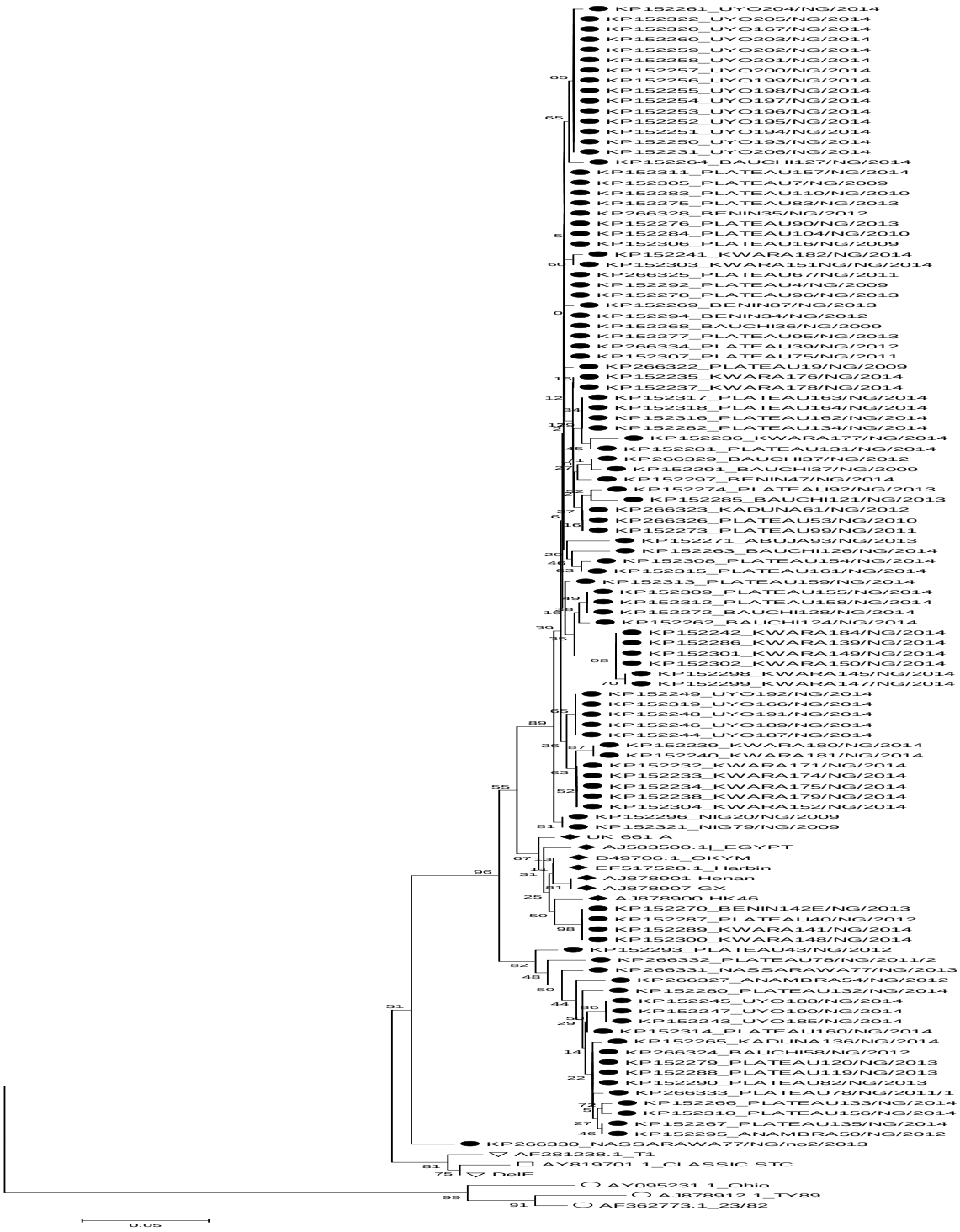

Fig 4: Phylogenetic tree produced using the NJ method with 1000 bootstrap replication based on nucleotides 701-1133 of the VP2 hypervariable region for Nigerian IBDVs, reference very virulent, variant, classic and serotype 2 IBDVs. Labels indicate strains: Nigerian viruses (black circle), very virulent strains (black diamond), variant (blank triangle), classic strain (blank square), serotype 2 (blank circle). GenBank accession numbers are followed by the common names of the virus.

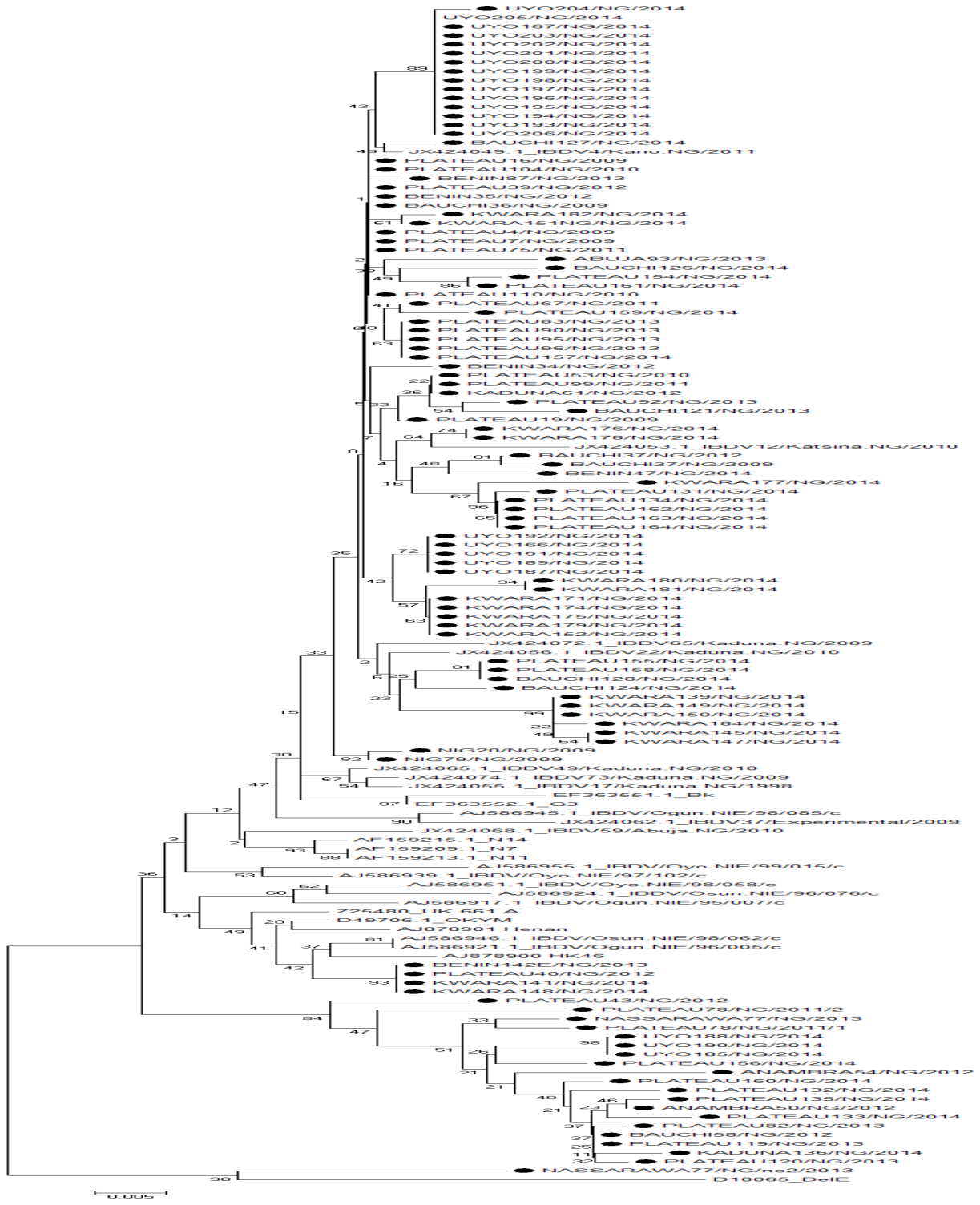

Fig 5: Phylogenetic tree produced using the NJ method with 1000 bootstrap replication based on nucleotides 704-1137 of the VP2 hypervariable region for Nigerian viruses used in this study with previously published Nigeria IBDVs. Labels: Nigerian viruses used in this study (black circle).

4.7.3 Phylogenetic analysis of the segment A of the Nigerian viruses with some African IBDV strains from GenBank

The phylogenetic tree constructed with the nucleotide sequences of the IBDV viruses used in this study and nucleotide sequences of some IBDV strains from Africa obtained from Genbank using the neighbor joining and UPGMA method with 1000 bootstrap replicates produced similar trees with three major branches. The first and largest branch has three distinct clusters; the first cluster with bootstrap value of 96% and 91% for NJ and UPGMA trees, respectively consists mainly of the IBDV viruses used in this study, the second cluster with bootstrap value of 69% and 81% for NJ and UPGMA trees, respectively consists of reference vvIBDVs, IBDV strains from South Africa, Tanzania, Egypt and four IBDV viruses used in this study, the third with bootstrap value of 85% and 86% for NJ and UPGMA, respectively consists of IBDV strains from Ethiopia and some IBDV viruses used in this study. The second branch consists of a cluster of IBDV from Ivory Coast only (bootstrap value 100% for NJ and UPGMA, respectively). The third major branch with bootstrap value of 71% and 51% for NJ and UPGMA, respectively consists of variant Del E and classic STC IBDV with IBDV strains from Senegal, Tanzania and Nassarawa77/NG/no2/2013. Generally, it was observed that IBDV viruses used in this study formed their own clusters within each branch (Figures 6, 7).

4.7.4 Phylogenetic analysis of the segment A of the Nigerian viruses with IBDV strains from other countries inGenBank

Phylogenetic analysis of the nucleotide sequences of the IBDV viruses used in this study and IBDV strains from other countries obtained from GenBank produced a tree that showed the IBDV viruses used in this study falling into four clusters, the first with a bootstrap value 86% consists of some of the Nigerian viruses used in this study, none of the other IBDV strains from other countries was found on this cluster, the second cluster with bootstrap value 22% consists of vvIBDV strains from United Kingdom, France, China, Brazil, Poland, Venezuela, Egypt, Hong Kong, Dominican Republic and USA plus four of the Nigerian viruses used in this study. The third cluster with bootstrap value 91% consists of only the Nigerian viruses used in this study, none of the other IBDV strains from other countries was seen on this cluster, while the fourth cluster consists of the variant, classic and one of the Nigerian virus used in this study (bootstrap

values of 81 and 54, respectively). The fifth cluster consists of the serotype 2 IBDV strains, none of the Nigerian strains were found in this cluster (Figure 8).

4.7.5 Phylogenetic analysis of the segment A of the Nigerian viruses with some IBDV vaccines from GenBank

Phylogenetic tree constructed using the nucleotide sequences obtained from the IBDV viruses used in this study and some vaccine strains obtained from Genbank using the neighbor joining methods with 1000 bootstrap replicate produced two distinct branches that had 104 out of 105 of the IBDV viruses used in this study on a branch with bootstrap value of 96% and within this branch two IBDV vaccine strains ABIC and MB were seen clustering with four of the IBDV strains used in this study (99% bootstrap value). The other branch with bootstrap value 64% comprised mainly of all the other vaccine strains except for one Nigerian IBDV virus used in this study that was seen in this branch (Figure 9).

Fig 6: Phylogenetic tree produced using the NJ method with 1000 bootstrap replication based on nucleotides 704-1136 of the VP2 hypervariable region for Nigerian IBDVs and some IBDV strains from Africa with reference very virulent, variant and classic IBDVs.

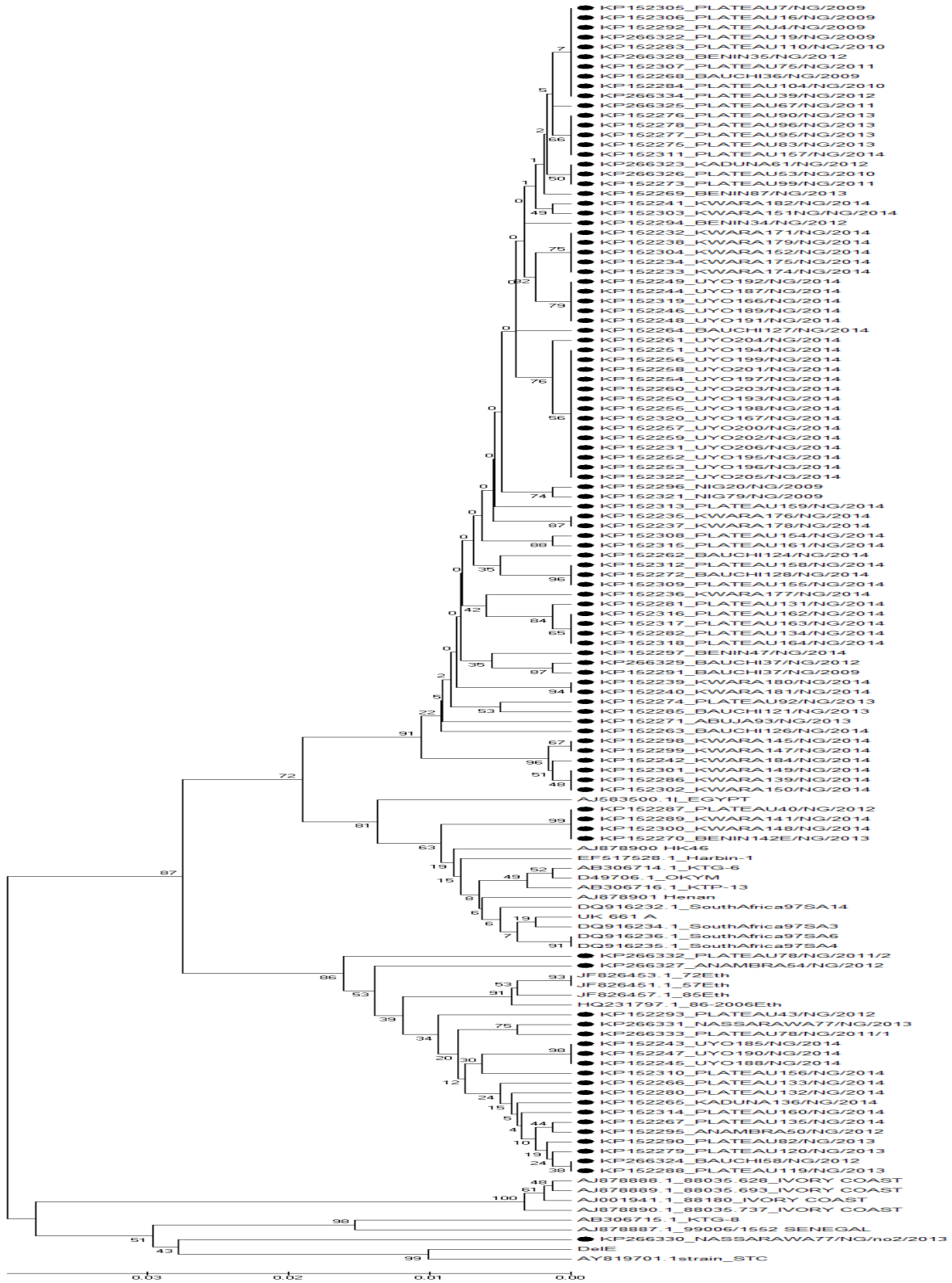

Fig 7:Phylogenetic tree produced using the UPGMA method with 1000 bootstrap replication based on nucleotides 704-1136 of the VP2 hypervariable region for Nigerian IBDVs and some IBDV strains from Africa with reference very virulent, variant and classic IBDVs. Labels indicate strains: Nigerian viruses used in this study (black circle)

Fig 8: Phylogenetic tree produced using the NJ method with 1000 bootstrap replication based on nucleotides 704-1099 of the VP2 hypervariable region for Nigerian IBDVs and some IBDV strains from other countries with reference very virulent, variant, classic and serotype 2 IBDVs. Labels indicate strains: Nigerian viruses used in this study (black circle).

4.8 Results for segment B

4.8.1 RT-PCR for segment B and gel electrophoresis

Out of the ninety-seven (97) samples selected to test for the presence of infectious bursal disease VP1 gene using RT-PCR and primers that amplify a 722bp of the viral protein (VP1), eighty-seven (87) showed corresponding bands of 722bp compared to the ladder and known IBDV VP1 positive control indicating a positive result and presence of IBD VP1 gene in the samples, while ten (10) were negative. Plates 5a and 5b show examples of amplicons of the expected 722bp that were obtained by RT-PCR.

4.9 Sequence Analysis

4.9.1 Sequence analysis of segment B nucleotide and amino acid sequences of the Nigerian IBD viruses and other segment B IBDV strains from GenBank

The VP1 of IBDV genome segment B of the Nigerian viruses was amplified in the RT-PCR assay and the nucleotide sequences were determined. Their GenBank accession numbers are KP 172084-KP172144 and KP266335-KP266340 (Appendix 5). Summary of the amino acid substitutions that occurred in the VP1 segment B genome of the Nigerian viruses compared with some IBDV from GenBank and their positions are shown in appendix 6. The nucleotide sequences of the VP1 of the Nigerian viruses were aligned and compared with the nucleotide sequences of eight reference IBDV VP1 sequences comprising of 4 vvIBDV, 4 non-vvIBDV from nucleotides position 312 – 854 obtained from GenBank, (Appendix 14). The following percentage identity ranges between the Nigerian viruses and the reference strains were obtained; with HK46→ 83.8 – 99.1, UK661→ 83.8 – 99.3, OKYM→ 83.4 – 98.3, Del E→ 86.2 – 87.7, T09→ 93.6 – 96.6, HENAN→ 84.9 – 88.6, TY89→ 85.8 – 87.8, 23-82→ 86.2 – 88.9. When the nucleotide sequences of the Nigerian IBDV VP1 722bp region was examined and compared with the eight reference IBDV VP1 sequences, mutations observed in the Nigerian sequences were uniform. Some of the mutations were silent or synonymous whereas others were not silent or non-synonymous. The silent substitution mutations observed did not alter the amino acids obtained when the nucleotides were translated and corresponded with the amino acids at the same positions with the reference strains used for comparism. However, non-synonymous

mutations were observed at nucleotides positions 396-398 (AAC) that translated to N (asparagine) in three of the Nigerian viruses while the reference strains had ACT (T-threonine), nucleotide position 402-404 of one of the Nigerian viruses had ACT (T) while other had TCA (S) at these positions. Forty six (46) of the Nigerian viruses had GAC (D-aspartic acid) at nucleotides positions 464-467 instead of GAA (E-glutamic acid) seen in the remaining Nigeria viruses and the reference strains. Nucleotide position 477-479 of three Nigerian viruses and one serotype 2 IBDV (23-82) had ACG (T) while the others had GCG (A-alanine), nucleotides positions 531-533 of 61 of the Nigerian viruses had ATT (I), 3 had ACT (T) while the remaining 3 and the other IBDV strains had GTT (V). At nucleotide positions 543-545, 51 of the Nigerian viruses had CAA, 13 had CAC while 3 had ACA like the vvIBDV, and non-vvIBDVs included for comparison had GAG at these positions. Nucleotides positions 546-548 showed 62 Nigerian viruses having GAA, 2 had GAC while 3 had GAT, and positions 549-551, 64 Nigerian viruses had GGG while the remaining 3 had AAC like the vvIBDV. Sixteen (16) of the Nigerian viruses used a combination of nucleotides CCA and CCT at positions 552-554 for amino acid proline while the rest had nucleotides CTC for lysine at these positions. Nucleotide positions 558-560 showed 61 of the Nigerian viruses having GAA (E) while the rest had GAT (D). At nucleotides position 567-569, one Nigerian virus had GCT while it was conserved for the others which had ACC and ACT that translated to the same amino acid threonine. Nucleotide position 570-572 showed 4 Nigerian viruses having ATC while the rest had CTA or CTC that translated to the same amino acid while at positions 591-593, 3 Nigerian viruses had GCT as against GAT or GAC found in the others. Nucleotides positions 597-599 showed 52 Nigerian viruses having GTC instead of GCC seen in the others. One Nigerian virus had an ACG at nucleotides positions 618-620 instead of the ACT or ACA seen in the rest. Likewise 2 Nigerian viruses at nucleotide positions 630-632 had TCA and GCA respectively instead of ACT or ACA like the others. Also one Nigerian virus at nucleotide position 735-737 had CCT and not ACT, ACC or ACA like the rest. Sixty four (64) of the Nigerian viruses had nucleotides GAG at positions 765-767 while the other IBDV strains including 3 Nigerian viruses had GAC instead at these positions. The remaining substitution mutations in the Nigerian viruses were seen at nucleotide positions 810-812 where one Nigerian virus had ATG instead of GTT or GTG like the others, and at positions 828-830 where another single Nigerian virus had TAT and not GAT like the others. Nucleotides

at positions 834-836 of 64 of the Nigerian viruses and one IBDV vaccine strain used for comparison had GAC while the rest had GAA. All these mutations observed were not silent.

The percentage identities when the Nigerian viruses were aligned and compared with each other ranged from 93.4 – 100. When the deduced amino acid sequences (aa 68-248) of the 67 Nigerian IBDV genome segment B were aligned and compared with the segment B sequences of some reference IBDV strains obtained from GenBank consisting of four vvIBDV (HK46, UK661, OKYM, T09) and four non-vvIBDV (HENAN, DEL E, TY89, 23-82), (Appendix 15) their percentage identity with the Nigerian viruses ranged as follows; with HK46= 94.5 – 98.9, with UK661= 94.5 – 98.9, with OKYM= 93.9 – 97.8, with T09= 94.5 – 98.9, with DEL E= 95.6 – 96.7, with HENAN= 95 – 96.7, TY89= 93.7 – 96.7 and with 23-82= 93.7 – 96.7. A critical look at the Nigerian IBDV genome segment B sequences starting from amino acid positions 68 – 248 compared with some IBDV segment B mentioned above obtained from GenBank, showed that amino acid positions 68 – 117 of both the Nigerian viruses and other strains were conserved except for two amino acid substitutions at position 69 of two IBDV used for comparison (OKYM and TY89), two amino acid substitutions seen at positions 96 (T→N) in three Nigerian viruses and at positions 98 (S→T) in one Nigerian virus. At positions 119, amino acid substitution E→D were seen in 46 out of 67 of the Nigerian strains but conserved in the remaining 21 and the other IBDV strains used for comparison. A123 was conserved in all except in three Nigerian viruses and 23/82 that had a T at this position. V141 was conserved in the non-vvIBDV and vvIBDV used for comparism and in 3 out of 67 Nigerian strains but an amino acid substitution V141I was seen in 61 of the Nigerian strains and V141T seen in the remaining three. At amino acid positions 145, 146 and 147 known as the triplet amino acids, the Nigerian viruses showed the following motifs; QEG in 50, HEG in 12, QDG in 1, HDG in 1 and TDN in 3. vvIBDVs included in this study have TDN at these positions while the non-vvIBDVs have NED, NEG or TEG at these three positions. Another amino acid substitution was observed in position 148 (L→P) in 16 out of 67 of the Nigerian viruses but it was conserved in the remaining and the other IBDV strains used for comparison. Positions 150 was conserved in all the IBDV strains used for comparison and in 6 out of 67 Nigerian viruses but a substitution (D→E) was observed in the remaining 61. Positions T153 was conserved in all except in one Nigerian virus. Likewise, positions 154 (L) was conserved in all except in four Nigerian viruses. D161 was conserved in

all except in three Nigerian viruses that had an A at this position. Amino acid substitution A163V was observed in 51 out of 67 of the Nigerian strains while this position was conserved in the remaining 16 and the other IBDV strains used for comparison. D219 was conserved in all the IBDV strains used for comparison and three Nigerian viruses but a substitution D219E was observed in the remaining 64 Nigerian strains. Position 242 was conserved in all the IBDV strains used for comparison and three Nigerian strains but an amino acid substitution E242D was observed in the remaining 64 Nigerian viruses. Tables 17a-h show the comparison of amino acid substitution and their positions between the Nigerian segment B viruses used in this study with reference very virulent HK46 and variant Del E strains of IBDV according to states. Tables 18 – 21 show the distribution of the amino acid substitutions that occurred in the 67 Nigerian segment B IBDV compared with reference very virulent HK46 and variant Del E IBDV strains according to states and years.

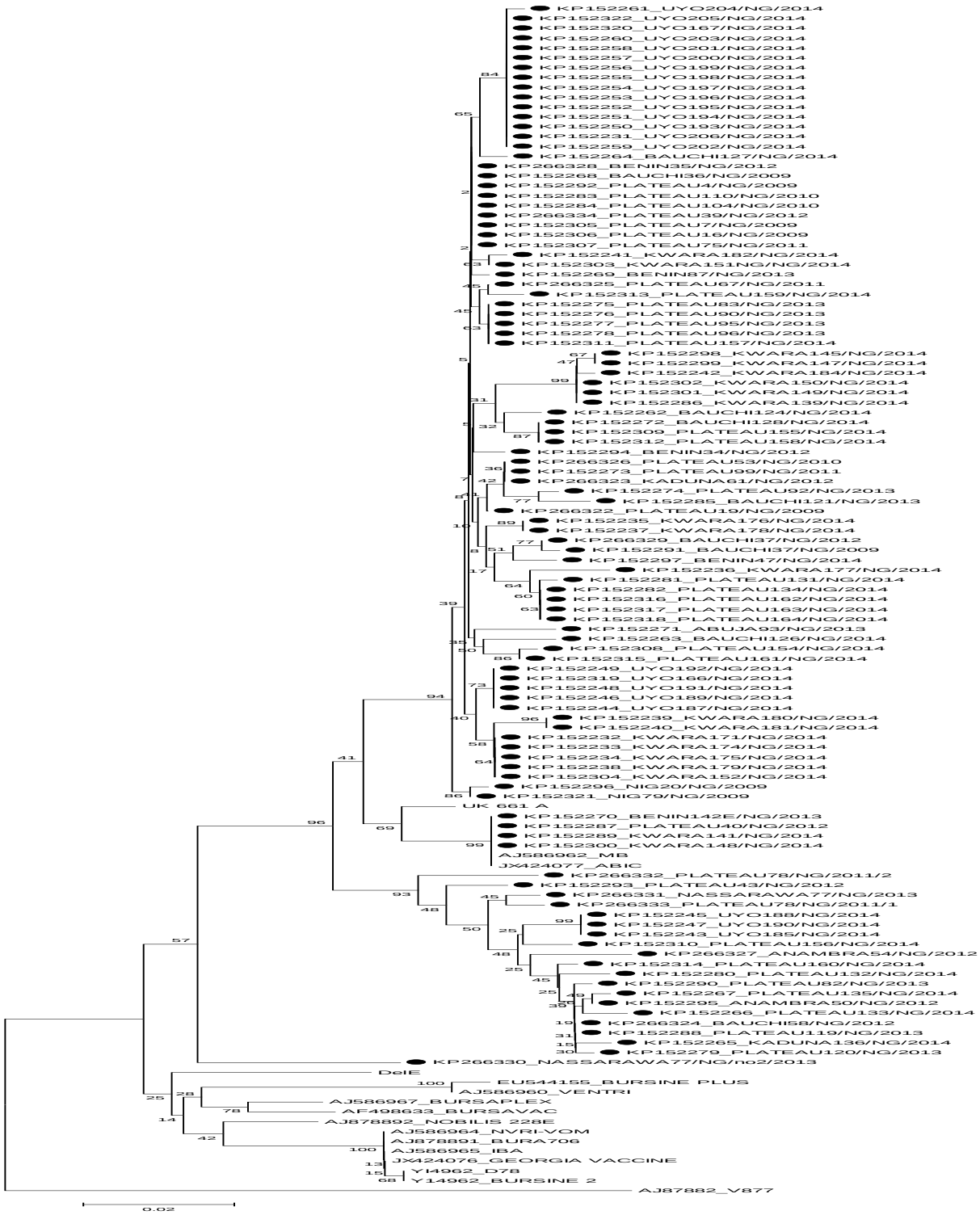

Fig 9: Phylogenetic tree produced using the NJ method with 1000 bootstrap replication based on nucleotides 704-1133 of the VP2 hypervariable region for Nigerian IBDVs and some IBDV vaccine strains. Labels indicate strains: Nigerian viruses used in this study (black circle).

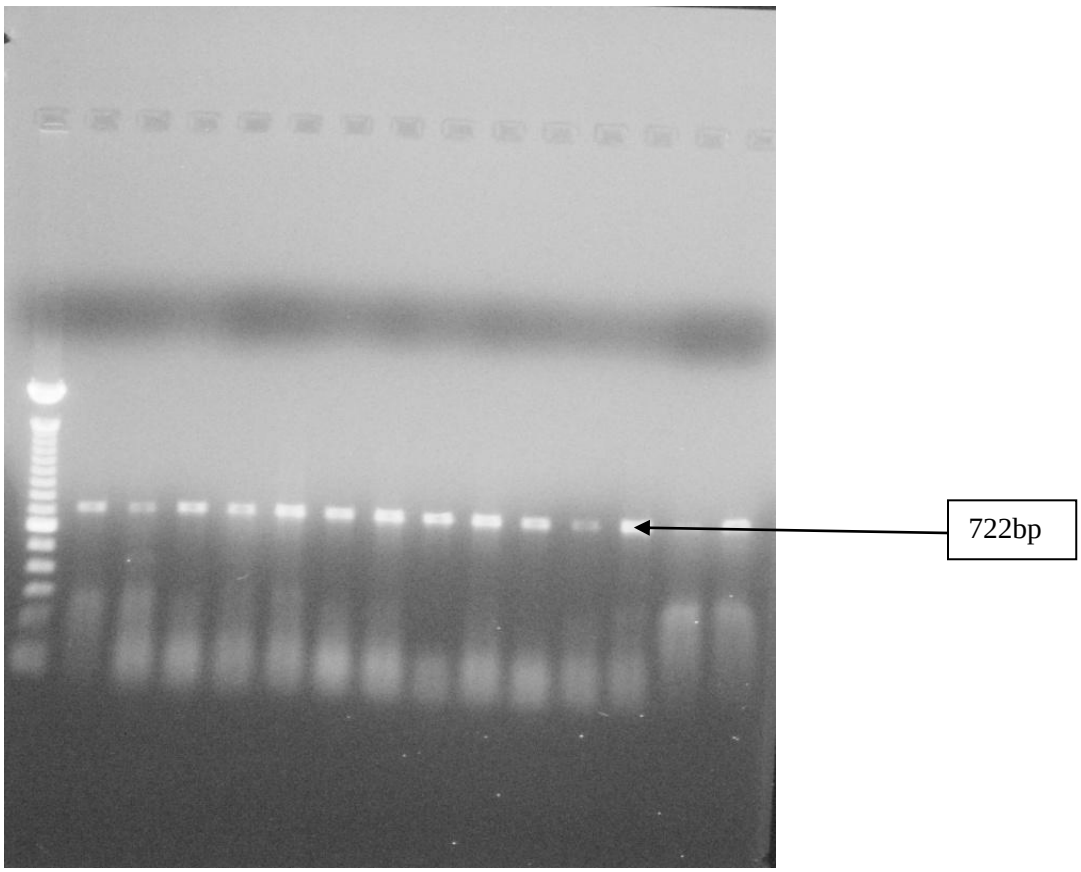

Plate 5a: Positive RT-PCR of the IBDV VP1 gene of bursa homogenate samples indicated by a 722bp band (arrowed). Lane 1 – Molecular marker (100bp), lane 2 – known positive IBDV sample, lane 3-13 – bursa samples.

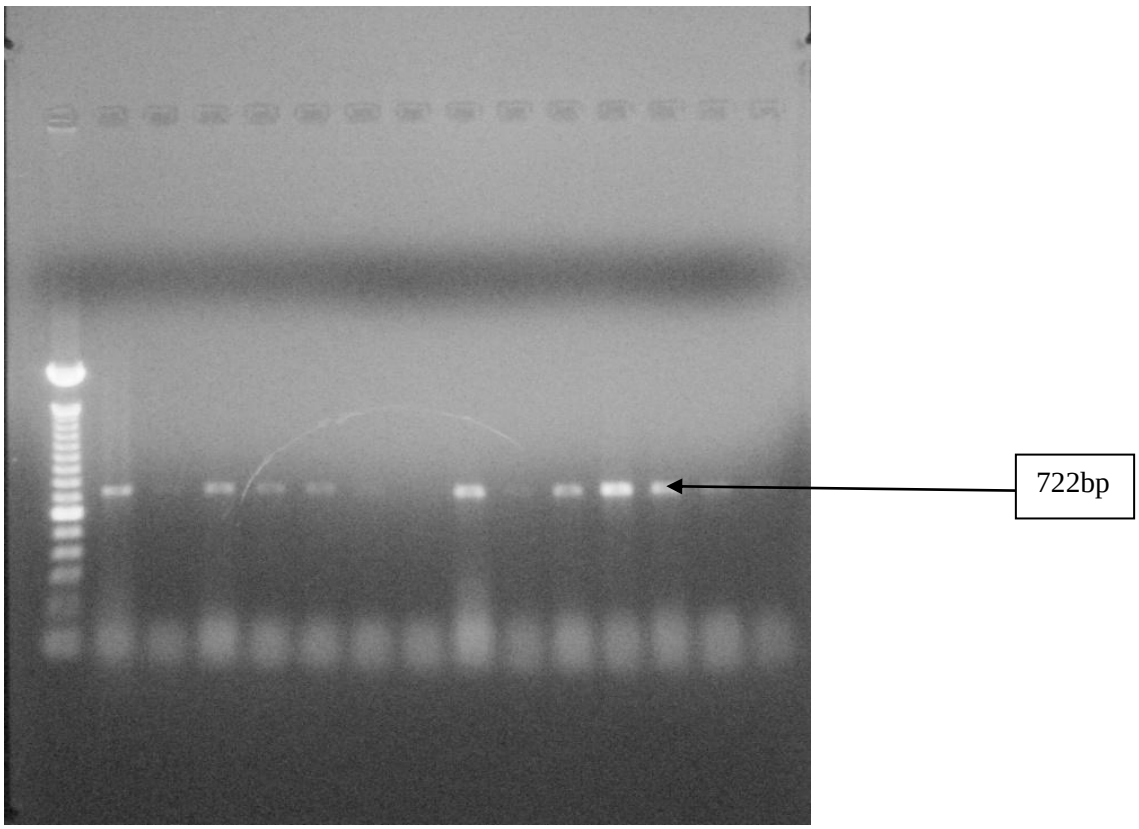

Plate 5b: Positive and negative RT-PCR of the IBDV VP1 gene of bursa homogenate samples indicated by a 722bp band (arrowed). Lane 1 – Molecular marker (100bp), lane 2 – known positive IBDV sample, lane 3-13 – bursa samples.

Table 17a:Comparison of amino acid substitutions at different positions between viruses from Plateau state, very virulent (vv) HK46 and variant Del E strains of IBDV for segment B

STRAIN/ISOLATE	AMINO ACID SUBSTITUTION AT POSITIONS													
	96	**119**	**123**	**141**	**145**	**146**	**147**	**148**	**150**	**154**	**161**	**163**	**219**	**242**
AJ878668_HK46	T	E	A	V	T	D	N	L	D	L	D	A	D	E
PLATEAU161/NG/2014	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
PLATEAU163/NG/2014	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
PLATEAU164/NG/2014	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
PLATEAU154/NG/2014	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
PLATEAU157/NG/2014	.	D	.	I	Q	D	G	.	E	I	.	V	E	D
PLATEAU162/NG/2014	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
PLATEAU158/NG/2014	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
PLATEAU90/NG/2013	.	D	.	I	Q	E	G	.	E	I	.	V	E	D
PLATEAU92/NG/2013	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
PLATEAU104/NG/2010	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
PLATEAU110/NG/2010	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
PLATEAU131/NG/2014	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
PLATEAU132/NG/2014	.	D	.	I	Q	E	G	.	E	I	.	V	E	D
PLATEAU134/NG/2014	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
PLATEAU135/NG/2014	.	D	.	I	Q	E	G	.	E	I	.	V	E	D
PLATEAU53/NG/2010	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
PLATEAU7/NG/2009	.	D	.	I	H	D	G	.	E	.	.	V	E	D
PLATEAU19/NG/2009	.	D	.	I	H	E	G	.	E	.	A	V	E	D
PLATEAU40/NG/2012	N	.	.	.	.	.	.	.	.	.	A	.	.	.
PLATEAU156/NG/2014	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
PLATEAU39/NG/2012	.	.	.	I	Q	E	G	.	E	.	.	V	E	D
PLATEAU43/NG/2012	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
PLATEAU160/NG/2014	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
PLATAEU96/NG/2013	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
PLATEAU82/NG/2013	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
PLATEAU83/NG/2013	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
PLATEAU95/NG/2013	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
PLATEAU119/NG/2013	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
PLATEAU120/NG/2013	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
PLATEAU133/NG/2014	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
AJ878676_DEL_E	.	.	.	.	N	E	D	.	.	.	.	.	.	D

- **NOTE**: A- Alanine, Q- Glutamine, G- Glycine, I- Isoleucine, T- Threonine, D- Aspartic acid, E- Glutamic acid, V- Valine, N- Asparagine

Table 17b:Comparison of amino acid substitutions at different positions between viruses from Kwara state, very virulent (vv) HK46 and variant Del E strains of IBDV for segment B

STRAIN/ISOLATE	AMINO ACID SUBSTITUTION AT POSITIONS													
	96	**119**	**123**	**141**	**145**	**146**	**147**	**148**	**150**	**154**	**161**	**163**	**219**	**242**
AJ878668_HK46	T	E	A	V	T	D	N	L	D	L	D	A	D	E
KWARA175/NG/2014	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
KWARA177/NG/2014	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
KWARA178/NG/2014	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
KWARA180/NG/2014	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
KWARA181/NG/2014	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
KWARA171/NG/2014	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
KWARA184/NG/2014	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
KWARA148/NG/2014	N	.	.	.	.	.	.	.	.	.	.	.	.	.
KWARA141/NG/2014	N	.	.	.	.	.	.	.	.	.	A	.	.	.
AJ878676_DEL_E	.	.	.	.	N	E	D	.	.	.	.	.	.	D

- **NOTE**: A- Alanine, Q- Glutamine, G- Glycine, I- Isoleucine, T- Threonine, D- Aspartic acid, E- Glutamic acid, V- Valine, N- Asparagine

Table 17c:Comparison of amino acid substitutions at different positions between viruses from Akwa Ibom (Uyo) state, very virulent (vv) HK46 and variant Del E strains of IBDV for segment B

STRAIN/ISOLATE	AMINO ACID SUBSTITUTION AT POSITIONS													
	96	**119**	**123**	**141**	**145**	**146**	**147**	**148**	**150**	**154**	**161**	**163**	**219**	**242**
AJ878668_HK46	T	E	A	V	T	D	N	L	D	L	D	A	D	E
UYO167/NG/2014	.	D	.	I	H	E	G	.	E	.	.	V	E	D
UYO187/NG/2014	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
UYO189/NG/2014	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
UYO192/NG/2014	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
UYO193/NG/2014	.	D	.	I	H	E	G	.	E	.	.	V	E	D
UYO194/NG/2014	.	D	.	I	H	E	G	.	E	.	.	V	E	D
UYO195/NG/2014	.	D	.	I	H	E	G	.	E	.	.	V	E	D
UYO196/NG/2014	.	D	.	I	H	E	G	.	E	.	.	V	E	D
UYO197/NG/2014	.	D	.	I	H	E	G	.	E	.	.	V	E	D
UYO198/NG/2014	.	D	.	I	H	E	G	.	E	.	.	V	E	D
UYO200/NG/2014	.	D	.	I	H	E	G	.	E	.	.	V	E	D
UYO202/NG/2014	.	D	.	I	H	E	G	.	E	.	.	V	E	D
UYO203/NG/2014	.	D	.	I	H	E	G	.	E	.	.	V	E	D
UYO207/NG/2014	.	D	.	I	H	E	G	.	E	.	.	V	E	D
UYO190/NG/2014	.	.	T	T	Q	E	G	P	E	.	.	V	E	D
UYO191/NG/2014	.	.	T	T	Q	E	G	P	E	.	.	V	E	D
UYO188/NG/2014	.	.	T	T	Q	E	G	P	E	.	.	V	E	D
AJ878676_DEL_E	.	.	.	.	N	E	D	.	.	.	.	.	.	D

- **NOTE**: A- Alanine, Q- Glutamine, G- Glycine, I- Isoleucine, T- Threonine, D- Aspartic acid, E- Glutamic acid, V- Valine, N- Asparagine, P- Proline, H- Histidine, L- Leucine.

Table 17d:Comparison of amino acid substitutions at different positions between viruses from Bauchi state, very virulent (vv) HK46 and variant Del E strains of IBDV for segment B

STRAIN/ISOLATE	AMINO ACID SUBSTITUTION AT POSITIONS													
	96	**119**	**123**	**141**	**145**	**146**	**147**	**148**	**150**	**154**	**161**	**163**	**219**	**242**
AJ878668_HK46	T	E	A	V	T	D	N	L	D	L	D	A	D	E
BAUCHI121/NG/2013	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
BAUCHI124/NG/2014	.	D	.	I	Q	E	G	.	.	.	.	V	E	D
BAUCHI128/NG/2014	.	D	.	I	Q	E	G	.	.	.	.	V	E	D
BAUCHI37/NG/2012	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
BAUCHI126/NG/2014	.	.	.	I	Q	E	G	.	E	.	.	V	E	D
BAUCHI58/NG/2012	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
AJ878676_DEL_E	.	.	.	.	N	E	D	.	.	.	.	.	.	D

- **NOTE**: A- Alanine, Q- Glutamine, G- Glycine, I- Isoleucine, T- Threonine, D- Aspartic acid, E- Glutamic acid, V- Valine, N- Asparagine, P- Proline, L-Leucine.

Table 17e:Comparison of amino acid substitutions at different positions between viruses from Anambra state, very virulent (vv) HK46 and variant Del E strains of IBDV for segment B

STRAIN/ISOLATE	AMINO ACID SUBSTITUTION AT POSITIONS													
	96	**119**	**123**	**141**	**145**	**146**	**147**	**148**	**150**	**154**	**161**	**163**	**219**	**242**
AJ878668_HK46	T	E	A	V	T	D	N	L	D	L	D	A	D	E
ANAMBRA50/NG/2012	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
AJ878676_DEL_E	.	.	.	.	N	E	D	.	.	.	.	.	.	D

- **NOTE**: A- Alanine, Q- Glutamine, G- Glycine, I- Isoleucine, T- Threonine, D- Aspartic acid, E- Glutamic acid, V- Valine, N- Asparagine, P- Proline, L-Leucine.

Table 17f:Comparison of amino acid substitutions at different positions between viruses from Abuja (FCT), very virulent (vv) HK46 and variant Del E strains of IBDV for segment B

STRAIN/ISOLATE	AMINO ACID SUBSTITUTION AT POSITIONS													
	96	**119**	**123**	**141**	**145**	**146**	**147**	**148**	**150**	**154**	**161**	**163**	**219**	**242**
AJ878668_HK46	T	E	A	V	T	D	N	L	D	L	D	A	D	E
ABUJA93/NG/2013	.	D	.	I	Q	E	G	.	.	.	.	V	E	D
AJ878676_DEL_E	.	.	.	.	N	E	D	.	.	.	.	.	.	D

- **NOTE**: A- Alanine, Q- Glutamine, G- Glycine, I- Isoleucine, T- Threonine, D- Aspartic acid, E- Glutamic acid, V- Valine, N- Asparagine.

Table 17g:Comparison of amino acid substitutions at different positions between viruses from Edo (Benin) state, very virulent (vv) HK46 and variant Del E strains of IBDV for segment B

STRAIN/ISOLATE	AMINO ACID SUBSTITUTION AT POSITIONS													
	96	**119**	**123**	**141**	**145**	**146**	**147**	**148**	**150**	**154**	**161**	**163**	**219**	**242**
AJ878668_HK46	T	E	A	V	T	D	N	L	D	L	D	A	D	E
BENIN87/NG/2013	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
AJ878676_DEL_E	.	.	.	.	N	E	D	.	.	.	.	.	.	D

- **NOTE**: A- Alanine, Q- Glutamine, G- Glycine, I- Isoleucine, T- Threonine, D- Aspartic acid, E- Glutamic acid, V- Valine, N- Asparagine.

Table 17h: Comparison of amino acid substitutions at different positions between viruses from Kaduna state, very virulent (vv) HK46 and variant Del E strains of IBDV for segment B

STRAIN/ISOLATE	AMINO ACID SUBSTITUTION AT POSITIONS													
	96	**119**	**123**	**141**	**145**	**146**	**147**	**148**	**150**	**154**	**161**	**163**	**219**	**242**
AJ878668_HK46	T	E	A	V	T	D	N	L	D	L	D	A	D	E
KADUNA136/NG/2014	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
KADUNA61/NG/2012	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
AJ878676_DEL_E	.	.	.	.	N	E	D	.	.	.	.	.	.	D

- **NOTE**: A- Alanine, Q- Glutamine, G- Glycine, I- Isoleucine, T- Threonine, D- Aspartic acid, E- Glutamic acid, V- Valine, N- Asparagine, P- Proline.

Table 18: Comparative spatial distribution of IBDV segment B genome amino acid substitutions and their positions between the Nigerian viruses and reference very virulent (vv) HK46 IBDV strain

S/no	States vs Hk46	No of Isolates	No of Amino Acid Substitutions	Amino Acid Positions
1	Plateau	30	13	96, 119, 141, 145, 146, 147, 148, 150, 154,161, 163, 219, 242
2	Akwa Ibom (Uyo)	17	11	119, 123, 141, 145, 146, 147, 148, 150, 161, 163, 219, 242
3	Kwara	9	11	96, 119, 141, 145, 146, 147, 150, 161, 163, 219, 242
4	Bauchi	6	10	119, 141, 145, 146, 147, 148, 150, 163, 219, 242
5	Anambra	1	8	141, 145, 146, 147, 148, 150, 219, 242
6	Abuja	1	8	119, 141, 145, 146, 147, 163, 219, 242
7	Edo (Benin)	1	9	119, 141, 145, 146, 147, 150, 163, 219, 242
8	Kaduna	1	10	119, 141, 145, 146, 147, 148, 150, 163, 219, 242

Table 19: Comparative spatial distribution of IBDV segment B genome amino acid substitutions and their positions between the Nigerian viruses and reference variant Del E IBDV strain

S/no	States vs Del E	No of Isolates	No of Amino Acid Substitutions	Amino Acid Positions
1	Plateau	30	15	96, 98, 119, 141, 145, 146, 147, 148, 150, 154, 161, 163, 219, 240, 242
2	Akwa Ibom (Uyo)	17	9	119, 123, 141, 145, 147, 148, 150, 163, 219
3	Kwara	9	9	96, 119, 141, 145, 146, 147, 150, 161, 163, 219, 234, 242
4	Bauchi	6	9	119, 141, 145, 147, 148, 150, 153, 163, 219
5	Anambra	1	7	141, 145, 147, 148, 150, 209, 219
6	Abuja	1	7	119, 141, 145, 147, 163, 170, 219
7	Edo (Benin)	1	7	119, 141, 145, 147, 150, 163, 219
8	Kaduna	1	9	119, 141, 145, 147, 148, 150, 163, 174, 219

Table 20: Comparative temporal distribution of amino acid substitutions and their positions between the Nigerian viruses and reference very virulent (vv) HK46 IBDV strain

S/no	Year	No of Isolates	No of Amino Acid Substitutions	Amino Acid Positions
1	2009	2	9	119, 141, 145, 146, 147, 150, 163, 219, 242
2	2010	3	9	119, 141, 145, 146, 147, 150, 163, 219, 242
3	2011	-	-	
4	2012	7	14	96, 119, 141, 145, 146, 147, 148, 150, 161, 163, 174, 209, 219, 242
5	2013	11	11	119, 141, 145, 146, 147, 148, 150, 154, 163, 170, 174
6	2014	44	18	96, 98, 119, 123, 141, 145, 146, 147, 148, 150, 153, 154, 161, 163, 219, 234, 240, 242

Table 21: Comparative temporal distribution of amino acid substitutions and their positions between the Nigerian viruses and reference variant Del E IBDV strain

S/no	Year	No of Isolates	No of Amino Acid Substitutions	Amino Acid Positions
1	2009	2	8	119, 141, 145, 146, 147, 150, 163, 219
2	2010	3	6	119, 141, 145, 147, 150, 219
3	2011	-	-	-
4	2012	7	13	96, 141, 145, 146, 147, 148, 161, 163, 174, 209, 219, 242
5	2013	11	10	119, 141, 145, 147, 148, 150, 154, 163, 170, 174, 219
6	2014	44	17	96, 98, 119, 123, 141,145, 146, 147, 148, 150, 154,, 161, 163, 219, 234, 240, 242

4.9.2 Sequence analysis of segment B nucleotide and amino acid sequences of the 6 Nigerian IBD viruses and others from GenBank

The nucleotide sequences of the 684bp of six (6) Nigerian viruses; Plateau19/NG/2009, Kaduna61/NG/2012, Bauchi58/NG/2012, Plateau53/NG/2010, Plateau7/NG/2009 and Bauchi37/NG/2012 starting from nucleotide position 186 – 869 were aligned and compared with each other, their percentage identities ranged from 96.1 – 99.9. Amino acid substitution in the six (6) Nigerian viruses and their positions compared with some reference IBDV strains from GenBank are shown in appendix 7. Likewise, when they were aligned and compared with some nucleotide sequences of reference IBDV VP1 obtained from GenBank comprising of four vvIBDV (HK46, UK661, OKYM, T09) and two non-vvIBDV (Del E and 23-82), the percentage identities had the following ranges; with HK46= 84 – 86.6, with UK661= 84.2 – 84.7, with OKYM= 84.3 – 84.9, with T09= 84.5 – 85.4, with Del E= 84.9 – 85.3 and with 23-82= 87.5 – 88 (Appendix 16). When the amino acid sequences obtained from the 684bp of segment B of six (6) Nigerian viruses that translated into 228 amino acids were aligned and compared with each other, the percentage identities obtained amongst them ranged from 97.8 – 99.6. The amino acid sequences of the six Nigerian viruses were aligned with some reference IBDV VP1 from the GenBank comprising of four vvIBDV (HK46, UK661, OKYM and T09) and two non-vvIBDV (Del E and 23-82) and the percentage identities between the Nigerian viruses and the reference IBDVs when calculated gave the following percentage ranges; with HK46= 95.6 – 96.5, with UK661= 95.2 – 96.1, with OKYM= 94.7 – 95.6, with T09= 95.2 – 96.1, with Del E= 95.2 – 96.1 and with 23-82= 96.1 – 96.9.

When the deduced amino acid sequences of the Nigerian viruses (aa 26 – 253) were compared with other IBDV VP1 from GenBank comprising of IBDV strains from Europe, Asia, Africa, North and South America, (Appendix 17) positions 26 – 60 were conserved in all except at position 29 where one Nigerian virus had a V instead of E. I61 was conserved in the six Nigerian viruses and in some of the others included for comparison while the rest had a V. E119 was conserved in all the IBDV strains included for comparison and one Nigerian virus but the remaining five Nigerian viruses had a D at this position which was due to a substitution mutation that occurred at the 3rd codon in the nucleotide sequence of the five Nigerian viruses where they had a GAC while the others had a GAA. V141 was conserved except in the six Nigerian viruses

where a substitution mutation occurred and in two Ethiopian viruses used for comparison, the Nigerian viruses had an I at this position while the Ethiopia viruses had an A. At positions 145, 146 and 147 different amino acid motifs were seen, while the vvIBDVs had a TDN at these three positions, the Nigerian viruses displayed three different motifs; QED, HEG and HDG. The non-vvIBDVs had SEG, NEG, TDS, NED and TEG at these positions. L148 was conserved except in one Nigerian virus that had a P at this position. Likewise D150 was conserved except in the six Nigerian and Gx from China that had an E. A163 was also conserved except in 5 out of 6 of the Nigerian viruses and Gx that have a V. D219 was conserved in all the IBDV strains used for comparism but the Nigerian viruses had a E at this position. E242 was conserved in all the vvIBDV included for comparism while the Nigerian viruses and other non-vvIBDVs had a D at this position.

4.10 Phylogenetic analysis

4.10.1 Phylogenetic analysis of segment B of the Nigerian viruses and reference segment B IBDV strains from GenBank

The phylogenetic trees constructed using the neighbor joining and UPGMA methods with 1000 bootstrap with the nucleotide sequences of the segment B of the 67 Nigerian IBDV strains used in this study and nucleotide sequences of some reference vvIBDV (OKYM, HK46 and UK661), variant (Del E), vaccine (Burs 706), serotype 2 (TY89, 23-82 and U20950-OH) and a West African turkey IBDV isolate (T09, Nigeria), produced two major branches. On the NJ tree (Figure 10), the first and largest branch with bootstrap value 99% consisted exclusively of a cluster of 64 out of the 67 Nigerian IBDV strains used in this study. The second branch showed two significant clusters, the first with 74% bootstrap value consists of the variant, vaccine, serotype 2 and Chinese IBDV strains while the second cluster on a branch with 99% bootstrap value consists of the West African turkey isolate, vvIBDV and three of the Nigerian IBDV strains used in this study. On the UPGMA tree (Figure 11), two branches were produced with the Nigerian, variant, vaccine, serotype 2 and Chinese IBDV strains seen on the first major branch but forming distinct clusters from each other with bootstrap values of 99% and 96% respectively. The second major branch consists of the West African turkey isolate, vvIBDV and three of the Nigerian IBDV strains used in this study. Interestingly, the Nigerian IBDV strains on the two

trees (NJ and UPGMA) formed their own distinct clusters. On the branch containing the vvIBDV where three Nigerian IBDV strains were found, they formed a cluster of their own within the vvIBDVs (bootstrap value 96 and 98 respectively). On the NJ and UPGMA trees, five significant genome segment B lineages were observed. Segment B sequences from IBD viruses in Nigeria were found in two of these five lineages. The first lineage with a bootstrap value of 99% consisted of 64 out of the 67 Nigerian IBDV strains exclusively but found on the same branch with variant, vaccine and serotypes 2 IBDV strains. The second lineage with a bootstrap value of 98% and 96% respectively consists of the variant and vaccine IBDV (Del E and bursa 706). The third lineage consists of the serotype 2 IBD viruses, with the fourth lineage containing IBDV from China (Henan) and none of the Nigerian IBDVs were seen on the second, third and fourth lineage. The fifth lineage contains vvIBDV with the turkey isolate from Nigeria and within this lineage, Nigerian IBDV strains used in this study were also found here. Within the vvIBDV, two significant lineages (bootstrap value of 97%) were observed. IBDV strains from Nigeria used in this study were found exclusively clustered in one of those lineages while the vvIBDV from China, Japan and UK were in the other. All 64 Nigerian viruses that were seen here on the same branch with the variant, vaccine, serotype 2 and Chinese IBDV strains though forming a unique cluster of their own and distant from them, had a genome segment A that indicated that they could be vvIBDV. Their exclusive clustering may not be unconnected with the observations made on the triplet amino acids at positions 145-147 coupled with other substitution mutations seen on their genome segment B amino acid sequences where four different motifs of amino acids QEG, HEG, HDG and QDG was observed. The non-vvIBDVs have the motif NEG at the triplet position (145-147). All vvIBDV have TDN at amino acid 145, 146 and 147 respectively and the three Nigerian IBDV strains that clustered with the vvIBDV had these amino acids at these positions. When phylogenetic trees constructed using the neighbor joining and UPGMA methods with 1000 bootstrap replicates with the nucleotide sequences of segment B of the 67 Nigerian IBDV strains used in this study and 50 IBDV segment B sequences consisting of thirty-eight wild-type strains representing scIBDV, cvIBDV and vvIBDV, seven vaccine strains, three naturally reassorted IBDV strains from Venezuela and two serotype 2 strains from Europe submitted by Le Nouën *et al*, 2006 with two others; turkey IBDV isolate from Nigeria and OH from US obtained from GenBank were analyzed, two major branches were also produced. On

both trees, the first branch showed two sub branches with the Nigerian IBDV strains forming a cluster containing exclusively again of 64 out of the 67 of the IBDV strains used in this study (bootstrap value 100% for NJ and UPGMA trees respectively). The second sub branch with bootstrap values of 74% and 69% (for NJ and UPGMA trees respectively), a large cluster consisting of IBDV strains from Australia, Venezuela, China, serotype 2, variant, classic and vaccine was seen. The second major branch (bootstrap value 100% for NJ and UPGMA trees respectively) consists of vvIBDV from various countries of the world with three of the Nigerian IBDV strain used in this study. On both trees, seven IBDV genome segment B lineages were observed. On the both trees, the first lineage consists of the Nigerian IBDV strains alone with none of the other IBDV strains from other countries seen here (bootstrap value 100%). The second lineage consists of the Australian and Venezuela IBDV strains on the NJ tree but on the UPGMA tree it was Australia and China. The third lineage on the NJ tree is IBDV from China while on the UPGMA it is IBDV from Venezuela. The fourth lineage on both trees were the serotype 2 IBDVs, fifth lineage consists of the variant, classic and vaccine IBDV strains, sixth lineage consists of IBDV strains from Ivory Coast and the seventh lineage consists of all the vvIBDV from various countries including three Nigerian IBDV strains.

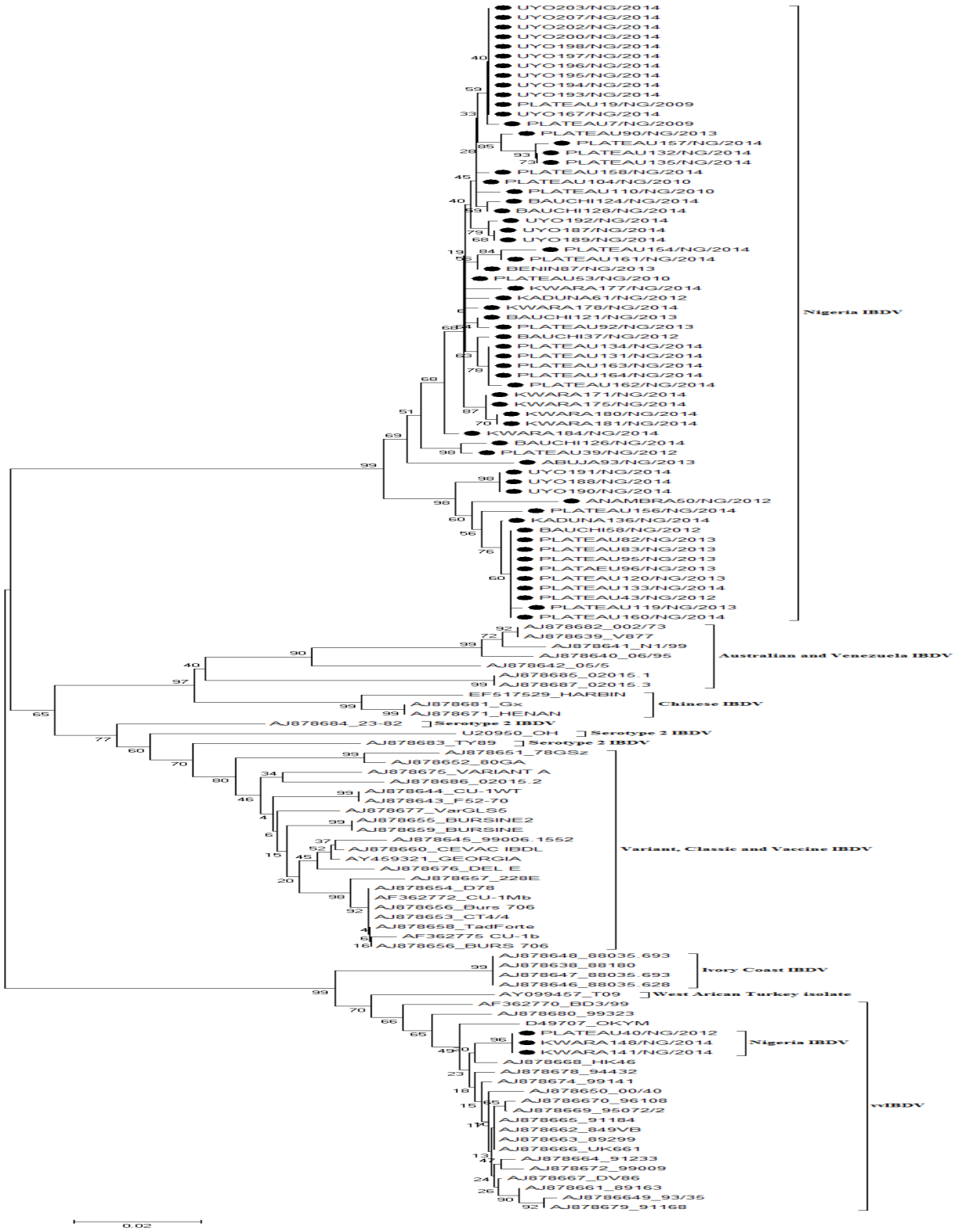

Fig 10: NJ of Nigerian viruses with IBDV segment B from other countries, variant, classic, vaccine and serotype 2.

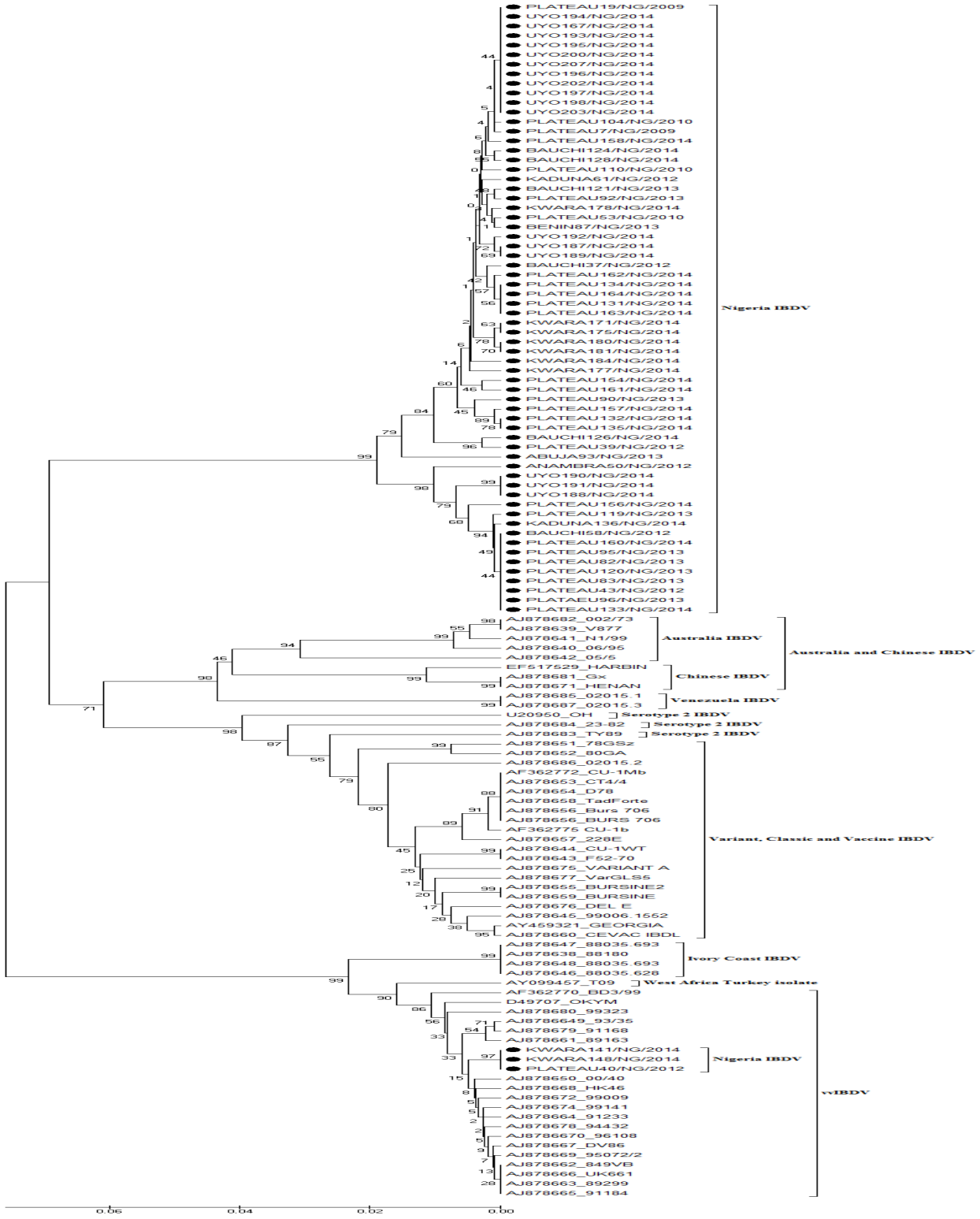

Fig 11: UPGMAof Nigerian viruses with IBDV segment B from other countries, variant, classic, vaccine and serotype 2.

4.10.2 Phylogenetic analysis of segment B of six Nigerian viruses and some segment B IBDV strains from other countries in GenBank

Phylogenetic trees constructed with the segment B nucleotide sequences (nt312 – 854) of the six Nigerian viruses and nucleotide sequence of some IBDV VP1 obtained from the GenBank consisting of IBDV strains from North and South America, Europe, Asia and Africa using the neighbor joining and UPGMA methods with 1000 bootstrap replicates produced two major branches on both the NJ and UPGMA trees with sub branches. On the NJ tree (Figure 12), the first major branch with sub branch (82% bootstrap value) showed the vvIBDV forming a cluster with 100% bootstrap value and HN04 from China on the same but separate branch. On the branch with bootstrap value of 84%, IBDVs from China, Brazil, Taiwan, Zambia, classic, variant and serotype 2 clustered on this branch. The first major branch on the UPGMA tree (Figure 13) consists of sub branches with the IBDVs from USA, China, Taiwan, Brazil, Zambia, classic, variant and serotype 2 forming a cluster (80% bootstrap value), the next sub branch consists of IBDV from Tanzania (100% bootstrap value). IBDV from China and USA formed the next cluster (98% bootstrap value) followed by IBDV from China and vvIBDV with bootstrap value of 48% although the IBDV strain from China formed its own cluster. The second major branch on the NJ tree consists of IBDV from Tanzania (100% bootstrap value), IBDV from China and USA (99% bootstrap value) and the six IBDV from Nigeria formed a separate cluster (100% bootstrap value). Also the major second branch on the UPGMA tree with 100% bootstrap value consisted exclusively of the six IBDV from Nigeria, none of the other IBDV included in this study was found on this branch.

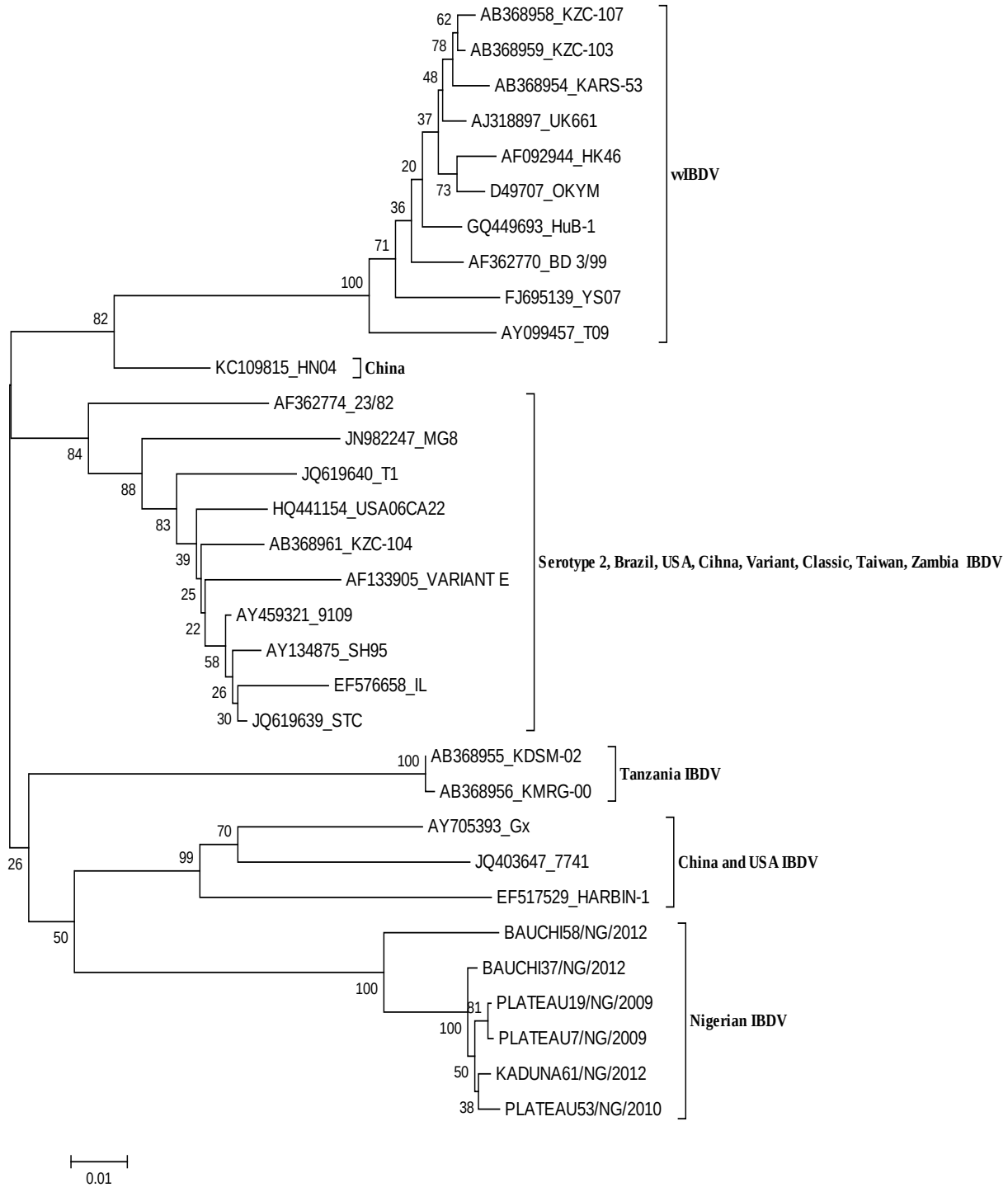

Fig 12: NJ of the segment B nucleotide sequences of six Nigerian viruses with IBDV from some countries.

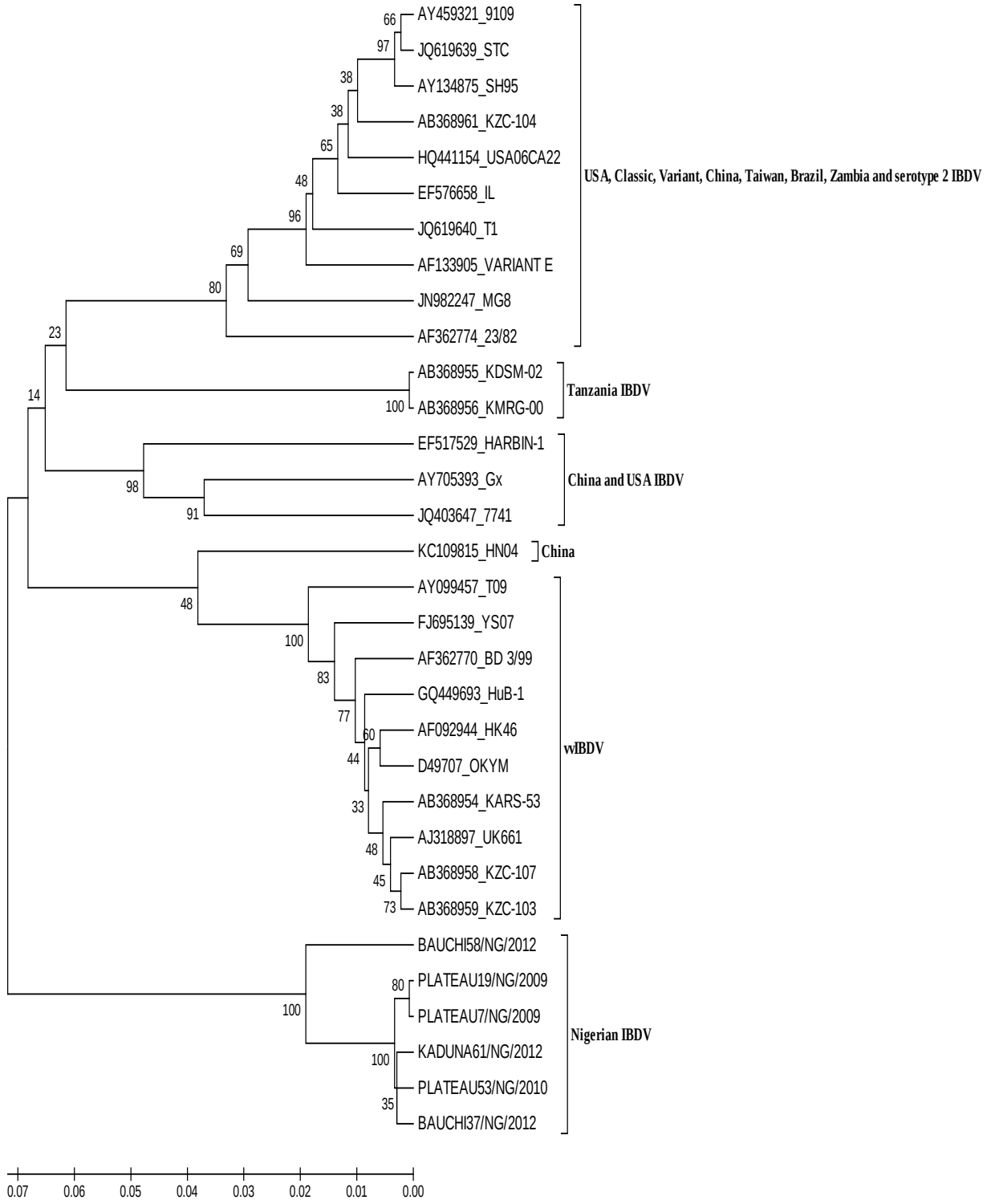

Fig 13: UPGMA of the segment B nucleotide sequences of six Nigerian viruses with IBDV from some countries

CHAPTER 5

DISCUSSION

Data collected from the field (appendix 1) and the results of the agar gel immunodiffusion (AGID) tests and reverse transcriptase-polymerase chain reaction (RT-PCR) showed that outbreaks of IBDV occurred mostly in birds between the age ranges of 3 – 19 weeks. The documented age for the infection of IBDV is 3 – 6 weeks when the bursa of Fabricius is well developed, mature and differentiated producing B-lymphocytes in adequate number thereby creating an enabling environment for IBD virus to replicate in and cause disease (Lukert and Saif, 2004; Khan *et al.*, 2007; Anru *et al.*, 2011). In birds aged 1 – 2 weeks, IBDV does not cause any clinical disease but renders the birds susceptible due to immunosuppression to other infectious agents at a later age (Mahgoub, 2012). IBDV antigen detected in older birds in this study is in agreement with the report of Shekaro and Josiah (2015) that detected IBDV in 15 weeks old pullets. Outbreaks of IBDV in vaccinated birds aged 14 – 20 weeks have also been reported in Nigeria (Okoye and Uzoukwu, 1981; Owoade and Durojaiye, 1995; Igbokwe *et al.*, 1996; Musa *et al.*, 2010). This shows an increase in the incidence of IBDV in older birds which can negatively affect the poultry industry in Nigeria. The high occurrence of IBDV outbreak in Plateau state as compared with other states sampled may probably be due to the willingness of the veterinary clinics where the samples were obtained in reporting outbreaks and subsequent collection and submission of bursae for analysis. From the data collected, all the sampled species populations were affected. Okoye and Okpe (1989) reported that IBDV was not detected in experimentally infected guinea fowl while Nwagu *et al*. (1997) reported the detection of low prevalence rate of IBDV in guinea fowl. The pullets and broilers were the most affected species followed by cockerels. These first two chicken types are known for their huge turnover and income they generate for poultry farmers hence their large population and prominence in poultry farming and the chance that during an outbreak of IBDV they are likely to be most affected. The breed of chickens most affected by IBDV apart from the unspecified group was the Isa brown breed. These breed of chickens are very popular with poultry farmers in Nigeria due to their ability to adapt to many condition, high demand of their eggs and their high performance (95%) (Poultry manual, 2016). Outbreaks of IBDV was significantly higher in the year 2014 compared

to the other years sampled. The reason for this increase is not known. IBDV outbreaks were observed more during the month of May followed by October, June and July from the data. This is in agreement with Mbuko *et al*. (2010), who reported a high outbreak of IBDV in the month of May. The seasonal distribution of IBDV indicated high occurrence of IBDV during the rainy season (May – November) with least occurrence during the dry harmattan season period (December – January). These observations were also in agreement with Mbuko *et al*. (2010) and Anosa and Eze (2010). The higher percentage use of foreign IBD vaccine by farmers in the field may probably be due to the inability of the nation owned veterinary vaccine production unit in the National Veterinary Research Institute situated in Vom, Plateau state to meet up with the high demands for IBD vaccines. Likewise, from the data, IBDV occurred in all the types of husbandry system used by the farmers irrespective of whether it was backyard, medium scale or large scale intensive system. The attempt to isolate field IBDV in embryonated chicken eggs and chicken embryo fibroblast (CEF) cell cultures proved abortive although only two blind passages were attempted using both host system. Interestingly, at the first blind passage in embryonated eggs, lesions typical of IBDV which includes swollen livers that were gray or greenish in color (OIE, 2008) were observed in some of the infected embryos while the livers of the embryos used as controls were normal. The effect of the virus on the sizes of the embryos in terms of dwarfing was unable to be ascertained since some of the control embryos were of the same size as the infected. The infected CEF did not show any cytopathogenic effect (CPE) and were the same as the controls. The results obtained in this study were partially in agreement to that of some workers that stated that field IBDV is difficult to isolate but can only be achieved after several passages which will subsequently lead to attenuation of the virus (McFerran *et al*, 1980;Eterradossi and Saif, 2008). Since only two passages were attempted in this study we cannot say if further passages would have yielded some virus. Interestingly, a particular field sample at second passage in embryonated egg yielded IBDV that was detected via RT-PCR contrary to assumptions that it will take several passages to isolate field IBDV in eggs. Ahmad *et al*. (2005) were able to detect IBDV at 1-3rd passage in embryonated eggs using the chorioallantoic fluid route but at a low titer with subsequent passages yielding higher titers. More interesting was the fact that when IBDV from the original bursa sample and its subsequent embryo isolate were sequenced, their sequences were characterized by mutations especially on

the embryo isolate and they clustered differently on the phylogenetic tree. The observation made here reemphasizes the need/fact that isolation of field IBDV are best done using specific pathogen free (SPF) embryonated eggs, chickens or chicken cell line originating from SPF flock to minimize mutations (Khan *et al.*, 2007). The embryonated eggs and CEF used were from poultry flocks that are suspected to have been vaccinated against IBDV and failure to isolate IBDV from the other bursa samples may be due to the presence of maternally derived antibodies in the embryonated eggs used in this study (Begum *et al.*, 2004), but the ability to isolate one after second passage needs to be investigated further.

In this study, agar gel immunodiffusion (AGID) was used for the initial screening (antigen detection) of 139 out of a total 216 IBDV samples obtained before subjecting the same number of samples to RT-PCR. Although AGID test has been reported to be less sensitive, not quantitative and does not detect serotypic differences and measures only group-specific soluble antigen (Lukert and Saif, 2004), it was observed in this study that AGID detected 93 out of the initial 139 (66.9%) screened for IBDV antigens compared to the 79 out of 139 (56.8%) detected by RT-PCR in the same number of samples, while AGID did not detect IBDV antigen in 46 out of 139 (33.1%) compared to 61 out of 139 (43.8%) by RT-PCR. Although, it was observed that some samples that were positive for AGID (19 samples) were negative by RT-PCR, while 5 samples that were negative for IBDV antigen by AGID were positive by RT-PCR. From the results, RT-PCR proved to be the test of choice in terms of sensitivity. Other workers have reported the usefulness of RT-PCR in the detection of IBDV (Parthiban *et al*, 2001; Elankumaran *et al*, 2002; Tahiri *et al*, 2013)

Jackwood and Sommer (1999) developed a method that differentiates IBDV vaccine strains into six different molecular groups by a RT-PCR-RFLP assay using specific primers that amplify a 743-bp region of the VP2 gene. RFLP has been used to divide IBDV strains into molecular groups that are either related or dissimilar antigenically. The molecular groups of IBDV are grouped thus; groups 1 and 2 contain variant viruses, 3 and 4 contain classical viruses, group 5 contains Lukert/Edgar strain classic viruses and 6 contains classical and very virulent viruses (Jackwood and Sommer, 1999). Analysis of IBDV strains outside the US revealed that molecular group six also contained the vvIBDV strains (Jackwood and Sommer, 1999) and the

characterization of more than 200 field IBDV isolates from 22 different countries added more molecular groups (Jackwood and Sommer, 1999.; Jackwood *et al.*, 2001.; Meir *et al.*, 2001) and so analysis of field IBDV strains by RFLP showed significant genetic diversity (Sapats and Ignjatovic, 2002). Several researchers have used this method (Jackwood and Sommer, 1997; Sapats and Ignjatovic, 2002; Ozbey *et al.*, 2003; Abdel-Alim *et al.*, 2003; Sareyyüpoğlu and Akan, 2006; Luka *et al.*, 2014). Study of the VP2 gene by different workers and the choice to amplify and compare different regions of the VP2 have further been compounded with the use of different restriction enzymes by these workers (Spats and Ignjatovic, 2002).Variant viruses have been described as those that can replicate in the presence of immunity to classic viruses and cause lesions in the bursa of Fabricius (Lukert and Saif, 1997). RFLP patterns have been used to promptly identify IBDV strains with the sequence potential to be variant viruses (Jackwood *et al.*, 1997). Jackwood *et al*, (2001) reported that *Bst*N1 enzyme can detect amino acid changes on the VP2 gene which can result in viruses with similar amino acids especially at position 222 having different *Bst*N1 restriction fragment length polymorphism profile. A *Bst*NI RFLP pattern with a 424-bp shows the amino acid at position 222 is not prolinebecause proline at this position is a distinctive feature of classic IBDV strains in North America and useful for the development of variant viruses. Researchers have used this RFLP pattern to detect non-classic IBDV strains in North America from field viruses. Since in the rest of the world, vvIBDV strains have the 424-bp RFLP pattern, additional screening steps were needed to distinguish potentially variant viruses from vvIBDV strains and this gave rise to the use of the restriction enzyme *Ssp*I and although not without its own fault, majority of the time vvIBDV strains are *Ssp*I positive and have molecular group 6 RFLP pattern (Jackwood and Sommer, 1999; Hoque *et al.*, 2001; Zierenberg *et al.*, 2001; Banda and Villegas, 2004).

The hypervariable region of VP2 of the Nigerian IBDV viruses was studied because it has been shown to mutate frequently and contains amino acid sequences important for tropism and antigenicity (Schnitzler *et al.*, 1993). With the result obtained when RFLP was used to differentiate some of the Nigerian viruses, it showed the existence of vvIBDV-like strains in Nigeria. A specific *Ssp*I site has previously been identified in all vvIBDV strains (Hoque *et al.*, 2001) with only two known exceptions (88180 and HK46) (Eterradossi *et al.*, 1999; Lim *et al.*, 1999). Consequently, this *Ssp*I site has been used as a genetic marker to predict a very virulent

phenotype that must be confirmed by *in vivo* studies (Sapats and Ignjatovic, 2002). The results of this study showed that this vvIBDV-specific *Ssp*I site was present in all except two out of the eleven IBDV field samples tested. Earlier reports by researchers from other parts of the world reported the presence of *Ssp*I site from many parts of the world except in Central and South America (Jackwood and Sommer, 1999), Europe (Jackwood *et al.,* 2006) as well as the absence of this site in Australia (Sapats and Ignjatovic, 2002). This was in agreement with an earlier report of the presence of vvIBDV in Nigeria by Luka *et al*. (2014) when they used RT-PCR-RFLP to differentiate both field and vaccine samples, although the restriction enzymes used in their study were different from the ones used in this study. They used *Taq*I, *Mva*I and *Sac*I. When the RFLP pattern obtained from their work was compared with the patterns obtained in this study, a similar pattern (424, 172, and 119) was observed with *Mva*I restriction enzyme they used and *Bst*NI restriction enzyme used in this study in 4 out of the 11 field IBDV samples used in this study. This may be so because *Mva*I is an isoschizomer of *Bst*NI. Generally, it was observed that all the IBDV samples used in this study had the same amino acid at position 222 (Alanine) but different *Bst*NI profiles were observed. This was in agreement with an observation by Jackwood *et al.* (2001) who showed that viruses with the same amino acid at position 222 could have a different *Bst*NI profiles. The molecular profiles of the Nigerian isolates were compared with other previously published RFLP profiles carried out by workers from different countries that used similar restriction enzymes (*Bst*NI, *Mbo*I and *Ssp*I) on 743bp RT-PCR products as used in this study. A comparison of the *Bst*NI RFLP patterns (424,172,119) generated by the Nigerian strains revealed a number of similarities to those identified by other researchers from Spain, France, Turkey, US, Puerto Rico, Guantemala, Costa Rica and variant viruses (Jackwood and Sommer, 1999; Jackwood *et al.,* 2006; Sareyyüpoğlu and Akan, 2006), *Mbo*I profiles (362,229) similar to IBDV strains from Turkey (Sareyyüpoğlu and Akan, 2006), (289, 229) similar to IBDV strains from Taiwan (Ture *et al.*, 1998). The IBDV viruses used in this study fall into molecular group 6 but it was also noted that new RFLP profile that were not seen in other IBDV strains in the world were present in the Nigerian strains. Detection of different RFLP profiles in IBDV strains can mean that they are not the same and detection of similar profile does not mean they are the same. The RFLP profile of a number of vaccine strains are recognized (Jackwood and Sommer, 1999), therefore whenever a new RFLP pattern is

observed there is likelihood that the virus is a wild-type (Gomma *et al*., 2003). Jackwood *et al*, (2001) observed that amino acid mutation occurred with viruses that have different RFLP profile than those with the same profile. These mutations were seen mostly at position 222, 254 and 318 which fall within hydrophilic peaks A and B. Schnitzler *et al*, (1993) also hypothesized that when amino acid mutations occurs in the A or B regions of the hydrophilic peaks but not both, it can cause antigenic drift but mutations in both regions can cause an antigenic shift. When the region A and B of the Nigeria IBDV amino acid sequences were examined (Fig 8), mutations were seen at position 219 of two viruses but position 222 was conserved in all within region A except when compared with Del-E mutations were seen at position 213 and 222, while the region B was conserved in all except that when compared with the amino acid sequences of the Del-E IBDV strains mutations were observed at position 318 and 323.When the RFLP profile obtained in this study were compared by states of origin of the samples, samples from Kaduna and Plateau were observed to have the same new RFLP pattern different from the same new RFLP pattern seen in samples from Bauchi, Anambra and Plateau. It should be noted that these three states: Bauchi, Kaduna and Plateau are in the northwestern part of Nigeria while Anambra is in the Eastern part of Nigeria. This is an indication that IBDV strains in Nigeria can circulate from one part of the country to another with ease aided by the transportation of chickens from one state to another.

Conventional RT-PCR, amplifying the VP2 hypervariable region in combination with DNA sequencing of the PCR product can be a very effective means for detecting and differentiating IBDV subtypes (Wu *et al.*, 2007). Variant and vvIBDV strains have characteristic amino acid substitutions in the VP2 hypervariable region relative to classic subtypes which can be detected quickly by RT-PCR and DNA sequencing. Expression/deletion studies have shown that amino acid positions 206 to 350 of the VP2 represents a major conformational, neutralizing antigenic domain known as the hypervariable region (HVR) (Xu *et al.*, 2011). It consists of the most variable region and is responsible for antigenic variation, tissue culture adaptation and viral virulence. The capsid regions that interact with the immune system are responsible for the evolution taking place in IBDV due to selective pressure (Durairaj *et al.*, 2011). The hypervariable VP2 region of IBDV is known for its high mutation rate but in vvIBDV strains, this region is conserved. Comparison of the VP2 region offers researchers an opportunity for

molecular epidemiology and phylogenetic study of IBDV. Very virulent IBDV display unique amino acids in the VP2 hypervariable region at amino acid residues 222 (Ala), 242 (Ile), 256 (Ile), 294 (Ile) and 299 (Ser) (Hoque *et al.*, 2001; Rudd *et al.*, 2002; Banda and Villegas, 2004; Jackwood *et al.*, 2008; Islam *et al.*, 2012; He *et al.*, 2012; Xu *et al*., 2015). Other amino acids conserved in vvIBDV are 249 (Glu), 253 (Glu), 254 (Gly), 279 (Asp), 284 (Ala), and 330 (Ser) (Xu *et al*., 2015). Avirulent IBDV have the following conserved amino acids; 222P, 256V, 279N, 284T, 294L and 299N while 249Q and 254G are seen in classical IBDV strains (He *et al.*, 2012), 222T, 249K and 254S are seen in variant strains while attenuated strains have 253H, 279N, 284T and 330R/K (Xia *et al.*, 2008) . The standard criterion for identification of vvIBDV phenotype is by mortality rates in specific-pathogen free (SPF) chickens (Jackwood and Sommer, 2007). Ignjatovic *et al*. (2004) reported an isolate of IBDV that caused high mortality as non vvIBDV after using molecular assay and pathogenicity studies. Hoque *et al*. (2001) reported a Malaysian IBDV with the characteristic virulent markers for vvIBDV but produced only 10% mortality in susceptible chickens. These *in vivo* studies are expensive, time consuming and sometimes not feasible (Rudd *et al.*, 2002; Mardassi*et al.*, 2004; Jackwood and Sommer, 2006). Due to the import restriction on live viruses into the United States where the analysis was done and lack of facilities in Nigeria to carry out pathogenicity studies, confirmation of the pathogenicity of the Nigerian viruses was not possible. Therefore the molecular characteristics consistent with the very virulent, variant and classic IBDV phenotype as listed above were used for identification and classification of the strain(s) of IBDV (Jackwood and Sommer, 2007) circulating within the states where the samples were obtained.

The VP2 region of IBDV has the highest mutation frequency compared to other regions of IBDV genome and can be used to uniquely identify variant, classic and very virulent IBDV strains (Jackwood and Sommer-Wagner, 2007). The nucleotide sequence of segment A of the Nigerian viruses were aligned and compared amongst themselves and between states and the nucleotide percentage identities showed varying ranges from 100% to as low as 93.4%. The sequence diversity of IBDV among and between some states was up to 6.1% with a sequence diversity of 6.6% between some IBDV from Kwara and Nassarawa states. This is higher than the 5.6% sequence diversity reported by Owoade *et al*, (2004). The 6.6% may be because the IBDV from Nassarawa had sequence similar to avirulent/attenuated virus. With the reference IBDV strains

consisting of 2 very virulent strains from Europe (UK661) and Asia (OKYM), though the nucleotide percentage identities was not 100%, they showed low to high sequence identities (95.0 – 98.9%). These percentages obtained show that though there are some mutations that occurred in the nucleotide sequences of the Nigerian viruses, they still share a close relationship with the very virulent IBDV strains used for comparison. The highest percentage (98.9%) nucleotide identity was observed to be from 4 Nigerian viruses consisting of IBDV from Kwara (2), Plateau (1) and Edo (1) (which was an isolate obtained at second passage in embryonated egg). Previous studies have shown a link between IBDV from Africa and Europe/Asia (Eterradossi *et al.*, 1999; Zierenberg *et al.*, 2000). The deduced amino acid sequence results of the hvVP2 region of the Nigerian viruses (amino acid positions 210 – 443) revealed that the amino acid sequences characteristic of vvIBDVs (A222, I242, I256, I294 and S299) (Jackwood *et al.*, 2008; Islam *et al.*, 2012; He *et al.*, 2012; Xu *et al*., 2015) were seen in the Nigerian viruses except in one Nasarawa77/NG/no2/2013 though this virus had A at position 222 like the vvIBDV. A critical look at the amino acid sequences of the Nigerian viruses showed that a total of twenty-one (21) amino acid substitution mutations occurred in their variable VP2 regions with some of them silent (synonymous) while others were not silent (non-synonymous). Six (6) of the substitution mutations occurred at the critical sites conserved for vvIBDV. Substitutions were seen mostly in IBD viruses collected from 2011-2014, with none in viruses collected during 2010 and a single mutation in viruses collected in 2009. Substitution mutation seen at position 219 of the Nigerian viruses were due to nucleotide substitutions seen at the 1st codon of the 16 Nigerian viruses mentioned and 2nd codon of the single Nigerian virus. Interestingly, this mutation at position 219 made its appearance from 2011. Since exchange of amino acid residues in VP2 occurs mostly in the four hydrophilic loops of the viral capsid, which is also important for cell antigenic and pathogenic variation (Coulibaly, 2005), this change could affect antigenicity of the Nigerian viruses since it occurred on the first hydrophilic loop (major hydrophilic peak A) (Jackwood and Sommer-Wagner, 2011). Also, substitution mutations were observed as seen in two of the Nigerian viruses that had an A222T which was due to a single non-synonymous A to T mutation at nucleotide position 737. Otherwise this position was conserved in the remaining Nigerian IBD viruses.Position 254 (G→S) was seen in all the Nigerian viruses and Del E, except in 4 that had the same amino acid like the vvIBDV UK661

(254G) used for comparison. The substitution seen here was due to a non-synonymous mutation where the vvIBDV UK661 used nucleotides GGC (for glutamine), the Nigerian viruses at this position used a combination of nucleotides AGT and AGC to form the amino acid serine (S). Adamu *et al*. (2013) noted that the non-synonymous mutations at amino acid position 254 and other synonymous mutations affected phylogenetic clustering of Nigerian and other IBDV strains in the tropical region and that different clusters of IBDV strains with identical VP2 amino acid sequences were formed based on a specific pattern of nucleotide mutations. Hoque *et al*, (2001) reported 10% mortality with a Malaysian IBDV that had markers for vvIBDV because of a mutation G→S at position 254 and A→E at position 270. Amino acid 253 is a residue shown to be involved in both cell tropism and virulence (Vakharia *et al.*, 1994; Kwon and Kim, 2004). Likewise mutations were observed at positions 284 (A→T) and 279 (D→N) (Kwon and Kim, 2004) of one Nigerian virus Nasarawa77/NG/no2/2013 as seen in avirulent/attenuated IBDV but this same virus had an A222 and S330 as seen in the vvIBDV and not 222Tor P for variant and classic strain and 330R or K that marks an attenuated strain. Amino acids 284 and 279 are linked to propagation in cell culture, which will mean that this virus is avirulent. Reverse genetic studies showed that amino acids at positions 253, 279 and 284 control virulence and cell tropism (Brandt *et al.*, 2001). Interestingly two other viruses (Nasarawa77/NG/2013 and Plateau78/NG/2011/1) had Q253L in this position with the nucleotide substitution occurring in the 2nd position of the codon used and it was non-synonymous but maintained the D279 and A284 seen in the vvIBDVs. Non-synonymous mutation seen at amino acid position 269 (T→S) of 19 Nigerian viruses was due to nucleotide substitution at position nt 878 – 880, where UK661 and some Nigerian viruses used a combination of ACT and ACC for threonine, while the 19 viruses used TCT and TCA for serine. The mutations seen at positions 272 (I→T) and 279 (D→N) of 4 Nigerian viruses were due to non-synonymous mutation that occurred at the 2nd and 1st codons of their nucleotides respectively. At positions 272, vvIBDV UK661 used ATC for isoleucine while the 4 Nigerian viruses used a combination of ACC and ACT for threonine and at position 279, vvIBDV UK661 used GAD for aspartic acid while the 4 viruses used AAC for asparagine. T272 and N279 are seen mostly in classic, variant and attenuated IBDV strains, and it would have been expected that the 4 viruses will share some close relationship with the avirulent strains but a nucleotide percentage identity of 94.8% and amino acid percentage of

94.9% showed a low relationship. Interestingly, 3 out of the 4 viruses were obtained from bursa samples and only one (Benin142E/NG/2014) was propagated in egg. Two out of the four hydrophilic peaks in the vVP2 of IBDV known as minor peak 1 that spans amino acid 249 – 254 and minor peak 2 that spans amino acid 279 – 290 are responsible for IBDV antigenicity (van den Berg, 1996). The mutations seen occurring within these regions in the Nigerian viruses could affect their antigenicity. At position 300, five Nigerian viruses had a similar amino acid with the vvIBDVs (E) but 81 and 19 Nigerian viruses had A and Q at this positions respectively. The 81 Nigerian viruses used the codons GCG and GCT for alanine, while 19 used CAG for glutamine and the mutations were not silent. This amino acid position 299 and 300 has been proposed by Adamu *et al.* (2013) to be used as a regional marker to differentiate IBDV strains isolated from different regions in the world. Many regional markers at the 299 and 300 amino acid position have been proposed for the identification of vvIBDV. The SE marker is seen in vvIBDV from Europe and Asia, NE marker for IBDVs from North America, Canada, South America and Mexico variant, SA marker for IBDV strains from tropical regions in Africa, India and Caribbean Islands while the ND, NK and NG is seen in IBDV from Venezuela, South Africa and China. One Nigerian virus had NE at positions 299 and 300. Mohamed *et al*, (2014), noted that amino acid 300A was seen in geographically distant vvIBDV isolates from Bangladesh, Nigeria and Nepal and that since glutamic acid is acidic while alanine is hydrophobic, this swap from acidic to hydrophobic could cause a change in the topology of the neutralizing epitopes in the VP2 thereby leading to vaccination failure. Amino acid substitution at position 300 could alter the biological characteristics of IBD viruses in terms of pathogenicity and virulence (Kasanga *et al.*, 2013).The serine-rich heptapeptide region found next to the second hydrophilic region 326 – 332 of the Nigerian viruses were conserved in the very virulent, variant and classical virulent IBDV strains as seen in the strains used for comparison except for 2 Nigerian viruses that had R330 as in the attenuated IBDV strains. These regions have been proposed by many workers as a confirmation of the highly virulent nature or virulence signature of vvIBDV (Mohamed *et al.*, 2014.;, Xu *et al*., 2015). Interestingly, two viruses used in this study Benin87/NG/2013 (bursal origin) and Benin142E/NG/2013 (an isolate obtained after second passage of bursal homogenate of Benin87/NG/2013 in embryonated eggs) though derived from the same IBDV sample had nucleotide and amino acid differences of 2.5% and 1.7%, respectively. This could be attributed

to passage in egg which may have caused the difference. Some workers have reported differences in the nucleotide and amino acid sequences of IBD viruses passaged in SPF chickens, eggs or continuous cell lines (Cao *et al*, 1998). It will be noted that vvIBDV is difficult to grow in cell culture and adaptation in cell culture or embryonated eggs can be achieved after several blind passages which will result in attenuation of the virus (Spies *et al*, 1987; Yamaguchi *et al.*, 1996) and mutations which is a characteristics of RNA viruses (Domingo and Holland, 1997). Reason for isolating the virus after second passage is not known and the mutations observed in the amino acid sequence of Benin142E/NG/2013 may be due to selection pressure.

The nucleotide sequences of the Nigerian viruses were aligned and compared with nucleotide sequences from previously published Nigerian IBDV strains from the GenBank. Their nucleotide percentage identities ranged from 92.7 – 100%. The 100% nucleotide identity obtained were with two previously published Nigeria IBDV strains (Accession nos JX424071.1 and JX424073.1) isolated from Kaduna state with eleven viruses used in this study from 3 states (Akwa Ibom, Edo and Plateau) showing that the viruses were related which indicates that they have the same origin. It also points at the ease with which IBD virus spreads within Nigeria. Akwa Ibom and Edo states are located in the Midwestern and Southeastern region of Nigeria while Plateau and Kaduna states are located in the North central and Northwestern region.

When the deduced amino acid sequences of the Nigerian viruses were compared with previously published Nigerian IBDVs, one thing that stood out was the mutation observed at position 219 (Q→T,P) seen in 17 of the viruses studied that was completely absent in the previous IBDVs which may point to an antigenic drift. Jackwood and Sommer-Wagner (2011) reported that antigenic drift in IBDV is possible by just a single point mutation in the vVP2. This will need further investigation to ascertain if this mutation will affect the antigenicity of IBD viruses circulating in Nigeria and the fact that 3 states sampled in this study (Plateau, Bauchi and Kaduna) all located within the same geographical zone had these mutation but none in the IBD viruses reported by Adamu *et al*, (2013) from 1 of the states mentioned (Kaduna state). The mutation at position 219 Q→T seen in the 17 Nigerian viruses used in this study but not in the other previously published Nigerian viruses may be attributed to an antigenic drift that occurred through time because it was observed that IBD virus from Kaduna state used in this study and

previously published IBDV strain obtained from the same Kaduna state between the years 1998 to 2011 did not have this mutation at position 219. Interestingly, one IBD virus from Kaduna used in this study that was collected in 2009 did not have this mutation at position 219 but the mutation appeared in an IBD virus from the same state in 2014. Another prominent mutation at position 269 (T→S) was also observed. This region was conserved in all the previously published Nigerian IBDV but not in 19 viruses used in this study that cuts across 5 out of the 9 states sampled. Since this particular mutation occurred outside the four hydrophilic regions, their contribution to the virulence and pathogenicity of IBDV in Nigeria is not known.Amino acid position 300 when compared between previous and the current IBDV studied showed that 300Q was not seen in any of the viruses from the northwestern part of Nigeria (Adamu *et al.*, 2013) and some of the current viruses that had this mutation were from the same or close by regions. From the southwest region, previously published IBDV from Osun and Ogun states had this mutation with Kwara in this study. Amino acids characteristic to vvIBDV were present in the previously published Nigerian strains and the viruses used in this study indicating that vvIBDV are present in the viruses studied except that at amino acid positions 222 a previously published Nigerian IBDV, accession number AJ586946.1 from Osun state and two viruses used in this study from Bauchi state had a “T” at this position. The serine-rich heptapeptide region was conserved in all except in two viruses used in this study. From these results, it can be said that the Nigerian viruses were relatively stable until 2011 when some mutations not seen previously started manifesting. He *et al*, (2012) observed a continuous heredity variation, with some IBDV mutation being stable in the population over 10 years.

When the nucleotide sequences of the Nigerian viruses were aligned with the nucleotide sequences of some IBDV strains from Africa (Tanzania, South Africa, Senegal, Ivory Coast, Egypt and Ethiopia), according to the nucleotide percentage identities obtained, the Nigerian viruses showed close relationship with IBDV from some African countries in this decreasing order; South Africa, Ethiopia, Egypt and Tanzania, Senegal, and Ivory Coast. The various high nucleotide percentage identities obtained with the African strains were with 4 Nigerian viruses Kwara141/NG/2014, Kwara148/NG/2014, Benin142E/NG/2013 and Plateau40/NG/2012 while the lowest nucleotide percentage identity was with one Nigerian virus Nassarawa77/NG/no2/2013 that was reported to have sequences similar to vaccinal/attenuated

IBDV. Generally, none of the Nigerian viruses had a 100% nucleotide identity with any of the African strains used for comparison. The deduced amino acid sequences of the Nigerian viruses (starting from amino acid 220-344) when compared with some of the African IBDV strains showed that the amino acid positions conserved for vvIBDV were present in both the Nigerian and African IBDVs with some exceptions. For example, at position 222, one IBDV from Tanzania (KTP-8) and Senegal had P at this position and strains from Ivory Coast had Q with two Nigerian viruses having a T. Position 242, had mutation for IBDV from Senegal, Tanzania, South Africa and two Nigerian viruses. At positions 256, mutations were observed in IBDV from Tanzania, Senegal, some from South Africa and one Nigerian virus while the position was conserved in the others. Position 294 also showed mutation L for IBDV from South Africa, Senegal, Ivory Coast and one Nigerian virus while mutation N was seen at position 299 in IBDV for the same countries. vvIBDVs have been reported in Egypt (Eterradossi *et al.*, 2004.; Mohamed *et al.*, 2014), Tanzania (Kasanga *et al.*, 2007), Ethiopia (Jenberie *et al.*, 2014), South Africa, Senegal (Eterradossi *et al.*, 1999) and Ivory Coast (Eterradossi *et al.*, 1999), Zambia (Kasanga *et al.*, 2013). Position 300 that is advocated to be used as one of the regional marker to differentiate the origin of IBDV was observed to be heterogeneous in the sequences compared. 300Q was seen in IBDVs from South Africa (1), Ivory Coast (3) with Nigerian viruses (19). None of the IBDV strains from other countries used for comparison had 300A as seen in the Nigerian viruses. A look at amino acid position 299 and 300 suggested to be used as a regional marker of both the Nigerian viruses and some of the African IBDVs used for comparison showed different amino acid motifs at these positions which are NE (South Africa, Nigeria, Tanzania and Senegal), ND (South Africa), NQ (Ivory Coast), SQ (Nigeria and South Africa), SA (Nigeria), and SE (South Africa and Nigeria). Position 330 was conserved in all the IBDV from the African countries used for comparison and in the Nigerian viruses except in two Nigerian viruses.

Percentage identities of the nucleotide and amino acid sequences of the Nigerian viruses and some IBDV strains from other countries outside Africa comprising of very virulent, variant and classical IBDV strains showed that though Nigerian viruses did not have a 100% identity with any of the IBD viruses used for comparison, high nucleotide and amino acid percentages were obtained with some vvIBDV strains. One of the Nigerian viruses Nassarawa77/NG/no2/2013 had high nucleotide and amino acid identity percentages with some variant and classic strains

included in the study. Comparison of the amino acid sequences of the Nigerian viruses and IBDV strains from other countries included in this study showed that the mutation at position 219 (Q→T and P) were unique to the Nigerian viruses only and that none of the other IBDV strain had this mutation. Also the mutation T269S seen in 19 Nigerian viruses was seen only in GLS5 a variant IBDV strain from USA. Amino acid position G254 that was conserved in the vvIBDVs and classic IBDV strains and 4 Nigerian viruses was not conserved in the remaining Nigerian viruses, they had a mutation substitution 254S at this position with the variant IBDV strains. The amino acids motifs at positions 299 and 300 of the IBDV strains used for comparison were NE, SE and SA. Amino acids at these positions in the Nigerian viruses were heterogeneous. The Nigerian viruses displayed 4 motifs; NE, SE, SA and SQ with SA being the most common (Appendix 11). None of the other IBDV strains used for comparison had SQ as seen in the Nigerian viruses. Amino acids at positions 299 and 300 have been suggested to be used as a regional marker for identification of IBDV (Adamu *et al.*, 2013). Amino acids characteristic for virulence seen in the vvIBDV strains included in this study were present in the Nigerian viruses except for 2 Nigerian viruses that had T at position 222 instead of A seen in vvIBDV (Hoque *et al.*, 2001; Rudd *et al.*, 2002; Banda and Villegas, 2004; Jackwood *et al.*, 2008; Islam *et al.*, 2012; He *et al.*, 2012; Xu *et al*., 2015). This result based on the amino acid sequences still go to confirm that the IBD viruses circulating within poultry flock in Nigeria is the very virulent type. Although the criteria for identification of the very virulent phenotype of IBDV is based on a combination of molecular, pathogenic and antigenic criteria (van den berg *et al*, 2004), analysis of nucleotide and amino acid sequences of the hypervariable region of the VP2 that are unique to classic, variant and very virulent IBDV strains can be used to differentiate and identify IBDV strains in the field (Jackwood and Sommer-Wagner, 2007). A222, I242, I256, I294 and S299 are unique and highly conserved to all vvIBDV strains (Hoque *et al.*, 2001; Rudd *et al.*, 2002; Banda and Villegas, 2004). Many worker have been able to identify and classify IBDV strains based on the study of the hypervariable region of the VP2 (Rudd *et al.*, 2002; Mardassi *et al.*, 2004; Owoade *et al.*, 2004; Adamu *et al.*, 2013; Owolodun *et al.*, 2015).

Different IBDV vaccines are used for vaccination of poultry flocks in Nigeria. Based on the data collected during this study, foreign IBD vaccine imported into the country was used mostly by the farmers whereas the locally manufactured NVRI Vom vaccine had just 4.35% usage. This

can be attributed to probably non availability and insufficient numbers of produced local IBD vaccine to meet the demands of farmers in the field hence the importation to augment the short fall. Sequences of the Nigerian viruses studied were aligned and compared with sequences of some IBDV vaccines used in Nigeria and some others from the GenBank to see if there are similarities between them. The analysis showed that the viruses circulating within the Nigerian poultry was not completely the same with the vaccine strains used except for two vaccines ABIC and MB (both intermediate plus vaccines) which had 100% identity in both their nucleotide and amino acid sequences with just four of the Nigerian viruses studied. It was suggested that MB vaccine can offer protection to chickens when used in the field (Owoade *et al.*, 2004) and from this study, ABIC may also provide protection but it is not known if it can protect for the other Nigerian viruses since their percentage identities were not up to 100% like the other four. This will need to be investigated. Some of the causes of vaccination failure includes difference between the field and vaccine strains, emergence of new antigenic variants, high level of maternally derived antibodies (Müller *et al.*, 2012), improper storage, handling and application of vaccine (Raue *et al*., 2004) or absence of certain antioxidant supplements in feeds (Shekaro *et al.*, 2012). From the farm data obtained in the course of this study, some farms that vaccinated their flock and submitted bursa samples when they suspected that the outbreak in their farms was due to IBDV, IBD virus were not detected from these farms. The inability to detect IBDV from this farm may be due to some of the factors mentioned above on the causes of vaccination failure especially improper storage, handling and application. A particular farm that used ABIC vaccine and reported an outbreak, the amino acid sequences of the viruses from the field showed some differences to the ABIC vaccine sequence. The same scenario was observed in another farm that used Georgia IBD vaccine, the sequence of the virus from the outbreak was different from that of the Georgia vaccine used. It would have been expected that in the vaccinated flock that vaccine viruses will be detected. Mutations/variations were observed at key neutralization epitopes in the deduced amino acid sequences of the Nigerian viruses. Some workers reported similarity between and phylogenetic clustering together of nucleotide sequences of both wild and vaccine virus strains in the same area (Jenberie *et al*., 2013; Amin and Jackwood, 2014), but in this study, the reverse was the case except in the four Nigerian viruses that clustered with the ABIC and MB vaccines but with mutations not seen in these two vaccines. In a poultry farm from Nassarawa

state, both field and attenuated-like IBD viruses were detected. Reports have indicated that IBD vaccinated birdscan die from IBDV infection than birds that are not vaccinated (Jakka *et al.*, 2014). From the results obtained in this study, it has become imperative to review the vaccines used in Nigeria's poultry industry and come up with a vaccine made solely from indigenous IBDV strain in Nigeria to curb the excessive losses encountered due to IBDV. It will also be necessary to formulate a vaccine that can break through maternal antibodies, due to the continuous use of IBD vaccine in the parent flock and the subsequent transfer of antibodies to the progeny which neutralizes vaccine viruses thereby resulting in vaccination failure. Based on the variability of IBDV strains in the field, immunization with vaccines that are antigenically similar to the circulating IBDV strains plays a key role in IBDV control.

The phylogenetic tree constructed with the nucleotide sequences of the Nigerian viruses and reference IBDV strains consisting of vv, classic, variant and serotype 2 retrieved from the GenBank to gain insight into the genetic relatedness of the Nigerian viruses to other IBDV strains showed that the Nigerian viruses were all serotype 1 IBDV, and were not classic or variant nor serotype 2 IBDVs except for one virus from Nassarawa state that showed a close relationship with the classic and variant IBDV. The tree revealed that the Nigerian viruses shared a close relationship with the vvIBDV but they formed unique clusters of their own on the tree and were related to vvIBDV from Europe and Asia. A look at the pattern of clustering of the Nigerian viruses in relation to their amino acid sequences revealed that they clustered according to the amino acids they have in common irrespective of state and location. On the tree, the largest cluster that consisted solely of 81 of the Nigerian viruses had this common amino acid A at position 300 plus S at position 254. The 4 Nigerian viruses that clustered together on the same branch with the vvIBDV used for comparison though forming their own unique cluster within that branch had T at position 272, N at position 279 which could have accounted for their forming the unique cluster though they had E at position 300 like the vvIBDV. Interestingly, the 3rd cluster made up exclusively of 19 Nigerian viruses had these amino acids in common; T (17 viruses), P (1 virus) at position 219, S at position 269 and Q at position 300. The various mutations seen on the amino acid sequences of the Nigerian viruses could be the reason for their forming the unique clusters observed on the tree while still sharing a close identity with the vvIBDV showing that the likely strain of IBDV circulating within Nigeria is the very virulent

strain. Adamu *et al*, (2013) commented that the non-synonymous mutations at amino acid position 254 and other synonymous mutations affected the clustering IBDV strains. It was observed that the clustering of the segment A of the Nigerian viruses used in this study was based on the combination of non-synonymous and synonymous mutations that occurred within the nucleotides of the Nigerian viruses. Results from the phylogenetic tree analysis based on the (702) sequences indicated that the Nigerian strains were closely related to the vvIBDV strains, forming a group clearly distinguished from other less virulent strains. The single Nigerian virus (Nassarawa77/NG/no 2/2013) seen clustering together with the variant and classic IBDVs but on a separate branch had the following amino acid mutations; A222 and T284 that were absent in the variant and classic IBDVs used for comparison that could have accounted for it forming its own branch within this cluster.

The phylogenetic tree constructed with nucleotide sequences of the Nigerian viruses and some nucleotide sequences of previously published Nigerian IBDV strains revealed that the 81 Nigerian viruses that formed a unique cluster were related to previous IBD viruses from Kano, Katsina and Kaduna which are located in the Northwestern region. Interestingly, the 81 Nigerian viruses comprised IBD viruses from 6 states and the federal capital territory (Uyo, Edo, Kwara, Plateau, Bauchi, Kaduna and Abuja). The states sampled in this study that have close proximity to the previous IBD viruses are Plateau, Bauchi and Kaduna while Uyo, Edo, Kwara and Abuja are located in the South-South, Midwest and North central. The similarity between these IBD viruses may be due to the movement of live birds from one location to another for brooding or sale in live bird markets. Ease in transportation in Nigeria has also aided the quick spread and endemicity of IBD virus in Nigeria. On the tree, the 4 Nigerian viruses were seen on the same branch with vvIBDVs from Europe, Asia and two previously published Nigerian viruses from Osun and Ogun state showing that they were more closely related to them than to the other Nigerian viruses but on this tree, the 4 viruses used in this study formed a separate cluster of their own within this branch. The reason for this may be due to the T and N seen at amino acid positions 272 and 279, respectively in the 4 viruses. Interestingly, the remaining 19 Nigerian viruses used in this study clustered on their own with none of the previously published Nigerian or other viruses included in this study found in this branch. It will be recalled that these Nigerian viruses had amino acid mutation at position 219 and 269 not seen in any of the IBDV strains

used for comparison. There was a degree of regional clustering observed in the Nigerian viruses with previously published Nigerian viruses with some exceptions. For example, all the IBD viruses from Kwara state used in this study would have been expected to cluster with previous Nigerian IBDV from Oyo and Osun states since it shares a boundary with them but it was not the case except on the branch where two viruses from Kwara were seen with the vvIBDV and IBDV from Ogun and Osun states. Majority of the viruses from Kwara clustered more with viruses from the North western region of Nigeria. On the branch with the 19 Nigerian viruses, IBDVs from Abuja, Edo and Kwara were not found here.

The phylogenetic tree constructed with the nucleotide sequences of the Nigerian viruses and some IBDV strains from Africa to access the genetic relatedness between them revealed that 4 Nigerian viruses were closely related to IBDV from South Africa, Tanzania and Egypt though IBDVs from each country formed sub clusters of their own within this branch, a bootstrap value of 69% showed that they were not significantly distinguished from each other and the vvIBDVs from Europe and Asia. The 19 Nigerian viruses that formed a unique cluster of their own were genetically related to IBDV from Ethiopia although viruses from the two countries were found on separate branches, but a bootstrap value of 85% suggests this branching may be significant. IBDVs from Ethiopia included in this study did not have the unique amino acid mutation T219 seen in the 19 Nigerian viruses that clustered in this branch. In the branch that had the 81 Nigerian viruses, none of the IBDV from some African countries included in this study was found on this branch although a bootstrap value of 46% showed that they were not significantly distinguished for the vvIBDVs and IBDVs from South Africa, Tanzania and Egypt. IBDV from Ivory Coast with a bootstrap value of 92% showed they were significantly different from the Nigerian viruses and that no IBD virus with sequences exactly like the IBDV strain from Ivory Coast is found in Nigeria. The IBD virus from Nassarawa state clustered with variant strains for US and classic IBDV strains from Senegal, Tanzania and the classic STC IBDV strain.

The phylogenetic tree constructed using the nucleotide sequences of the 105 Nigerian viruses and some IBDV strains from other countries representing Europe, Asia, North and South America and Australia retrieved from the Genbank showed 4 of the Nigerian viruses clustering on the same branch with vvIBDVs from these countries though forming a sub cluster of their

own. The 81 Nigerian viruses still formed their unique cluster on a separate branch from the vvIBDV with a bootstrap value of 86%, it could mean that the branching is significant. The remaining 19 Nigerian viruses still branched out but a bootstrap value of 34% means they were not significantly distinguished from the vvIBDVs from other countries. The tree showed that 104 out of 105 of the Nigerian viruses share a genetic relationship with other vvIBDVs from other countries outside Africa though with some differences and that they are not classic, variant or attenuated IBDVs except the single Nigerian virus that clustered with variant, classic and attenuated strain with a bootstrap value of 96% showing that it is significantly different from the remaining Nigerian and other vvIBDVs included for comparison. It also formed a separate cluster from the variant, classic and vaccine viruses. The tree also showed that none of the Nigerian viruses is a serotype 2 IBDV that they are all serotype 1 IBDV. Serotype 2 IBDV is seen only in turkeys and not chickens and are apathogenic for chickens (Mahgoub, 2012).

The tree constructed with the nucleotide sequences of the 105 Nigerian viruses and nucleotide sequences of some IBDV vaccines retrieved from the GenBank clearly shows that the Nigerian viruses were not vaccine viruses. The single Nigerian virus that did not cluster with the other Nigerian viruses was on a separate branch and bootstrap value of 96% shows that it is significantly distinguished from the remaining Nigerian viruses. The vaccine viruses included for comparison formed a separate cluster but a bootstrap value of 57% between them and the single Nigerian virus indicates that they are not significantly distinguished from each other. On the tree, it was observed that two vaccine viruses, ABIC and MB clustered with 4 Nigerian viruses and vvIBDV UK661. Unfortunately, data collected on the IBD vaccine(s) used were not available. One of the Nigerian virus (Benin142E/NG/2014) that clustered with ABIC and MB was an isolate derived after second passage of bursa homogenate from an unvaccinated cockerel that died during IBDV outbreak in embryonated egg. Commercial vaccines are divided into 3 categories and according to the virulence of the strain used for producing them; mild, intermediate and intermediate-plus or “hot” vaccine. Mild and intermediate vaccines are easily neutralized by maternal derived antibodies (MDA) and are only effective in chicks with low MDA (Tsukamoto *et al*., 1995). Intermediate-plus or “hot” IBD vaccines are used mostly in flock with high MDA and they can break through maternal immunity and provide protection to the chicks (Chansiripornchai and Wanasawaeng, 2009).Bursa samples collected for these studies

were from vaccinated and non-vaccinated chicken flocks. Data collected showed that different types of vaccines ranging from mild to intermediate strains were used. vvIBDV are known to cause clinical diseases even in vaccinated flocks. Jakka *et al*. (2014) reported that IBD vaccinated birds will easily give in to field IBD virus outbreak than unvaccinated birds. As was earlier reported, on a farm that used ABIC vaccine that experienced an outbreak, there were some differences noticed in the sequences of the virus from this outbreak and the ABIC vaccine sequence. Their nucleotide sequence identity was 96.1%. The same scenario played out in some farms whereby outbreaks of IBDV occurred despite vaccination with some of the vaccinated flock showing negative results for IBDV antigen by RT-PCR. The reasons for the failure of the vaccines used in these farms to mount antibodies against field IBDV and to protect the flock may be due to improper handling and application of the vaccines or that the vaccine viruses were no longer viable or that the strains encountered in this study were able to affect the chickens despite vaccination.

Information gleaned from the field in the course of this study revealed that poultry farmers were sometimes given already reconstituted IBD vaccines to administer to their flock if by the veterinarian estimation that the time it will take the farmer to get back home is about 30 minutes with prior instruction to withdraw water before application of vaccine. Some vaccine manufacturers recommend administration of vaccines within one hour after reconstitution (Aliu, 2004) and some of the prior reconstituted vaccines are given to the farmers in syringes which they carry to their farms on their person without maintaining a cold chain. If the poultry farm is far away, the vaccine is handed over to the farmer with instructions on how to apply it. All these are done without supervision so the integrity of the vaccine and how it is applied is doubtful. IBD vaccines are advised to be applied with chlorine free water and in situations where the water source is doubtful, skimmed milk should be added (Aliu, 2004) but this is not the case by some farmers (Bosha and Nongo, 2012). Some poultry farmers who due to the fact that they have been in the business for a long time do not bother to consult veterinarians but go ahead and administer the vaccines on their own. All of the above mentioned practice can lead to vaccination failure.

IBDV VP1, the RNA-dependent RNA polymerase (RdRp) is responsible for the replication of the genome and synthesis of mRNAs (von Einem *et al.*, 2004.; Pan *et al.*, 2007). Several recent

reports have shown that VP1 on segment B may play a role in viral pathogenesis and vvIBDV expansion (Liu and Vakharia, 2004; Boot *et al.*, 2005; Hon *et al.*, 2006; Le Nouen *et al.*, 2012; Escaffre *et al.*, 2013; Yu *et al.*, 2013; Gao *et al.*, 2014). Double-stranded RNA viruses have a high rate of mutation, short generation time, and high progeny yield (Domingo and Holland, 1997). Coupled with mutations introduced by their error-prone polymerases, RNA viruses generate genomic variation by homologous and non-homologous recombination and reassortment in viruses with segmented genomes (Pita *et al.*, 2001; Kew *et al.*, 2002; Wei *et al.*, 2006). Partial sequence analysis of the IBDV genome has suggested that genome recombination and / or reassortment may occur among pathotypes of IBDV. Evidence of reassortant IBDV have been reported by different workers (Le Nouën *et al.*, 2006; Hon *et al.*, 2006; Wu *et al.*, 2007; Gao *et al.*, 2007; Jackwood *et al.*, 2011; Jackwood *et al.*, 2012; Chen *et al.*, 2012). Hon *et al.* (2006) showed that very virulent IBDV emerged after a reassorting of genome segment A with an undocumented segment B and further studies indicated that the genome segment B of vvIBDV was highly conserved and clearly different from non-vvIBDV (Gao *et al.*, 2007; Le Nouën *et al.*, 2006; Hon *et al.*, 2008; Jackwood *et al.*, 2012). This study investigated the possibility of the existence of reassortant IBD viruses in chicken population in Nigeria which hitherto had never been reported. Partial sequence and phylogenetic relationships between the VP1 genes of IBDV from Nigerian chicken flocks were compared with other VP1 genes of IBDV strains worldwide. Partial sequencing of the VP2 of segment A and VP1 of segment B is adequate to give accurate phylogenetic information just like the full genome sequencing will and equally enough to assess their genetic relatedness (Le Nouen *et al.*, 2005). So the same region was examined in this study and phylogenetic analyses were conducted using those sequences available in GenBank listed by Le Nouen *et al.* (2006) and some others. The sequences obtained from GenBank that were used for comparison consists of thirty-eight wild-type strains representing scIBDV, cvIBDV and vvIBDV, seven vaccine strains, three naturally reassorted IBDV strains from Venezuela and two serotype 2 strains from Europe plus turkey IBDV isolate from Nigeria and OH (serotype 2 IBDV) from US.The sequence results showed that 64 out of 67 of the Nigerian IBDV VP1 gene shared similar amino acid sequences with the non-vvIBDV like sequences obtained from the GenBank while the remaining 3 shared similar amino acid sequences with vvIBDV like sequences of some vvIBDV reference IBDV strains (UK661, HK46

and OKYM) from the GenBank but with obvious substitution mutations occurring along the Nigerian sequences. The nucleotide sequences of the 64 Nigerian viruses were less closely related to the reference non-vvIBDV used in this study with nucleotide identities of between 83.4 – 88.9%, but the remaining 3 viruses showed between 98.3 – 99.1% nucleotide identity with the vvIBDV UK661, HK46 and OKYM suggesting a close relationship but a 96.6% identity with T09, a turkey IBDV isolate from Nigeria. It will be recalled that the VP2 of the 104 out of 105 of the Nigerian IBDV used in this study had VP2 sequences similar to vvIBDV with all the markers for virulence present in the amino acid sequences. These results are in agreement with that of some worker that reported reassortant strains of IBDV with a vvIBDV-like segment A and a non-vvIBDV like segment B (Gao *et al.*, 2007; Le Nouën *et al.*, 2006; Kasanga *et al.*, 2012). Jackwood *et al*. (2012), also reported IBD viruses isolated from California that contained a genome segment B sequence consistent with vvIBDV. These findings on the other hand, are in contrast with Owoade *et al*. (2004) findings. They reported that there was no evidence of reassortment between the IBDV segments of different serotypes or species associated with the turkey isolate from Nigeria, indicating the prolonged co-evolution of both segments A and B implying that there are no reassortant IBDV present in Nigeria. It should be recalled that this study used isolates from chickens hence could be the reason for the different results obtained. Multiple sequence analysis revealed that VP1 of vvIBDV strains i.e. D6948, UK661, HK46, OKYM contains 17 characteristic amino acid residues (4V, 13K, 61I, 145T,146D,147N, 242E, 287A, 390M, 508K, 511S, 546P, 562P, 646S, 687P, 695R) (Cui *et al.*, 2013). Yu *et al*, 2010, reported similar sequences at positions 4, 61, 145, 287, 508, 511, 646 and 687 for seven vvIBDV isolated from China while the non-vvIBDV had 4I, 61V, 145N, 287T, 508R, 511R, 646G and 687S. In the partial VP1 sequences of the Nigerian IBDV, four amino acid residues (145, 146, 147 and 242) were examined. 64 out of 67 of the Nigerian IBDV isolates had the following motifs at these four positions 145Q and H, 146E and D, 147G (QEG (glutamine/glutamic acid/glycine), HEG (Histidine/glutamic acid/glycine), QDG (glutamic acid/aspartic acid/glycine), HDG (Histidine/aspartic acid/glycine), and 242D (aspartic acid) like the non-vvIBDV, with QEG been the most common motif. The remaining 3 had 145T, 146N, 147D i.e. TDN (threonine/aspartic acid/asparagine) and 242E like the vvIBDVs. Jackwood *et al*, (2012) reported 67 IBDV from nine states in the U.S with viruses from California containing segment B

consistent with vvIBDV and the remaining 54 had non-vvIBDV genome segment B. They also reported five non-vvIBDV motifs NEG, NED, SEG, NES, and TEG. In the nucleotide sequences of the Nigerian IBDV, the mutation observed at position 145 of the Nigerian viruses that gave rise to the amino acid Q and H observed at this position, occurred at the 1st and 2nd codon and they were not silent, the Nigerian viruses used CAA (Q) and CAC (H). Other non-vvIBDVs included in this study for comparison used the nucleotides AAC, AAT, and ACC which translated into N, N, T amino acids, respectively at position 145. At amino acid position 146, the Nigerian IBDV usedtwo nucleotides combination of GAG and GAA for E, and the mutations were silent while the other non-vvIBDV used for comparison used GAG mostly for E. At position 147, the Nigerian IBDV used GGG for G. The amino acid at position 147 for some of the non-vvIBDV included in this study were different, some had D while others had S. Generally, the motifs at positions 145, 146 and 147 of the non-vvIBDVs included in this study were NED (asparagine/glutamic acid/aspartic acid), NEG (asparagine/glutamic acid/glycine), TES (threonine/glutamic acid/serine) and TEG (threonine/glutamic acid/glycine).When the motifs observed in the 67 Nigerian viruses were examined at state of origin level, QEG which was the highest motif observed cuts across 8 out of the 9 states in Nigeria sampled. These 8 states include Plateau, Anambra, Abuja, Bauchi, Kwara, Benin, Kaduna and Uyo in Akwa Ibom state. It was observed that in Plateau state alone that apart from the motif QEG, four other motifs were seen; HEG, QDG, HDG and TDN. Uyo in Akwa Ibom state had two motifs; HEG and QEG. Kwara state also had two motifs QEG and TDN. In a Blast search carried out on 24/02/2015, to search for reassortant IBDV in GenBank with the same motifs as the Nigerian IBDVs, non was seen for the non-vvIBDV like viruses, mostly the matches were only at amino acid positions 146 (E) and 147 (G) but at positions 145, none of the sequences in the GenBank had the same amino acid like that found in the Nigerian viruses. Gao *et al*. (2007) and Le Nouën *etal.* (2006), reported an IBDV reassortant containing a vvIBDV genome segment A and a non-vvIBDV genome segment B less pathogenic in specific-pathogen-free (SPF) chickens while Wei *et al*, (2006), on the other hand described a novel reassortant strain, ZJ2000, that had a non-vvIBDV-like segment A but a vvIBDV-like segment B which caused high mortality in SPF chicken embryo at a 0.4ml dosage and in SPF chickens at a dosage of 10^5 ELD (embryo lethal dose). It would have been interesting to assess the pathogenicity of these Nigerian reassortants in

SPF chickens to determine their virulence during the course of this study but due to some obvious constrains such as lack of a containment facility, it was not possible to carry out this study. Control of IBDV is mostly by vaccination. Live vaccine offers poor protection when the IBDV strain during an outbreak is the vvIBDV strain (Abdel-Alim and Saif, 2001). It is proposed that the mechanism of IBDV reassortment in the field arises when very virulent strain of IBDV infects a vaccinated flock leading to exchange of genetic material in the bursa of Fabricius and giving rise to new reassorted viruses (Figure 2). Reporting of reassortant IBD virus in the field re-enforces the risk of the use of live IBD virus vaccine for vaccination (Wei *et al.*, 2006). An essential aspect of reassortment is the exchange of genetic information between the IBDV strain used in vaccination with other IBDV in circulation which plays a role in virus evolution, and giving rise to more virulent virus lineage. Vaccination of animals that have had contact with the wild virus can lead to the emergence of new virus lineage with new biological character (He *et al.*, 2009). Live vaccine could not only lead to virulence reversion due to continued passage in flock, but could also act as a genetic donor for genome reassortment. Unfortunately in Nigeria, different types of attenuated live vaccine strains of IBDV produced from different countries around the world that use indigenous IBDV strains found in those countries and unrelated to the IBDV strains found in Nigeria are used in the field for vaccination thereby creating a mixing pot for reassortment and/ or recombination between field and vaccine IBDV and can and may have put the country in a difficult position in the prevention and control of IBDV which have acquired an endemic status in Nigeria. Although a lot of IBDV strains have been isolated worldwide, almost all known strains were not reassortant virus, indicating the reassortment rate is low in nature but the discovery of reassortant IBDV strain in Nigeria for the first time in this study may be an indication that reassortment rate may likely be increasing, which confirms an observation made by Jackwood *et al*, (2012) on the possibility and concern of additional vvIBDV reassorted viruses and the need for routine examination of the genome segment B when conducting molecular diagnosis of IBD viruses. Also the identification of two unique amino acids at position 145 (Q and H) not seen in any other reassortant strain from other countries of the world in GenBank is a source of worry.

The nucleotide sequences of genome segment B (684bp) of six Nigerian viruses showed they were less closely related with the non-vvIBDVs included for comparison with nucleotide

identities of between 84.5 – 88% despite sharing similar amino acid sequences but not devoid of mutations and their amino acid sequence identities with non-vvIBDV was between 95.2 – 96.9%. The deduced amino acid sequences of the six Nigerian viruses at position 61 had an I as seen in the vvIBDV and not V that was commonly seen in non-vvIBDVs. Phylogenetic trees constructed with the nucleotide sequences of the six viruses with others obtained from the GenBank still showed the Nigerian viruses forming an exclusive clusters of their own from both the non-vvIBDV and vvIBDV included, and the similar amino acid with vvIBDV at position 61 did not alter their position on the tree (figure 4.10). On the NJ tree, the six Nigerian viruses were on the same branch with viruses from China (Harbin and Gx) and from USA (7741) though on significant branches from each other but with a bootstrap value of 56 the branching may not be significant. On the UPGMA tree, a different scenario was observed with the six Nigerian viruses on a significantly separate branch almost like an outgroup from the other viruses included in this study.

Phylogenetic approaches have been particularly useful for studying the origin and subsequent evolution of a virus. The phylogenetic trees constructed with the nucleotide sequences of the Nigerian IBDV with reference and other IBDV strains from other countries in the world confirm not only the presence of reassortant strains of IBDV in Nigeria but also a novel lineage. In a study by Gao *et al*. (2007), out of the 23 viruses, 8 vvIBDV had similar genome segment B sequences that clustered in one phylogenetic group and a second phylogenetic group contained classic, variant, and vaccine non-vvIBDV strains. Hon *et al*. (2008), reported the identification of three lineages of genome segment B where one lineage contained vvIBDV exclusively while the other two included a variety of non-vvIBDV strains. Le Nouën *et al*. (2006), showed that of the 50 viruses analyzed, the phylogenetic tree constructed using the parsimony method showed the vvIBDV clustering exclusively in one segment B lineage, and the non-vvIBDV were significantly divided into at least four additional genetic lineages.

The phylogenetic trees constructed with the nucleotide sequences of the Nigerian IBDV with reference and other IBDV strains from other countries in the world confirms the difference between the reassortant IBDV strains in Nigeria and others in that they formed a separate significant clusters of their own. Also the presence of these different motifs in Nigeria IBDV

reassortant strain may be a hindrance to the production of a single efficient IBDV vaccine but pathogenicity studies are needed to confirm this.

CONCLUSION

Since the first reported case of IBDV in Nigeria, the virus is now endemic in the country and is negatively affecting the poultry industry. Likewise, cases of post vaccinal outbreaks are on the increase. In this study therefore, molecular and phylogenetic analysis of the segment A and B of IBDV was done to characterize, identify and determine the strain of IBDV circulating in Nigeria poultry population, and to determine if reassortant IBDV strains are circulating in the field with the aim of establishing the reason for the failure of IBD vaccines used in the field in protecting chicken flocks from IBDV outbreaks when they occur. This present study therefore has shown that vvIBDV-like viruses are circulating within poultry population in the 9 states sampled and for the first time in 5 (Anambra, Akwa Ibom, Edo, Kwara and Nassarawa) out of the 9 states. The Nigerian IBD viruses had mutations on the VP2 not seen in other IBDV strains that may affect their antigenicity. The mutations observed signify an antigenic drift in the IBDV strain circulating in Nigerian poultry. The mutations also show that vvIBDV in Nigeria is evolving. The mutations observed placed them on separate phylogenetic clusters from other IBDVs but still share close relationship with the vvIBDVs. In addition, the presence of reassortant IBDV strains derived from a vvIBDV-like segment A and non-vvIBDV-like segment B were also confirmed with a motif not seen in any IBDV strain in the world. The reassortant IBDV strains detected in this study revealed a novel reassorted segment B lineage. The results from this study show that the cause(s) of vaccination failure in the poultry population in Nigeria are due to the presence of vvIBDV-like viruses with abilities to bypass maternally derived antibodies conferred on the flock by classic and variant IBD vaccines used in the field and also due to the presence of reassortant IBD viruses.

RECOMMENDATIONS

Based on the findings of this study, the following recommendations were made.

1. There is a need for continuous molecular and epidemiological study of Infectious bursal disease virus in Nigeria to keep track of the changes (mutations) that are constantly encountered in the field in order to protect birds from the sudden emergence of new predominant strains.
2. The mutations observed in this study need to be investigated to ascertain if they will affect the antigenicity of the Nigerian IBD viruses.

3. Infectious bursal disease vaccines used in the field needs to be reviewed. It is recommended that vaccines using indigenous Nigerian IBDV strains be produced.
4. Poultry farmers should be educated on the best IBD vaccines to use in the farm and discourage the use of just any IBD vaccine available. They are also to be educated on and monitored for the implementation of strict biosecurity measures to avoid IBDV outbreaks in their farms.
5. Indiscriminate movement and sales of sick/recovering birds especially during/after an outbreak should be discouraged.
6. Further studies are required to investigate the epidemiology and biological characteristics of reassortant strains so that the appropriate and safe IBDV vaccines can be recommended.

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APPENDICES

Appendix 1:Raw field data

S/NO	Sample code	State	Year	Specie of bird	Breed	Origin	Age	IBD vaccine used	Flock size
1	NIG1/NG/2009	Unknown	2009	Guinea fowl	N/A	Bursa	N/A	N/A	N/A
2	Plateau2/NG/2009	Plateau	2009	Pullet	Isa brown	Bursa	4 wks	V/N/A	N/A
3	Plateau3/NG/2009	Plateau	2009	Pullet	Isa brown	Bursa	4 wks	V/N/A	N/A
4	Plateau4/NG/2009	Plateau	2009	Pullet	N/A	Bursa	4 wks	V/N/A	N/A
5	Plateau5/NG/2009	Plateau	2009	Layer	N/A	Bursa	29 wks	V/N/A	N/A
6	Plateau6/NG/2009	Plateau	2009	Pullet	N/A	Bursa	4wks	V/N/A	50
7	Plateau7/NG/2009	Plateau	2009	Pullet	Isa brown	Bursa	12 wks	V/N/A	N/A
8	Plateau8/NG/2009	Plateau	2009	Pullets	N/A	Bursa	N/A	NVRI, Vom	50
9	Plateau9/NG/2009	Plateau	2009	Pullets	N/A	Bursa	N/A	NVRI, Vom	1,200
10	Plateau10/NG/2009	Plateau	2009	N/A	N/A	Bursa	N/A	N/A	N/A
11	Nig11/NG/2009	Unknown	2009	Pullet	N/A	Bursa	N/A	N/A	N/A
12	Plateau12/NG/2009	Plateau	2009	Pullets	Isa brown	Bursa	12 wks	V/N/A	120
13	Plateau13/NG/2009	Plateau	2009	Pullets	N/A	Bursa	12wk	N/A	N/A
14	Plateau14/NG/2009	Plateau	2009	Pullets	Isa brown	Bursa	N/A	V/N/A	1,050
15	Plateau15/NG/2009	Plateau	2009	Pullets	N/A	Bursa	9 wks	N/A	N/A
16	Plateau16/NG/2009	Plateau	2009	Pullets	N/A	Bursa	9 wks	NVRI Vom	101
17	Plateau17/NG/2009	Plateau	2009	Pullets	N/A	Bursa	19 wks	N/A	N/A
18	Plateau18/NG/2009	Plateau	2009	N/A	N/A	Bursa	N/A	N/A	N/A
19	Plateau19/NG/2009	Plateau	2009	Broilers	N/A	Bursa	4 wks	N/A	303
20	Nig20/NG/2009	Unknown	2009	N/A	N/A	Bursa	N/A	N/A	N/A
21	Nig21/NG/2009	Unknown	2009	N/A	N/A	Bursa	N/A	N/A	N/A
22	Nig22/NG/2009	Unknown	2009	N/A	N/A	Bursa	N/A	N/A	N/A
23	Plateau23/NG/2009	Plateau	2009	Pullets	N/A	Bursa	N/A	N/A	950
24	Plateau24/NG/2009	Plateau	2009	Pullets	N/A	Bursa	N/A	V/N/A	715
25	Nig25/NG/2009	Unknown	2009	N/A	N/A	Bursa	N/A	N/A	N/A
26	Nig26/NG/2009	Unknown	2009	N/A	N/A	Bursa	N/A	N/A	N/A
27	Plateau27/NG/2009	Plateau	2009	N/A	N/A	Bursa	N/A	N/A	N/A
28	Nig28/NG/2009	Unknown	2009	N/A	N/A	Bursa	N/A	N/A	N/A
29	Bauchi29/NG/2012	Bauchi	2012	Pullets	Isa brown	Bursa	N/A	V/N/A	2,498
30	Plateau30/NG/2012	Plateau	2012	Pullets	N/A	Bursa	N/A	Not vaccinated	60
31	Nig31/NG/2009	Unknown	2009	N/A	N/A	Bursa	N/A	N/A	N/A
32	Plateau32/NG/2012	Plateau	2012	Pullets	Isa brown	Bursa	N/A	V/N/A	2,000
33	Plateau33/NG/2012	Plateau	2012	Cockerel	N/A	Bursa	8 wks	N/A	170
34	Benin34/NG/2012	Edo	2012	Pullet	N/A	Bursa	8wks	V/N/A	N/A
35	Benin35/NG/2012	Edo	2012	Pullet	N/A	Bursa	10 wks	V/N/A	N/A
36	Bauchi36/NG/2012	Bauchi	2012	Pullet	N/A	Bursa	N/A	N/A	N/A
37	Bauchi37/NG/2012	Bauchi	2012	Pullet	N/A	Bursa	N/A	N/A	N/A
38	Bauchi38/NG/2012	Bauchi	2012	Pullet	N/A	Bursa	N/A	N/A	N/A
39	Plateau39/NG/2012	Plateau	2012	N/A	N/A	Bursa	N/A	N/A	N/A
40	Plateau40/NG/2012	Plateau	2012	Pullets	N/A	Bursa	8 wks	V/N/A	1,097
41	Kaduna41/NG/2012	Kaduna	2012	Pullet	N/A	Bursa	4 wks	Izovac	N/A
42	Plateau42/NG/2012	Plateau	2012	Pullets	Black harco	Bursa	3 wks	Biovac (Israel)	700
43	Plateau43/NG/2012	Plateau	2012	Pullets	Black harco	Bursa	5 wks	Ornibur (Czech)	500
44	Benin44/NG/2012	Edo	2012	N/A	N/A	Bursa	N/A	N/A	N/A
45	Benin45/NG/2012	Edo	2012	N/A	N/A	Bursa	5 wks	Abic	200
46	Benin46/NG/2012	Edo	2012	Broiler	N/A	Bursa	8 wks	Vom	150
47	Benin47/NG/2012	Edo	2012	Pullet	N/A	Bursa	7 wk	Abic	100
48	Benin48/NG/2012	Edo	2012	Pullet	N/A	Bursa	5 wks	Vom	100
49	Benin49/NG/2012	Edo	2012	Pullet	Black harco	Bursa	8 wks	Bur 706 & Vom	600

50	Anambra50/NG/2012	Anambra	2012	Pullet	Isa brown	Bursa	8 wks	Indovac (India)	4,200
51	Bauchi51/NG/2012	Bauchi	2012	Pullets	N/A	Bursa	N/A	N/A	N/A
52	Bauchi52/NG/2012	Bauchi	2012	Pullets	N/A	Bursa	N/A	N/A	N/A
53	Plateau53/NG/2010	Plateau	2010	Pullets	N/A	Bursa	N/A	N/A	N/A
54	Anambra54/NG/2012	Anambra	2012	Pullets	Isa brown	Bursa	7 wks	Not vaccinated	100
55	Anambra55/NG/2012	Anambra	2012	Pullets	Isa brown	Bursa	5 wks	N/A	N/A
56	Plateau56/NG/2012	Plateau	2012	N/A	N/A	Bursa	N/A	N/A	N/A
57	Plateau57/NG/2012	Plateau	2012	N/A	N/A	Bursa	N/A	N/A	N/A
58	Bauchi58/NG/2012	Bauchi	2012	Cockerel	N/A	Bursa	7 wks	V/N/A	170
59	Bauchi59/NG/2012	Bauchi	2012	Pullets	Isa brown	Bursa	4 wks	Ornibur	5,000
60	Plateau60/NG/2012	Plateau	2012	broilers		Bursa	4 wks	N/A	150
61	Kaduna61/NG/2012	Kaduna	2012	Pullets	Isa brown	Bursa	5 wks	Georgia strain	79
62	Kaduna62/NG/2012	Kaduna	2012	Pullets/Broilers	N/A	Bursa	5 wks	N/A	500
63	Plateau63/NG/2012	Plateau	2012	Pullets	N/A	Bursa	N/A	N/A	N/A
64	Nig64/NG/2011	Unknown	2011	N/A	N/A	Bursa	N/A	N/A	N/A
65	Nig65/NG/2011	Unknown	2011	N/A	N/A	Bursa	N/A	N/A	N/A
66	Nig66/NG/2011	Unknown	2011	N/A	N/A	Bursa	N/A	N/A	N/A
67	Plateau67/NG/2011	Plateau	2011	Broiler	N/A	Bursa	4 wks	Ornibur	N/A
68	Plateau68/NG/2011	Plateau	2011	Pullets	N/A	Bursa	6 wks	N/A	N/A
69	Plateau69/NG/2011	Plateau	2011	Broilers	N/A	Bursa	8 wks	N/A	N/A
70	Plateau70/NG/2011	Plateau	2011	Broilers	N/A	Bursa	8 wks	N/A	N/A
71	Plateau71/NG/2011	Plateau	2011	Broilers	N/A	Bursa	6 wks	N/A	N/A
72	Plateau72/NG/2011	Plateau	2011	Pullets	N/A	Bursa	12 wks	Ornibur	N/A
73	Plateau73/NG/2011	Plateau	2011	Pullets	N/A	Bursa	3 wks	Ornibur	N/A
74	Plateau74/NG/2011	Plateau	2011	Broilers	N/A	Bursa	5 wks	Ornibur	N/A
75	Plateau75/NG/2011	Plateau	2011	Broilers	N/A	Bursa	6 wks	Ornibur	N/A
76	Plateau76/NG/2011	Plateau	2011	Pullets	N/A	Bursa	9 wks	Not vaccinated	N/A
77	Nassarawa77/NG/2013	Nassarawa	2013	Pullets	Isa brown	Bursa	5wks	V/N/A	2,000
78	Plateau Vom Vaccine	Plateau	2013	N/A	N/A	Bursa	N/A	N/A	N/A
79	Nig79/NG/2009	Unknown	2009	N/A	N/A	Bursa	N/A	N/A	N/A
80	Plateau80/NG/2013	Plateau	2013	Cockerels	N/A	Bursa	12 wks	Not vaccinated	200
81	Plateau81/NG/2013	Plateau	2013	Pullets	Isa brown	Bursa	6 wks	V/N/A	300
82	Plateau82/NG/2013	Plateau	2013	Pullets	Isa brown	Bursa	6 wks	V/N/A	300
83	Plateau83/Ng/2013	Plateau	2013	Pullets	Isa brown	Bursa	4 wks	Ornibus intermediate	3175
84	Plateau84/NG/2013	Plateau	2013	Pullets	Isa brown	Bursa	20 wks	N/A	N/A
85	Plateau85/NG/2013	Plateau	2013	Pullets	N/A	Bursa	6 wks	V/N/A	800
86	Kaduna86/NG/2013	Kaduna	2013	Pullets	Isa brown	Bursa	3 wks	Izovac	2000
87	Benin87/NG/2013	Edo	2013	Cockerel	N/A	Bursa	12 wks	Not vaccinated	98
88	Benin88/NG/2013	Edo	2013	Cockerel	N/A	Bursa	5 wks	Biovac	200
89	Benin89/NG/2013	Edo	2013	Pullets	Isa brown	Bursa	8 wks	Foreign vaccine	3,000
90	Plateau90/NG/2013	Plateau	2013	Pullets	Isa brown	Bursa	9 wks	Ornibus intermediate	30
91	Plateau91/NG/2013	Plateau	2013	Pullets	Isa brown	Bursa	6 wks	Abic	600
92	Plateau92/NG/2013	Plateau	2013	Pullets	Isa brown	Bursa	N/A	Izovac	N/A
93	Abuja93/NG/2013	Abuja	2013	Pullets	Isa brown &black	Bursa	9 wks	V/N/A	3,000
94	Anambra94/NG/2013	Anambra	2013	Pullets	Isa brown	Bursa	8wks	V/N/A	N/A
95	Plateau95/NG/2013	Plateau	2013	Pullets	Black harco	Bursa	N/A	N/A	N/A
96	Plateau96/NG/2013	Plateau	2013	Pullet	Isa brown	Bursa	5 wks	N/A	N/A
97	Plateau97/NG/2011	Plateau	2011	Pullets	N/A	Bursa	N/A	Ornibus	350
98	Plateau98/NG/2011	Plateau	2011	Pullets	N/A	Bursa	N/A	N/A	550
99	Plateau99/NG/2011	Plateau	2011	Broilers	N/A	Bursa	N/A	V/N/A	100
100	Nig100/NG/2009	Unknown	2009	N/A	N/A	Bursa	N/A	N/A	n/a
101	Kaduna101/NG/2010	Kaduna	2010	Pullets	N/A	Bursa	N/A	V/N/A	300
102	Nig102/NG/2009	Unknown	2009	N/A	N/A	Bursa	N/A	N/A	N/A

103	Plateau103/NG/2010	Plateau	2010	Pullets	N/A	Bursa	6 wks	V/N/A	550
104	Plateau104/NG/2010	Plateau	2010	Layers	N/A	Bursa	18 wks	V/N/A	63
105	Plateau105/NG/2009	Plateau	2009	Pullets	N/A	Bursa	N/A	N/A	n/a
106	Plateau106/NG/2009	Plateau	2009	Broilers	N/A	Bursa	5 wks	V/N/A	61
107	Abuja107/NG/2010	Abuja	2010	Pullets	N/A	Bursa	N/A	V/N/A	3652
108	Plateau108/NG/2009	Plateau	2009	Broiler	N/A	Bursa	6 wks	V/N/A	13
109	Plateau109/NG/2010	Plateau	2010	Pullets	N/A	Bursa	5 wks	V/N/A	60
110	Plateau110/NG/2010	Plateau	2010	Pullets	N/A	Bursa	5 wks	V/N/A	300
111	Abuja111/NG/2010	Abuja	2010	N/A	N/A	Bursa	N/A	V/N/A	3,652
112	Plateau112/NG/2010	Plateau	2010	Pullets	Isa brown	Bursa	5 wks	V/N/A	2,000
113	Plateau113/NG/2009	Plateau	2009	Pullets	N/A	Bursa	N/A	NVRI, Vom	664
114	Plateau114/NG/2009	Plateau	2009	Grower	N/A	Bursa	13 wks	V/N/A	720
115	Plateau115/NG/2009	Plateau	2009	Layers	Isa brown	Bursa	24 wks	V/N/A	N/A
116	Plateau11/NG/2009	Plateau	2009	Broilers	N/A	Bursa	5 wks	V/N/A	N/A
117	Nassarawa117/NG/2009	Nassarawa	2009	Pullets	N/A	Bursa	14 wks	V/N/A	700
118	Plateau118/NG/2009	Plateau	2009	Layers	N/A	Bursa	N/A	N/A	N/A
119	Plateau119/NG/2013	Plateau	2013	Cockerels	N/A	Bursa	5 wks	Not vaccinated	200
120	Plateau120/NG/2013	Plateau	2013	Pullets	Isa brown	Bursa	4 wks	Izovac	1,000
121	Bauchi121/NG/2013	Bauchi	2013	Cockerels	N/A	Bursa	5 wks	N/A	N/A
122	Bauchi122/NG/2014	Bauchi	2014	Pullets	N/A	Bursa	N/A	N/A	N/A
123	Bauchi123/NG/2014	Bauchi	2014	Pullets	N/A	Bursa	N/A	N/A	N/A
124	Bauchi124/NG/2014	Bauchi	2014	Pullets	N/A	Bursa	N/A	N/A	N/A
125	Bauchi125/NG/2014	Bauchi	2014	Pullets	N/A	Bursa	N/A	N/A	N/A
126	Bauchi126/NG/2014	Bauchi	2014	Pullets	N/A	Bursa	N/A	N/A	N/A
127	Bauchi127/NG/2014	Bauchi	2014	Pullets	N/A	Bursa	N/A	N/A	N/A
128	Bauchi128/NG/2014	Bauchi	2014	Pullets	N/A	Bursa	N/A	N/A	N/A
129	Bauchi129/NG/2014	Bauchi	2014	Broilers	N/A	Bursa	3 wks	N/A	70
130	Bauchi130/NG/2014	Bauchi	2014	Broilers	N/A	Bursa	3 wks	Foreign vaccine	500
131	Plateau131/NG/2014	Plateau	2014	Broilers	N/A	Bursa	4 wks	NVRI vaccine	N/A
132	Plateau132/NG/2014	Plateau	2014	Pullets	N/A	Bursa	11 wks	V/N/A	N/A
133	Plateau133/NG/2014	Plateau	2014	Broiler	N/A	Bursa	4 wks	N/A	N/A
134	Plateau134/NG/2014	Plateau	2014	Pullets	White eggers	Bursa	6-7 wks	Not vaccinated	60
135	Plateau135/NG/2014	Plateau	2014	Pullets	Isa brown	Bursa	4 wks	V/N/A	52
136	Kaduna136/NG/2014	Kaduna	2014	Pullets	Isa brown	Bursa	4 wks	Not vaccinated	200
137	Kwara137/NG/2014	Kwara	2014	Pullets	Isa brown	Bursa	4 wks	FVN/A	3,000
138	Kwara138/NG/2014	Kwara	2014	Turkey	N/A	Bursa	3 wks	N/A	30
139	Kwara139/NG/2014	Kwara	2014	Pullets	Black	Bursa	6 wks	FV/N/A	99
140	Kwara140/NG/2014	Kwara	2014	Broiler	N/A	Bursa	4 wks	V/N/A	100
141	Kwara141/NG/2014	Kwara	2014	Pullets	Black harco	Bursa	3 wks	NVRI Vom	460
142	Benin142E/NG/2013	Edo	2014	Embryo passage	-	Embryo	-	-	-
143	Kwara143/NG/2014	Kwara	2014	Pullets	Isa brown	Bursa	4 wks	FV/N/A	60
144	Kwara144/NG/2014	Kwara	2014	Broiler	N/A	Bursa	7 wks	V/N/A	25
145	Kwara145/NG/2014	Kwara	2014	Pullets	Isa brown	Bursa	7 wks	FV/N/A	600
146	Kwara146/NG/2014	Kwara	2014	Pullets	Black harco	Bursa	4 wks	FV/N/A	60
147	Kwara147/NG/2014	Kwara	2014	Pullets	Isa brown	Bursa	5 wks	FV/N/A	300
148	Kwara148/NG/2014	Kwara	2014	Pullets	Isa brown	Bursa	15 wks	FV/N/A	100
149	Kwara149/NG/2014	Kwara	2014	Broilers	N/A	Bursa	7 wks	V/N/A	1,000
150	Kwara150/NG/2014	Kwara	2014	Broilers	N/A	Bursa	5 wks	V/N/A	150
151	Kwara151/NG/2014	Kwara	2014	Broilers	N/A	Bursa	4 wks	V/N/A	100
152	Kwara152/NG/2014	Kwara	2014	Pullets	Black harco	Bursa	4 wks	FV/N/A	500
153	Nassarawa153/NG/2014	Nassarawa	2014	Pullets	N/A	Bursa	N/A	N/A	N/A
154	Plateau154/NG/2014	Plateau	2014	Pullets	N/A	Bursa	3 wks	V/N/A	350
155	Plateau155/NG/2014	Plateau	2014	Broilers	N/A	Bursa	4 wks	N/A	254
156	Plateau156/NG/2014	Plateau	2014	Broiler	N/A	Bursa	N/A	N/A	N/A
157	Plateau157/NG/2014	Plateau	2014	Broiler	N/A	Bursa	N/A	N/A	N/A
158	Plateau158/NG/2014	Plateau	2014	Pullet	Isa brown	Bursa	N/A	N/A	N/A

159	Plateau159/NG/2014	Plateau	2014	Pullet	Isa brown	Bursa	N/A	N/A	N/A
160	Plateau160/NG/2014	Plateau	2014	Broiler	N/A	Bursa	N/A	N/A	N/A
161	Plateau161/NG/2014	Plateau	2014	Broiler	N/A	Bursa	N/A	N/A	N/A
162	Plateau162/NG/2014	Plateau	2014	N/A	N/A	Bursa	N/A	N/A	N/A
163	Plateau163/NG/2014	Plateau	2014	N/A	N/A	Bursa	N/A	N/A	N/A
164	Plateau164/NG/2014	Plateau	2014	N/A	N/A	Bursa	N/A	N/A	N/A
165	Plateau165/NG/2014	Plateau	2014	N/A	N/A	Bursa	N/A	N/A	N/A
166	Uyo166/NG/2014	Akwa Ibom	2014	Pullets	Isa brown	Bursa	6wks	FV/N/A	10,000
167	Uyo167/NG/2014	Akwa Ibom	2014	Pullets	Isa brown	Bursa	6wks	FV/N/A	10,000
168	Nassarawa168/NG/2014	Nassarawa	2014	Pullets	N/A	Bursa	N/A	N/A	N/A
169	Nassarawa169/NG/2014	Nassarawa	2014	Pullets	N/A	Bursa	N/A	N/A	N/A
170	Kwara170/NG/2014	Kwara	2014	Pullets	Isa brown	Bursa	3 wks	FV/N/A	850
171	Kwara171/NG/2014	Kwara	2014	Broilers	N/A	Bursa	5 wks	N/A	196
172	Kwara172/NG/2014	Kwara	2014	Pullets	Isa brown	Bursa	1 wk	FV/N/A	200
173	Kwara173/NG/2014	Kwara	2014	Pullets	Isa brown	Bursa	3 wks	FV/N/A	200
174	Kwara174/NG/2014	Kwara	2014	Pullets	Isa brown	Bursa	4 wks	FV/N/A	1,380
175	Kwara175/NG/2014	Kwara	2014	Pullets	Isa brown	Bursa	6 wks	N/A	100
176	Kwara176/NG/2014	Kwara	2014	Cockerel	N/A	Bursa	5 wks	V/N/A	1,380
177	Kwara177/NG/2014	Kwara	2014	Cockerel	N/A	Bursa	4 wks	V/N/A	1,380
178	Kwara178/NG/2014	Kwara	2014	Pullets	Isa brown	Bursa	5 wks	FV/N/A	500
179	Kwara179/NG/2014	Kwara	2014	Pullets	Isa brown	Bursa	4 wks	FV/N/A	500
180	Kwara180/NG/2014	Kwara	2014	Broilers	N/A	Bursa	5 wks	Not vaccinated	200
181	Kwara181/NG/2014	Kwara	2014	Cockerel	N/A	Bursa	7 wks	N/A	100
182	Kwara182/NG/2014	Kwara	2014	Cockerel	N/A	Bursa	12 wks	V/N/A	200
183	Kwara183/NG/2014	Kwara	2014	Pullets	Isa brown	Bursa	5 wks	V/N/A	500
184	Kwara184/NG/2014	Kwara	2014	Pullets	Isa brown	Bursa	9 wks	FV/N/A	200
185	Uyo185/NG/2014	Akwa Ibom	2014	Broilers	N/A	Bursa	4wks	FV/N/A	6,000
186	Uyo186/NG/2014	Akwa Ibom	2014	Broilers	N/A	Bursa	4wks	FV/N/A	6,000
187	Uyo187/NG/2014	Akwa Ibom	2014	Broilers	N/A	Bursa	4wks	FV/N/A	6,000
188	Uyo188/NG/2014	Akwa Ibom	2014	Broilers	N/A	Bursa	4wks	FV/N/A	6,000
189	Uyo189/NG/2014	Akwa Ibom	2014	Broilers	N/A	Bursa	4wks	FV/N/A	6,000
190	Uyo190/NG/2014	Akwa Ibom	2014	Broilers	N/A	Bursa	4wks	FV/N/A	6,000
191	Uyo191/NG/2014	Akwa Ibom	2014	Broilers	N/A	Bursa	4wks	FV/N/A	6,000
192	Uyo192/NG/2014	Akwa Ibom	2014	Broilers	N/A	Bursa	4wks	FV/N/A	6,000
193	Uyo193/NG/2014	Akwa Ibom	2014	Broilers	N/A	Bursa	4wks	FV/N/A	6,000
194	Uyo194/NG/2014	Akwa Ibom	2014	Broilers	N/A	Bursa	4wks	FV/N/A	6,000
195	Uyo195/NG/2014	Akwa Ibom	2014	Broilers	N/A	Bursa	4wks	FV/N/A	6,000
196	Uyo196/NG/2014	Akwa Ibom	2014	Broilers	N/A	Bursa	4wks	FV/N/A	6,000
197	Uyo197/NG/2014	Akwa Ibom	2014	Broilers	N/A	Bursa	4wks	FV/N/A	6,000
198	Uyo198/NG/2014	Akwa Ibom	2014	Broilers	N/A	Bursa	4wks	FV/N/A	6,000
199	Uyo199/NG/2014	Akwa Ibom	2014	Broilers	N/A	Bursa	4wks	FV/N/A	6,000
200	Uyo200/NG/2014	Akwa Ibom	2014	Broilers	N/A	Bursa	4wks	FV/N/A	6,000
201	Uyo201/NG/2014	Akwa Ibom	2014	Broilers	N/A	Bursa	4wks	FV/N/A	6,000
202	Uyo202/NG/2014	Akwa Ibom	2014	Broilers	N/A	Bursa	4wks	FV/N/A	6,000
203	Uyo203/NG/2014	Akwa Ibom	2014	Broilers	N/A	Bursa	4wks	FV/N/A	6,000
204	Uyo204/NG/2014	Akwa Ibom	2014	Broilers	N/A	Bursa	4wks	FV/N/A	6,000
205	Uyo205/NG/2014	Akwa Ibom	2014	Broilers	N/A	Bursa	4wks	FV/N/A	6,000
206	Uyo206/NG/2014	Akwa Ibom	2014	Broilers	N/A	Bursa	4wks	FV/N/A	6,000
207	Uyo207/NG/2014	Akwa Ibom	2014	Broilers	N/A	Bursa	4wks	FV/N/A	6,000
208	Uyo208/NG/2014	Akwa Ibom	2014	Broilers	N/A	Bursa	4wks	FV/N/A	6,000
209	CNT209/NG/2013	n/a	2013	n/a	-	CEF	n/a	n/a	-
210	CNT210/NG/2013	n/a	2013	n/a	-	CEF	n/a	n/a	-
211	Nassarawa211T/NG/2013	Nassarawa	2013	n/a	-	CEF	n/a	n/a	-
212	Bauchi212T/NG/2012	Bauchi	2012	n/a	-	CEF	n/a	n/a	-
213	Kaduna213E/NG/2012	Kaduna	2012	n/a	-	Embryo	n/a	n/a	-
214	Kaduna214E/NG/2013	Kaduna	2013	n/a	-	Embryo	n/a	n/a	-

215	Bauchi215E/NG/2012	Bauchi	2012	n/a	-	Embryo	n/a	n/a	-
216	Plateau216E/NG/2013	Plateau	2013	n/a	-	Embryo	n/a	n/a	-

Note: N/A- not available, n/a- not applicable, CEF- chicken embryo fibroblast, FV/N/A= Foreign IBD vaccine used but name not available , V/N/A= Vaccinated but whether foreign or local vaccine used not known.

Appendix 2: AGAR GEL IMMUNODIFFUSSION (AGID) AND RT-PCR RESULTS FOR SEGMENT A

S/NO	SAMPLE CODE	AGID	RT-PCR
1	NIG1/NG/2009	-ve	-ve
2	PLATEAU2/NG/2009	+ve	F+ve
3	PLATEAU3/NG/2009	+ve	+ve
4	PLATEAU4/NG/2009	+ve	F+ve
5	PLATEAU5/NG/2009	-ve	-ve
6	PLATEAU6/NG/2009	+ve	+ve
7	PLATEAU7/NG/2009	+ve	+ve
8	PLATEAU8/NG/2009	+ve	+ve
9	PLATEAU9/NG/2009	-ve	-ve
10	PLATEAU10/NG/2009	+ve	-ve
11	NIG11/NG/2009	-ve	-ve
12	PLATEAU12/NG/2009	+ve	+ve
13	PLATEAU13/NG/2009	+ve	F+ve
14	PLATEAU14/NG/2009	-ve	-ve
15	PLATEAU15/NG/2009	+ve	F+ve
16	PLATEAU16/NG/2009	+ve	+ve
17	PLATEAU17/NG/2009	-ve	-ve
18	PLATEAU18/NG/2009	-ve	-ve
19	PLATEAU19/NG/2009	+ve	+ve
20	NIG20/NG/2009	+ve	+ve
21	NIG21/NG/2009	-ve	-ve
22	NIG22/NG/2009	-ve	-ve
23	PLATEAU23/NG/2009	-ve	-ve
24	PLATEAU24/NG/2009	-ve	-ve
25	NIG25/NG/2009	-ve	-ve
26	NIG26/NG/2009	-ve	-ve
27	PLATEAU27/NG/2009	+ve	-ve
28	NIG28/NG/2009	-ve	-ve
29	BAUCHI29/NG/2012	-ve	-ve
30	PLATEAU30/NG/2012	+ve	F+ve
31	NIG31/NG/2009	-ve	-ve
32	PLATEAU32/NG/2012	+ve	+ve
33	PLATEAU33/NG/2012	+ve	f+ve
34	BENIN34/NG/2012	+ve	+ve
35	BENIN35/NG/2012	+ve	+ve
36	BAUCHI36/NG/2012	+ve	+ve
37	BAUCHI37/NG/2012	+ve	+ve
38	BAUCHI38/NG/2012	+ve	+ve
39	PLATEAU39/NG/2012	+ve	+ve
40	PLATEAU40/NG/2012	-ve	+ve
41	KADUNA41/NG/2012	-ve	-ve
42	PLATEAU42/NG/2012	+ve	+ve

43	PLATEAU43/NG/2012	+ve	+ve
44	BENIN44/NG/2012	-ve	-ve
45	BENIN45/NG/2012	-ve	-ve
46	BENIN46/NG/2012	+ve	+ve
47	BENIN47/NG/2012	-ve	+ve
48	BENIN48/NG/2012	+ve	-ve
49	BENIN49/NG/2012	-ve	-ve
50	ANAMBRA50/NG/2012	+ve	F+ve
51	BAUCHI51/NG/2012	+ve	-ve
52	BAUCHI52/NG/2012	+ve	-ve
53	PLATEAU53/NG/2010	+ve	+ve
54	ANAMBRA54/NG/2012	+ve	+ve
55	ANAMBRA55/NG/2012	+ve	-ve
56	PLATEAU56/NG/2012	+ve	+ve
57	PLATEAU57/NG/2012	+ve	-ve
58	BAUCHI58/NG/2012	+ve	+ve
59	BAUCHI59/NG/2012	+ve	+ve
60	PLATEAU60/NG/2012	-ve	-ve
61	KADUNA61/NG/2012	+ve	+ve
62	KADUNA62/NG/2012	+ve	+ve
63	PLATEAU63/NG/2012	-ve	-ve
64	NIG64/NG/2011	nt	+ve
65	NIG65/NG/2011	nt	-ve
66	NIG66/NG/2011	+ve	+ve
67	PLATEAU67/NG/2011	+ve	+ve
68	PLATEAU68/NG/2011	-ve	+ve
69	PLATEAU69/NG/2011	+ve	+ve
70	PLATEAU70/NG/2011	+ve	+ve
71	PLATEAU71/NG/2011	+ve	+ve
72	PLATEAU72/NG/2011	+ve	-ve
73	PLATEAU73/NG/2011	+ve	+ve
74	PLATEAU74/NG/2011	+ve	F+ve
75	PLATEAU75/NG/2011	+ve	+ve
76	PLATEAU76/NG/2011	+ve	+ve
77	NASSARAWA77/NG/2013	+ve	+ve
78	PLATEAU VOM VACCINE	+ve	Ff+ve
79	NIG79/NG/2009	+ve	+ve
80	PLATEAU80/NG/2013	+ve	+ve
81	PLATEAU81/NG/2013	+ve	-ve
82	PLATEAU82/NG/2013	+ve	+ve
83	PLATEAU83/NG/2013	+ve	+ve
84	PLATEAU84/NG/2013	-ve	-ve
85	PLATEAU85/NG/2013	-ve	-ve
86	KADUNA86/NG/2013	+ve	-ve
87	BENIN87/NG/2013	+ve	F+ve
88	BENIN88/NG/2013	+ve	+ve

89	BENIN89/NG/2013	+ve	+ve
90	PLATEAU90/NG/2013	+ve	+ve
91	PLATEAU91/NG/2013	-ve	-ve
92	PLATEAU92/NG/2013	+ve	+ve
93	ABUJA93/NG/2013	+ve	+ve
94	ANAMBRA94/NG/2013	+ve	F+ve
95	PLATEAU95/NG/2013	+ve	+ve
96	PLATEAU96/NG/2013	+ve	+ve
97	PLATEAU97/NG/2011	-ve	-ve
98	PLATEAU98/NG/2011	-ve	-ve
99	PLATEAU99/NG/2011	+ve	+ve
100	NIG100/NG/2009	NT	-ve
101	KADUNA101/NG/2010	+ve	F+ve
102	NIG102/NG/2009	NT	-ve
103	PLATEAU103/NG/2010	+ve	-ve
104	PLATEAU104/NG/2010	-ve	+ve
105	PLATEAU105/NG/2009	+ve	Ff+ve
106	PLATEAU106/NG/2009	+ve	+ve
107	ABUJA107/NG/2010	-ve	-ve
108	PLATEAU108/NG/2009	-ve	-ve
109	PLATEAU109/NG/2010	-ve	-ve
110	PLATEAU110/NG/2010	+ve	+ve
111	ABUJA111/NG/2010	-ve	-ve
112	PLATEAU112/NG/2010	-ve	-ve
113	PLATEAU113/NG/2009	-ve	-ve
114	PLATEAU114/NG/2009	-ve	-ve
115	PLATEAU115/NG/2009	-ve	-ve
116	PLATEAU11/NG/2009	+ve	Ff+ve
117	NASSARAWA/117/2009	-ve	-ve
118	PLATEAU118/NG/2009	-ve	-ve
119	PLATEAU119/NG/2013	+ve	+ve
120	PLATEAU120/NG/2013	+ve	+ve
121	BAUCHI121/NG/2013	+ve	+ve
122	BAUCHI122/NG/2014	-ve	-ve
123	BAUCHI123/NG/2014	-ve	-ve
124	BAUCHI124/NG/2014	+ve	+ve
125	BAUCHI125/NG/2014	-ve	-ve
126	BAUCHI126/NG/2014	+ve	+ve
127	BAUCHI127/NG/2014	+ve	+ve
128	BAUCHI128/NG/2014	-ve	F+ve
129	BAUCHI129/NG/2014	+ve	-ve
130	BAUCHI130/NG/2014	-ve	-ve
131	PLATEAU131/NG/2014	+ve	+ve
132	PLATEAU132/NG/2014	+ve	+ve
133	PLATEAU133/NG/2014	+ve	+ve
134	PLATEAU134/NG/2014	+ve	+ve

135	PLATEAU135/NG/2014	NT	+ve
136	KADUNA136/NG/2014	NT	+ve
137	KWARA137/NG/2014	NT	-ve
138	KWARA138/NG/2014	NT	-ve
139	KWARA139/NG/2014	NT	+ve
140	KWARA140/NG/2014	NT	-ve
141	KWARA141/NG/2014	NT	+ve
142	BENIN142E/NG/2013	+ve	+ve
143	KWARA143/NG/2014	NT	F+ve
144	KWARA144/NG/2014	NT	F+ve
145	KWARA145/NG/2014	NT	F+ve
146	KWARA146/NG/2014	NT	+ve
147	KWARA147/NG/2014	NT	F+ve
148	KWARA148/NG/2014	NT	+ve
149	KWARA149/NG/2014	NT	+ve
150	KWARA150/NG/2014	NT	+ve
151	KWARA151/NG/2014	NT	+ve
152	KWARA152/NG/2014	NT	+ve
153	NASSARAWA153/NG/2014	NT	-ve
154	PLATEAU154/NG/2014	NT	+ve
155	PLATEAU155/NG/2014	NT	+ve
156	PLATEAU156/NG/2014	NT	+ve
157	PLATEAU157/NG/2014	NT	+ve
158	PLATEAU158/NG/2014	NT	+ve
159	PLATEAU159/NG/2014	NT	+ve
160	PLATEAU160/NG/2014	NT	+ve
161	PLATEAU161/NG/2014	NT	+ve
162	PLATEAU162/NG/2014	NT	+ve
163	PLATEAU163/NG/2014	NT	+ve
164	PLATEAU164/NG/2014	NT	+ve
165	PLATEAU165/NG/2014	NT	-ve
166	UYO166/NG/2014	NT	+ve
167	UYO167/NG/2014	NT	+ve
168	NASSARAWA168/NG/2014	NT	-ve
169	NASSARAWA169/NG/2014	NT	-ve
170	KWARA170/NG/2014	NT	-ve
171	KWARA171/NG/2014	NT	+ve
172	KWARA172/NG/2014	NT	F+ve
173	KWARA173/NG/2014	NT	-ve
174	KWARA174/NG/2014	NT	+ve
175	KWARA175/NG/2014	NT	+ve
176	KWARA176/NG/2014	NT	+ve
177	KWARA177/NG/2014	NT	+ve
178	KWARA178/NG/2014	NT	+ve
179	KWARA179/NG/2014	NT	+ve
180	KWARA180/NG/2014	NT	+ve

181	KWARA181/NG/2014	NT	+ve
182	KWARA182/NG/2014	NT	+ve
183	KWARA183/NG/2014	NT	F+ve
184	KWARA184/NG/2014	NT	+ve
185	UYO185/NG/2014	NT	+ve
186	UYO 186/NG/2014	NT	F+ve
187	UYO187/NG/2014	NT	+ve
188	UYO188/NG/2014	NT	+ve
189	UYO189/NG/2014	NT	+ve
190	UYO190/NG/2014	NT	+ve
191	UYO191/NG/2014	NT	+ve
192	UYO192/NG/2014	NT	+ve
193	UYO193/NG/2014	NT	+ve
194	UYO194/NG/2014	NT	+ve
195	UYO195/NG/2014	NT	+ve
196	UYO196/NG/2014	NT	+ve
197	UYO197/NG/2014	NT	+ve
198	UYO198/NG/2014	NT	+ve
199	UYO199/NG/2014	NT	+ve
200	UYO200/NG/2014	NT	+ve
201	UYO201/NG/2014	NT	+ve
202	UYO202/NG/2014	NT	+ve
203	UYO203/NG/2014	NT	+ve
204	UYO204/NG/2014	NT	+ve
205	UYO205/NG/2014	NT	+ve
206	UYO206/NG/2014	NT	+ve
207	UYO207/NG/2014	NT	+ve
208	UYO208/NG/2014	NT	+ve
209	CNT209/NG/2013	+ve	-ve
210	CNT210/NG/2013	+ve	-ve
211	NASSARAWA211T/NG/2013	+ve	-ve
212	BAUCHI212T/NG/2012	+ve	-ve
213	KADUNA213E/NG/2012	+ve	-ve
214	KADUNA214E/NG/2013	+ve	-ve
215	BAUCHI215E/NG/2012	+ve	-ve
216	PLATEAU216E/NG/2013	+ve	-ve

- Nt- not tested, +ve- positive, -ve- negative

Appendix 3: GenBank accession numbers for Nigerian infectious bursal disease virus isolates segment A VP2

S/NO	CODE	ACCESSION NUMBER
1	UYO206/NG/2014	KP152231
2	KWARA171/NG/2014	KP152232
3	KWARA174/NG/2014	KP152233
4	KWARA175/NG/2014	KP152234
5	KWARA176/NG/2014	KP152235
6	KWARA177/NG/2014	KP152236
7	KWARA178/NG/2014	KP152237
8	KWARA179/NG/2014	KP152238
9	KWARA180/NG/2014	KP152239
10	KWARA181/NG/2014	KP152240
11	KWARA182/NG/2014	KP152241
12	KWARA184/NG/2014	KP152242
13	UYO185/NG/2014	KP152243
14	UYO187/NG/2014	KP152244
15	UYO188/NG/2014	KP152245
16	UYO189/NG/2014	KP152246
17	UYO190/NG/2014	KP152247
18	UYO191/NG/2014	KP152248
19	UYO192/NG/2014	KP152249
20	UYO193/NG/2014	KP152250
21	UYO194/NG/2014	KP152251
22	UYO195/NG/2014	KP152252
23	UYO196/NG/2014	KP152253
24	UYO197/NG/2014	KP152254
25	UYO198/NG/2014	KP152255
26	UYO199/NG/2014	KP152256
27	UYO200/NG/2014	KP152257
28	UYO201/NG/2014	KP152258
29	UYO202/NG/2014	KP152259
30	UYO203/NG/2014	KP152260
31	UYO204/NG/2014	KP152261
32	BAUCHI124/NG/2014	KP152262
33	BAUCHI126/NG/2014	KP152263
34	BAUCHI127/NG/2014	KP152264
35	KADUNA136/NG/2014	KP152265
36	PLATEAU133/NG/2014	KP152266
37	PLATEAU135/NG/2014	KP152267
38	BAUCHI36/NG/2009	KP152268
39	BENIN87/NG/2013	KP152269
40	BENIN142E/NG/2013	KP152270
41	ABUJA93/NG/2013	KP152271
42	BAUCHI128/NG/2014	KP152272

43	PLATEAU99/NG/2011	KP152273
44	PLATEAU92/NG/2013	KP152274
45	PLATEAU83/NG/2013	KP152275
46	PLATEAU90/NG/2013	KP152276
47	PLATEAU95/NG/2013	KP152277
48	PLATEAU96/NG/2013	KP152278
49	PLATEAU120/NG/2013	KP152279
50	PLATEAU132/NG/2014	KP152280
51	PLATEAU131/NG/2014	KP152281
52	PLATEAU134/NG/2014	KP152282
53	PLATEAU110/NG/2010	KP152283
54	PLATEAU104/NG/2010	KP152284
55	BAUCHI121/NG/2013	KP152285
56	KWARA139/NG/2014	KP152286
57	PLATEAU40/NG/2012	KP152287
58	PLATEAU119/NG/2013	KP152288
59	KWARA141/NG/2014	KP152289
60	PLATEAU82/NG/2013	KP152290
61	BAUCHI37/NG/2009	KP152291
62	PLATEAU4/NG/2009	KP152292
63	PLATEAU43/NG/2012	KP152293
64	BENIN34/NG/2012	KP152294
65	ANAMBRA50/NG/2012	KP152295
66	NIG20/NG/2009	KP152296
67	BENIN47/NG/2014	KP152297
68	KWARA145/NG/2014	KP152298
69	KWARA147/NG/2014	KP152299
70	KWARA148/NG/2014	KP152300
71	KWARA149/NG/2014	KP152301
72	KWARA150/NG/2014	KP152302
73	KWARA151NG/NG/2014	KP152303
74	KWARA152/NG/2014	KP152304
75	PLATEAU7/NG/2009	KP152305
76	PLATEAU16/NG/2009	KP152306
77	PLATEAU75/NG/2011	KP152307
78	PLATEAU154/NG/2014	KP152308
79	PLATEAU155/NG/2014	KP152309
80	PLATEAU156/NG/2014	KP152310
81	PLATEAU157/NG/2014	KP152311
82	PLATEAU158/NG/2014	KP152312
83	PLATEAU159/NG/2014	KP152313
84	PLATEAU160/NG/2014	KP152314
85	PLATEAU161/NG/2014	KP152315
86	PLATEAU162/NG/2014	KP152316
87	PLATEAU163/NG/2014	KP152317
88	PLATEAU164/NG/2014	KP152318

89	UYO166/NG/2014	KP152319
90	UYO167/NG/2014	KP152320
91	NIG79/NG/2009	KP152321
92	UYO205/NG/2014	KP152322
93	PLATEAU19/NG/2009	KP266322
94	KADUNA61/NG/2012	KP266323
95	BAUCHI58/NG/2012	KP266324
96	PLATEAU67/NG/2010	KP266325
97	PLATEAU53/NG/2010	KP266326
98	ANAMBRA54/NG/2012	KP266327
99	BENIN35/NG/2012	KP266328
100	BAUCHI37/NG/2012	KP266329
101	NASSARAWA77/NG/no2/2013	KP266330
102	NASSARAWA77/NG/2013	KP266331
103	PLATEAU78/NG/2011/2	KP266332
104	PLATEAU78/NG/2011/1	KP266333
105	PLATEAU39/NG/2012	KP266334

Appendix 4: Comparison of amino acid substitutions at different positions between Nigerian field isolates and other published strains of IBDV for segment A

STRAIN/ISOLATE	AMINO ACID SUBSTITUTION AT POSITIONS															
	219	222	242	253	254	256	261	269	272	279	284	294	299	300	315	330
vvIBDV_UK_661	Q	A	I	Q	G	I	I	T	I	D	A	I	S	E	S	S
NIG20/NG/2009	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA174/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU43/NG/2012	.	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
NIG79/NG/2009	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO167/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO166/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU164/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU163/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU162/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU161/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU160/NG/2014	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
PLATEAU159/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU158/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU157/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU156/NG/2014	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
PLATEAU155/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU154/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU16/NG/2009	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU4/NG/2009	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA152/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA151NG/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA150/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA149/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA148/NG/2014	.	.	.	.	.	.	.	.	T	N	.	.	.	.	.	.
KWARA147/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA145/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
BENIN47/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
BAUCHI37/NG/2012	.	T	.	.	S	.	.	.	.	.	.	.	.	A	.	.
BAUCHI36/NG/2009	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU19/NG/2009	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KADUNA61/NG/2009	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU39/NG/2012	.	.	.	.	S	.	.		.	.	.	.	.	A	.	.
BAUCHI58/NG/2012	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
PLATEAU67/NG/2011	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU53/NG/2010	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
ANAMBRA54/NG/2012	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
PLATEAU7/NG/2009	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
BENIN35/NG/2012	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
BAUCHI37/NG/2009	.	T	.	.	S	.	.	.	.	.	.	.	.	A	.	.
BAUCHI124/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
BAUCHI126/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
BAUCHI127/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KADUNA136/NG/2014	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
PLATEAU133/NG/2014	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
PLATEAU135/NG/2014	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
NASSARAWA77/NG/no2/_2013	.	.	V	H	S	V	.	.	.	N	T	L	N	.	.	.
NASSARAWA77/NG/2013	.	.	.	L	S	.	.	S	.	.	.	.	.	Q	.	R
PLATEAU78/NG/2011/2	P	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	R
PLATEAU78/ NG/2011/1	T	.	.	L	S	.	.	S	.	.	.	.	.	Q	.	.
BENIN142E/NG/2013	.	.	.	.	.	.	.	.	T	N	.	.	.	.	.	.

BENIN87/NG/2013	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
ABUJA93/NG/2013	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
BAUCHI128/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU99/NG/2011	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU92/NG/2013	.	.	.	.	S	.	T	.	.	.	.	.	.	A	.	.
PLATEAU83/NG/2013	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU90/NG/2013	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU95/NG/2013	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU96/NG/2013	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU131/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU134/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU110/NG/2010	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU104/NG/2010	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
BAUCHI121/NG/2013	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA139/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
PLATEAU40/NG/2012	.	.	.	.	.	.	.	.	T	N	.	.	.	.	.	.
PLATEAU119/NG/2013	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
KWARA141/NG/2014	.	.	.	.	.	.	.	.	T	N	.	.	.	.	.	.
PLATEAU120/NG/2013	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
PLATEAU132/NG/2014	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
PLATEAU82/NG/2013	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
PLATEAU75/NG/2011	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
ANAMBRA50/NG/2012	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
BENIN34/NG/2012	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA175/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA176/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	T	.
KWARA177/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA178/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	T	.
KWARA179/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA180/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA181/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA182/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA184/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO185/NG/2014	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
UYO187/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO188/NG/2014	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
UYO189/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO190/NG/2014	T	.	.	.	S	.	.	S	.	.	.	.	.	Q	.	.
UYO191/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO192/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO193/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO194/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO195/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO196/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO197/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO198/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO199/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO200/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO201/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO202/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO203/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO204/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO205/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
UYO206/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
KWARA171/NG/2014	.	.	.	.	S	.	.	.	.	.	.	.	.	A	.	.
VVIBDV OKYM	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
VARIANT DEL E	.	T	V	.	S	V	.	.	.	N	.	L	N	.	.	.

VARIANT T1	.	T	V	.	N	V	.	.	.	N	.	L	N	.	.	.
CLASSIC STC	.	P	V	.	.	V	.	.	.	.	.	L	N	.	.	.

- Amino acid sequences of Nigerian IBDV segment A viruses and published strains of IBDV segment A were compared with UK661 strain sequence. Dots indicate sequences identical to those of the UK661 strain.

Appendix 5: GenBank accession numbers for Nigerian infectious bursal disease virus isolates segment B

S/NO	CODE	ACCESSION NUMBER
1	ABUJA93/NG/2013	KP172084
2	BAUCHI121/NG/2013	KP172085
3	BAUCHI124/NG/2014	KP172086
4	BAUCHI126/NG/2014	KP172087
5	KWARA171/NG/2014	KP172088
6	KWARA175/NG/2014	KP172089
7	KWARA177/NG/2014	KP172090
8	KWARA178/NG/2014	KP172091
9	KWARA180/NG/2014	KP172092
10	KWARA181/NG/2014	KP172093
11	UYO187/NG/2014	KP172094
12	UYO189/NG/2014	KP172095
13	UYO190/NG/2014	KP172096
14	UYO191/NG/2014	KP172097
15	UYO192/NG/2014	KP172098
16	UYO193/NG/2014	KP172099
17	UYO194/NG/2014	KP172100
18	UYO195/NG/2014	KP172101
19	UYO196/NG/2014	KP172102
20	UYO197/NG/2014	KP172103
21	UYO198/NG/2014	KP172104
22	UYO200/NG/2014	KP172105
23	UYO202/NG/2014	KP172106
24	UYO203/NG/2014	KP172107
25	UYO207/NG/2014	KP172108
26	KWARA184/NG/2014	KP172109
27	UYO188/NG/2014	KP172110
28	BAUCHI128/NG/2014	KP172111
29	BENIN87/NG/2013	KP172112
30	KADUNA136/NG/2014	KP172113
31	KWARA141/NG/2014	KP172114
32	PLATEAU134/NG/2014	KP172115
33	PLATEAU40/NG/2012	KP172116
34	PLATEAU82/NG/2013	KP172117
35	PLATEAU83/NG/2013	KP172118
36	PLATEAU90/NG/2013	KP172119
37	PLATEAU92/NG/2013	KP172120
38	PLATEAU95/NG/2013	KP172121
39	PLATAEU96/NG/2013	KP172122
40	PLATEAU104/NG/2010	KP172123
41	PLATEAU110/NG/2010	KP172124
42	PLATEAU119/NG/2013	KP172125

43	PLATEAU154/NG/2014	KP172126
44	PLATEAU120/NG/2013	KP172127
45	PLATEAU131/NG/2014	KP172128
46	PLATEAU132/NG/2014	KP172129
47	PLATEAU133/NG/2014	KP172130
48	PLATEAU135/NG/2014	KP172131
49	KWARA148/NG/2014	KP172132
50	PLATEAU156/NG/2014	KP172133
51	PLATEAU163/NG/2014	KP172134
52	PLATEAU164/NG/2014	KP172135
53	PLATEAU39/NG/2012	KP172136
54	PLATEAU43/NG/2012	KP172137
55	PLATEAU157/NG/2014	KP172138
56	PLATEAU160/NG/2014	KP172139
57	UYO167/NG/2014	KP172140
58	PLATEAU161/NG/2014	KP172141
59	PLATEAU162/NG/2014	KP172142
60	ANAMBRA50/NG/2012	KP172143
61	PLATEAU158/NG/2014	KP172144
62	PLATEAU19/NG/2009	KP266335
63	KADUNA61/NG/2012	KP266336
64	BAUCHI58/NG/2012	KP266337
65	PLATEAU53/NG/2010	KP266338
66	PLATEAU7/NG/2009	KP266339
67	BAUCHI37/NG/2012	KP266340

Appendix 6: **Comparison of amino acid substitutions at different positions between Nigerian viruses and other published strains of IBDV for segment B (aa 68 – 248)**

STRAIN/ISOLATE	PHENO TYPE	AMINO ACID SUBSTITUTION AT POSITIONS													
		96	**119**	**123**	**141**	**145**	**146**	**147**	**148**	**150**	**154**	**161**	**163**	**219**	**242**
AJ878668_HK46	VV	T	E	A	V	T	D	N	L	D	L	D	A	D	E
D49707_OKYM	VV	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PLATEAU19/NG/2009	ND	.	D	.	I	H	E	G	.	E	.	A	V	E	D
KWARA148/NG/2014	“	N	.	.	.	.	.	.	.	.	.	.	.	.	.
PLATEAU156/NG/2014	“	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
PLATEAU161/NG/2014	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
PLATEAU163/NG/2014	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
PLATEAU164/NG/2014	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
PLATEAU154/NG/2014	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
UYO167/NG/2014	,,	.	D	.	I	H	E	G	.	E	.	.	V	E	D
PLATEAU39/NG/2012	,,	.	.	.	I	Q	E	G	.	E	.	.	V	E	D
PLATEAU43/NG/2012	,,	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
PLATEAU157/NG/2014	,,	.	D	.	I	Q	D	G	.	E	I	.	V	E	D
PLATEAU160/NG/2014	,,	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
PLATEAU162/NG/2014	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
ANAMBRA50/NG/2012	,,	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
PLATEAU158/NG/2014	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
ABUJA93/NG/2013	,,	.	D	.	I	Q	E	G	.	.	.	.	V	E	D
BAUCHI121/NG/2013	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
KWARA175/NG/2014	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
KWARA177/NG/2014	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
KWARA178/NG/2014	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
KWARA180/NG/2014	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
KWARA181/NG/2014	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
UYO187/NG/2014	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
UYO189/NG/2014	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
UYO190/NG/2014	,,	.	.	T	T	Q	E	G	P	E	.	.	V	E	D
UYO191/NG/2014	,,	.	.	T	T	Q	E	G	P	E	.	.	V	E	D
UYO192/NG/2014	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
UYO193/NG/2014	,,	.	D	.	I	H	E	G	.	E	.	.	V	E	D
UYO194/NG/2014	,,	.	D	.	I	H	E	G	.	E	.	.	V	E	D
UYO195/NG/2014	,,	.	D	.	I	H	E	G	.	E	.	.	V	E	D
UYO196/NG/2014	,,	.	D	.	I	H	E	G	.	E	.	.	V	E	D
UYO197/NG/2014	,,	.	D	.	I	H	E	G	.	E	.	.	V	E	D
UYO198/NG/2014	,,	.	D	.	I	H	E	G	.	E	.	.	V	E	D
UYO200/NG/2014	,,	.	D	.	I	H	E	G	.	E	.	.	V	E	D
UYO202/NG/2014	,,	.	D	.	I	H	E	G	.	E	.	.	V	E	D
UYO203/NG/2014	,,	.	D	.	I	H	E	G	.	E	.	.	V	E	D
UYO207/NG/2014	,,	.	D	.	I	H	E	G	.	E	.	.	V	E	D
KWARA184/NG/2014	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
UYO188/NG/2014	,,	.	.	T	T	Q	E	G	P	E	.	.	V	E	D
KWARA171/NG/2014	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
BAUCHI124/NG/2014	,,	.	D	.	I	Q	E	G	.	.	.	.	V	E	D
BAUCHI126/NG/2014	,,	.	.	.	I	Q	E	G	.	E	.	.	V	E	D
BAUCHI128/NG/2014	,,	.	D	.	I	Q	E	G	.	.	.	.	V	E	D
BENIN87/NG/2013	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
KADUNA136/NG/2014	,,	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
KWARA141/NG/2014	,,	N	.	.	.	.	.	.	.	.	.	A	.	.	.
PLATAEU96/NG/2013	,,	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
PLATEAU40/NG/2012	,,	N	.	.	.	.	.	.	.	.	.	A	.	.	.

PLATEAU82/NG/2013	,,	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
PLATEAU83/NG/2013	,,	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
PLATEAU90/NG/2013	,,	.	D	.	I	Q	E	G	.	E	I	.	V	E	D
PLATEAU92/NG/2013	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
PLATEAU95/NG/2013	,,	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
PLATEAU104/NG/2010	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
PLATEAU110/NG/2010	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
PLATEAU119/NG/2013	,,	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
PLATEAU120/NG/2013	,,	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
PLATEAU131/NG/2014	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
PLATEAU132/NG/2014	,,	.	D	.	I	Q	E	G	.	E	I	.	V	E	D
PLATEAU133/NG/2014	,,	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
PLATEAU134/NG/2014	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
PLATEAU135/NG/2014	,,	.	D	.	I	Q	E	G	.	E	I	.	V	E	D
AJ878666_UK661	VV	.	.	.	.	.	.	.	.	.	.	.	.	.	.
AJ878676_DEL_E	Variant	.	.	.	.	N	E	D	.	.	.	.	.	.	D
KADUNA61/NG/2012	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
BAUCHI58/NG/2012	,,	.	.	.	I	Q	E	G	P	E	.	.	.	E	D
PLATEAU53/NG/2010	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
PLATEAU7/NG/2009	,,	.	D	.	I	H	D	G	.	E	.	.	V	E	D
BAUCHI37/NG/2012	,,	.	D	.	I	Q	E	G	.	E	.	.	V	E	D
AJ878671_HENAN	VV	.	.	.	.	.	E	G	.	.	.	.	.	.	D
AY099457_T09	VV	.	.	.	.	.	.	.	.	.	.	.	.	.	.
AJ878656_BURS706	Vaccine	.	.	.	.	N	E	G	.	.	.	.	.	.	D

- Amino acid sequences of Nigerian IBD viruses segment B and published strains of IBDV segment B were compared with HK46 strain sequence. Dots indicate sequences identical to those of the HK46 strain.
- VV- very virulent
- ND means not determined.

Appendix 7:Comparison of amino acid substitutions at different positions between six Nigerian field IBDV isolates and other published strains of IBDV for segment B (aa 26 – 253)

STRAIN/ISOLATE	PHENOTYPE	AMINO ACID SUBSTITUTION AT POSITIONS										
		29	**61**	**69**	**90**	**91**	**104**	**112**	**113**	**119**	**141**	**143**
AF092944_HK46	VV	E	I	E	A	G	Q	R	T	E	V	E
BAA23207_OKYM	VV	.	.	D	.	.	.	.	.	.	.	.
AAM45385_T09	VV	.	.	.	.	.	.	.	.	.	.	.
AAK69714_23/82	Serotype 2	.	V	.	.	.	.	.	.	.	.	.
Q86230_UK661	VV	.	.	.	.	P	.	.	.	.	.	.
PLATEAU19/NG/2009	ND	.	.	.	.	.	.	.	.	D	I	.
KADUNA61/NG/2012	ND	.	.	.	.	.	.	.	.	D	I	.
BAUCHI58/NG/2012	ND	.	.	.	.	.	.	.	.	.	I	.
PLATEAU53/NG/2010	ND	V	.	.	.	.	.	.	.	D	I	.
PLATEAU7/NG/2009	ND	.	.	.	.	.	.	.	.	D	I	.
BAUCHI37/NG/2012	ND	.	.	.	.	.	.	.	.	D	I	.
AB368955.1_TANZANIA	VV	.	.	.	.	.	.	.	.	.	.	.
AB368957.1_TANZANIA	VV	.	.	.	.	.	.	.	.	.	.	.
AB368961.1_ZAMBIA	VV	.	V	.	.	.	.	.	.	.	.	.
AB368958.1_ZAMBIA	VV	.	.	.	.	.	.	.	.	.	.	.
JQ241031.1_87ETH	VV	.	.	.	.	.	.	.	.	.	A	D
JQ241031.1_86ETH	VV	.	.	.	.	.	.	.	.	.	A	D
AY705393.2_Gx	VV	.	.	.	.	.	.	.	.	.	.	D
EU544149.1_BRAZIL	VV	.	V	.	.	.	.	.	.	.	.	.
AM167551.1_94432	VV	.	V	.	.	.	.	S	.	.	.	.
FJ95139.1_YS07	VV	.	.	.	.	.	.	.	A	.	.	.
JF907705.1_CAK785		.	V	.	.	.	.	.	.	.	.	.
AF133905_VARIANT_E	Variant	.	V	.	R	.	H	.	.	.	.	.
AF32806_STC	Classic	.	V	.		.	.	.	.	.	.	.

- vv= very virulent, ND= not determined

Appendix 7 Continued

STRAIN/ISOLATE	PHENOTYPE	AMINO ACID SUBSTITUTION AT POSITIONS									
		145	**146**	**147**	**148**	**150**	**163**	**174**	**219**	**242**	**251**
AF092944_HK46	VV	T	D	N	L	D	A	T	D	E	K
BAA23207_OKYM	VV	.	.	.	.	.	.	.	.	.	.
AAM45385_T09	VV	.	.	.	.	.	.	.	.	.	.
AAK69714_23/82	Serotype 2	N	E	G	.	.	.	.	.	D	.
Q86230_UK661	VV	.	.	.	.	.	.	.	.	.	.
PLATEAU19/NG/2009	ND	H	E	G		E	V		E	D	.
KADUNA61/NG/2012	ND	Q	E	G		E	V	S	E	D	.
BAUCHI58/NG/2012	ND	Q	E	G	P	E		.	E	D	.
PLATEAU53/NG/2010	ND	Q	E	G	.	E	V	.	E	D	.
PLATEAU7/NG/2009	ND	H	.	G	.	E	V	.	E	D	.
BAUCHI37/NG/2012	ND	Q	E	G	.	E	V	.	E	D	.
AB368955.1_TANZANIA	VV	S	E	G	.	.	.	.	E	D	.
AB368957.1_TANZANIA	VV	S	E	G	.	.	.	.	E	D	.
AB368961.1_ZAMBIA	VV	N	E	G	.	.	.	.	.	D	.
AB368958.1_ZAMBIA	VV	.	.	.	.	.	.	.	.	.	
JQ241031.1_87ETH	VV	.	.	S	.	.	.	.	.	.	R
JQ241031.1_86ETH	VV	.	.	.	.	.	.	.	.	.	R
AY705393.2_Gx	VV	.	E	G	.	E	V	.	.	D	.
EU544149.1_BRAZIL	VV	N	E	D	.	.	.	.	.	D	.
AM167551.1_94432	VV	.		.	.	.	.	.	.	.	.
FJ95139.1_YS07	VV	.	E	.	.	.	.	.	.	.	.
JF907705.1_CAK785		N	E	G	.	.	.	.	.	D	.
AF133905_VARIANT_E	Variant	N	E	G	.	.	.	.	.	D	.
AF32806_STC	Classic	N	E	D	.	.	.	.	.	D	.

- Amino acid sequences of Nigerian IBDV segment B field isolates and published strains of IBDV segment B were compared with HK46 strain sequence. Dots indicate sequences identical to those of the HK46 strain.
- ND means not determined

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                                         701                                                                        760
#UK_661_A                                  GCA GCC GAC GAT TAC CAA TTC TCA TCA CAG TAC CAA GCA GGT GGG GTA ACA ATC ACA CTG
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#DelE                                ... ... ..T A.. ... ... ... ... ... ... ... ... ... A.. ... ... ... ... ... ...
                                   761                                                                                 820
#UK_661_A                            TTC TCA GCT AAT ATC GAT GCC ATC ACA AGC CTC AGC ATC GGG GGA GAA CTC GTG TTT CAA
#KP152231_UYO206/NG/2014             ... ... ..C ..C ... ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ...
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#KP152236_KWARA177/NG/2014           ... ... ..C ..C ..T ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ...
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#KP266323_KADUNA61/NG/2012           ... ... ... ... ... ... T.. ... ... ... ..C ... ... ...
#KP266324_BAUCHI58/NG/2012           ... ... ... ... ..A ... ... ... ... ... ... ... ... ...
#KP266325_PLATEAU67/NG/2011          ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP266326_PLATEAU53/NG/2010          ... ... ... ... ... ... T.. ... ... ... ..C ... ... ...
#KP266327_ANAMBRA54/NG/2012          ... ... ... ... ..A ... ... ... ... ... ... ... ... ...
#KP266328_BENIN35/NG/2012            ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP266329_BAUCHI37/NG/2012           ... ... ... ... ... ... T.. ... ... ... ..C ... ... ...
#KP266330_NASSARAWA77/NG/no2/2013    ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#KP266331_NASSARAWA77/NG/2013        ... ... ... ... ..A ... ... ... ... ... ... ... ... ...
#KP266332_PLATEAU78/NG/2011/2        ... ... ... ... ..A ... ... ... ... ... ... ... ... ...
#KP266333_PLATEAU78/NG/2011/1        ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#KP152268_BAUCHI36/NG/2009           ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152292_PLATEAU4/NG/2009           ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152241_KWARA182/NG/2014           ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152242_KWARA184/NG/2014           ... ... ... ... ... ... ..A ... ... ... ..C ... ... ...
#KP152243_UYO185/NG/2014             ... ... ... ... ..A ... ... ... ... ... ... ... ... ...
#KP152244_UYO187/NG/2014             ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152245_UYO188/NG/2014             ... ... ... ... ..A ... ... ... ... ... ... ... ... ...
#KP152246_UYO189/NG/2014             ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152247_UYO190/NG/2014             ... ... ... ... ..A ... ... ... ... ... ... ... ... ...
#KP152248_UYO191/NG/2014             ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152249_UYO192/NG/2014             ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152250_UYO193/NG/2014             ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152251_UYO194/NG/2014             ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152252_UYO195/NG/2014             ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152253_UYO196/NG/2014             ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152254_UYO197/NG/2014             ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
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#KP152255_UYO198/NG/2014          ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152256_UYO199/NG/2014          ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152257_UYO200/NG/2014          ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152258_UYO201/NG/2014          ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152259_UYO202/NG/2014          ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152260_UYO203/NG/2014          ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152261_UYO204/NG/2014          ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152262_BAUCHI124/NG/2014       ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152263_BAUCHI126/NG/2014       ..T ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152264_BAUCHI127/NG/2014       ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152265_KADUNA136/NG/2014       ... ... ... ... ..A ... ... ... ... ... ... ... ... ...
#KP152266_PLATEAU133/NG/2014      ... ... ... ... ..A ... ... ... ... ... ... ... ... ...
#KP152267_PLATEAU135/NG/2014      ... ... ... ... ..A ... ... ... ... ... ... ... ... ...
#KP152269_BENIN87/NG/2013         ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152270_BENIN142E/NG/2013       ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#KP152271_ABUJA93/NG/2013         ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152272_BAUCHI128/NG/2014       ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152273_PLATEAU99/NG/2011       ... ... ... ... ... ... T.. ... ... ... ..C ... ... ...
#KP152274_PLATEAU92/NG/2013       ... ... ... ... ... ... T.. ... ... ... ..C ... ... ...
#KP152275_PLATEAU83/NG/2013       ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152276_PLATEAU90/NG/2013       ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152277_PLATEAU95/NG/2013       ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152278_PLATEAU96/NG/2013       ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152279_PLATEAU120/NG/2013      ... ... ... ... ..A ... ... ... ... ... ... ... ... ...
#KP152280_PLATEAU132/NG/2014      ... ... ... ... ..A ... ... ... ... ... ... ... ... ...
#KP152281_PLATEAU131/NG/2014      ... ... ... ... ... ... T.. ... ... ... ..C ... ... ...
#KP152282_PLATEAU134/NG/2014      ... ... ... ... ... ... T.. ..A ... ... ..C ... ... ...
#KP152283_PLATEAU110/NG/2010      ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152284_PLATEAU104/NG/2010      ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152285_BAUCHI121/NG/2013       ... ... ... ... ... ... T.. ... ... ... ..C ... ... ...
#KP152286_KWARA139/NG/2014        ... ... ... ... ... ... ..A ... ... ... ..C ... ... ...
#KP152287_PLATEAU40/NG/2012       ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#KP152288_PLATEAU119/NG/2013      ... ... ... ... ..A ... ... ... ... ... ... ... ... ...
#KP152289_KWARA141/NG/2014        ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#KP152290_PLATEAU82/NG/2013       ... ... ... ... ..A ... ... ... ... ... ... ... ... ...
#KP152291_BAUCHI37/NG/2009        ... ... ... ... ... ... T.. ... ... ... ..C ... ... ...
#KP266334_PLATEAU39/NG/2012       ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152293_PLATEAU43/NG/2012       ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152294_BENIN34/NG/2012         ... ... ... ... ... ... T.. ... ... ... ..C ... ... ...
#KP152295_ANAMBRA50/NG/2012       ... ... ... ... ..A ... ... ... ... ... ... ... ... ...
#KP152296_NIG20/NG/2009           .A. ... ... ... ..A ... ... ... ... ... ... ... ... ...
#KP152297_BENIN47/NG/2014         ... ... ... ... ... ... T.. ... ... ... ..C ... ... ...
#KP152298_KWARA145/NG/2014        ... ... ... ... ... ... ..A ... ... ... ..C ... ... ...
#KP152299_KWARA147/NG/2014        ... ... ... ... ... ... ..A ... ... ... ..C ... ... ...
#KP152300_KWARA148/NG/2014        ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#KP152301_KWARA149/NG/2014        ... ... ... ... ... ... ..A ... ... ... ..C ... ... ...
#KP152302_KWARA150/NG/2014        ... ... ... ... ... ... ..A ... ... ... ..C C.. ... ...
#KP152303_KWARA151NG/NG/2014      ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152304_KWARA152/NG/2014        ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152305_PLATEAU7/NG/2009        ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152306_PLATEAU16/NG/2009       ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152307_PLATEAU75/NG/2011       ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152308_PLATEAU154/NG/2014      ... ... ... ... ... ... ... ... ... ... ..C ..T ... ...
#KP152309_PLATEAU155/NG/2014      ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152310_PLATEAU156/NG/2014      ... ... ... ... ..A ... ... ... ... ... ... ... ... ...
#KP152311_PLATEAU157/NG/2014      ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152312_PLATEAU158/NG/2014      ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152313_PLATEAU159/NG/2014      ... ... ... ... ... ... T.. ... ... ... ..C ... ... ...
#KP152314_PLATEAU160/NG/2014      ... ... ... ... ..A ... ... ... ... ... ... ... ... ...
#KP152315_PLATEAU161/NG/2014      ... ... ... ... ... ... ... ... ... ... ..C ..T ... ...
#KP152316_PLATEAU162/NG/2014      ... ... ... ... ... ... T.. ... ... ... ..C ... ... ...
#KP152317_PLATEAU163/NG/2014      ... ... ... ... ... ... T.. ... ... ... ..C ... ... ...
#KP152318_PLATEAU164/NG/2014      ... ... ... ... ... ... T.. ... ... ... ..C ... ... ...
#KP152319_UYO166/NG/2014          ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152320_UYO167/NG/2014          ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152321_NIG79/NG/2009           ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#KP152322_UYO205/NG/2014          ... ... ... ... ... ... ... ... ... ... ..C ... ... ...
#DelE                             ... ... ... ... ... ... ... ... ... ... ... ... ... ...
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Appendix 8: Nucleotide sequence data for Nigerian IBDV viruses

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                                   210        220        230        240        250
#vvIBDV_UK_661                     AADDYQFSSQ YQAGGVTITL FSANIDAITS LSIGGELVFQ TSVQGLILGA
#KP152296_NIG20/NG/2009            .......... .......... .......... .......... .....S.....
#KP152233_KWARA174/NG/2014         .......... .......... .......... .......... .....S.....
#KP152293_PLATEAU43/NG/2012        .......... .......... .......... .......... .....S.....
#KP152321_NIG79/NG/2009            .......... .......... .......... .......... .....S.....
#KP152320_UYO167/NG/2014           .......... .......... .......... .......... .....S.....
#KP152319_UYO166/NG/2014           .......... .......... .......... .......... .....S.....
#KP1522318_PLATEAU164/NG/2014      .......... .......... .......... .......... .....S.....
#KP152317_PLATEAU163/NG/2014       .......... .......... .......... .......... .....S.....
#KP152316_PLATEAU162/NG/2014       .......... .......... .......... .......... .....S.....
#KP152315_PLATEAU161/NG/2014       .......... .......... .......... .......... .....S.....
#KP152314_PLATEAU160/NG/2014       ........T. .......... .......... .......... .....S.....
#KP152313_PLATEAU159/NG/2014       .......... .......... .......... .......... .....S.....
#KP152312_PLATEAU158/NG/2014       .......... .......... .......... .......... .....S.....
#KP152311_PLATEAU157/NG/2014       .......... .......... .......... .......... .....S.....
#KP152310_PLATEAU156/NG/2014       ........T. .......... .......... .......... .....S.....
#KP152309_PLATEAU155/NG/2014       .......... .......... .......... .......... .....S.....
#KP152308_PLATEAU154/NG/2014       .......... .......... .......... .......... .....S.....
#KP152306_PLATEAU16/NG/2009        .......... .......... .......... .......... .....S.....
#KP152292_PLATEAU4/NG/2009         .......... .......... .......... .......... .....S.....
#KP152304_KWARA152/NG/2014         .......... .......... .......... .......... .....S.....
#KP152303_KWARA151NG/NG/2014       .......... .......... .......... .......... .....S.....
#KP152302_KWARA150/NG/2014         .......... .......... .......... .......... .....S.....
#KP152301_KWARA149/NG/2014         .......... .......... .......... .......... .....S.....
#KP152300_KWARA148/NG/2014         .......... .......... .......... .......... ..........
#KP152299_KWARA147/NG/2014         .......... .......... .......... .......... .....S.....
#KP152298_KWARA145/NG/2014         .......... .......... .......... .......... .....S.....
#KP152297_BENIN47/NG/2014          .......... .......... .......... .......... .....S.....
#KP266329_BAUCHI37/NG/2012         .......... ..T....... .......... .......... .....S.....
#KP152268_BAUCHI36/NG/2009         .......... .......... .......... .......... .....S.....
#KP266322_PLATEAU19/NG/2009        .......... .......... .......... .......... .....S.....
#KP266323_KADUNA61/NG/2009         .......... .......... .......... .......... .....S.....
#KP266334_PLATEAU39/NG/2012        .......... .......... .......... .......... .....S.....
#KP266324_BAUCHI58/NG/2012         ........T. .......... .......... .......... .....S.....
#KP266325_PLATEAU67/NG/2011        .......... .......... .......... .......... .....S.....
#KP266326_PLATEAU53/NG/2010        .......... .......... .......... .......... .....S.....
#KP266327_ANAMBRA54/NG/2012        ........T. .......... .......... .......... .....S.....
#KP152305_PLATEAU7/NG/2009         .......... .......... .......... .......... .....S.....
#KP266328_BENIN35/NG/2012          .......... .......... .......... .......... .....S.....
#KP152291_BAUCHI37/NG/2009         .......... ..T....... .......... .......... .....S.....
#KP152262_BAUCHI124/NG/2014        .......... .......... .......... .......... .....S.....
#KP152263_BAUCHI126/NG/2014        .......... .......... .......... .......... .....S.....
#KP152264_BAUCHI127/NG/2014        .......... .......... .......... .......... .....S.....
#KP152265_KADUNA136/NG/2014        ........T. .......... .......... .......... .....S.....
#KP152266_PLATEAU133/NG/2014       ........T. .......... .......... .......... .....S.....
#KP152267_PLATEAU135/NG/2014       ........T. .......... .......... .......... .....S.....
#KP266330_NASSARAWA77/NG/no2/_2013 .......... .......... .......... ..V....... ...HS.V...
#KP266331_NASSARAWA77/NG/2013      .......... .......... .......... .......... ...LS.....
#KP266332_PLATEAU78/NG/2011/2      ........P. .......... .......... ..V....... .....S.....
#KP266333_PLATEAU78/NG/2011/1      ........T. .......... .......... .......... ...LS.....
#KP152270_BENIN142E/NG/2013        .......... .......... .......... .......... ..........
#KP152269_BENIN87/NG/2013          .......... .......... .......... .......... .....S.....
#KP152271_ABUJA93/NG/2013          .......... .......... .......... .......... .....S.....
#KP152272_BAUCHI128/NG/2014        .......... .......... .......... .......... .....S.....
#KP152273_PLATEAU99/NG/2011        .......... .......... .......... .......... .....S.....
#KP152274_PLATEAU92/NG/2013        .......... .......... .......... .......... .....S.....
#KP152275_PLATEAU83/NG/2013        .......... .......... .......... .......... .....S.....
#KP152276_PLATEAU90/NG/2013        .......... .......... .......... .......... .....S.....
#KP152277_PLATEAU95/NG/2013        .......... .......... .......... .......... .....S.....
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#KP152278_PLATEAU96/NG/2013          ..........  .....  ..........  .........  .  ...S.....
#KP152281_PLATEAU131/NG/2014         ..........  .....  ..........  .........  .  ...S.....
#KP152282_PLATEAU134/NG/2014         ..........  .....  ..........  .........  .  ...S.....
#KP152283_PLATEAU110/NG/2010         ..........  .....  ..........  .........  .  ...S.....
#KP152284_PLATEAU104/NG/2010         ..........  .....  ..........  .........  .  ...S.....
#KP152285_BAUCHI121/NG/2013          ..........  .....  ..........  .........  .  ...S.....
#KP152286_KWARA139/NG/2014           ..........  .....  ..........  .........  .  ...S.....
#KP152287_PLATEAU40/NG/2012          ..........  .....  ..........  .........  .  .........
#KP152288_PLATEAU119/NG/2013         ........T.  .....  ..........  .........  .  ...S.....
#KP152289_KWARA141/NG/2014           ..........  .....  ..........  .........  .  .........
#KP152279_PLATEAU120/NG/2013         ........T.  .....  ..........  .........  .  ...S.....
#KP152280_PLATEAU132/NG/2014         ........T.  .....  ..........  .........  .  ...S.....
#KP152290_PLATEAU82/NG/2013          ........T.  .....  ..........  .........  .  ...S.....
#KP152307_PLATEAU75/NG/2011          ..........  .....  ..........  .........  .  ...S.....
#KP152295_ANAMBRA50/NG/2012          ........T.  .....  ..........  .........  .  ...S.....
#KP152294_BENIN34/NG/2012            ..........  .....  ..........  .........  .  ...S.....
#vvIBDV_OKYM                         ..........  .....  ..........  .........  .  .........
#Variant_DelE                        ...N......  ..T...  ..........  ..V.....  K  ...S.V...
#Variant_T1                          ...N......  ..T...  ..........  ..V.....  K  ...N.V...
#Classic_STC                         ..........  ..P...  ..........  ..V.....  .  ....V...
#KP152234_KWARA175/NG/2014           ..........  .....  ..........  .........  .  ...S.....
#KP152235_KWARA176/NG/2014           ..........  .....  ..........  .........  .  ...S.....
#KP152236_KWARA177/NG/2014           ..........  .....  ..........  .........  .  ...S.....
#KP152237_KWARA178/NG/2014           ..........  .....  ..........  .........  .  ...S.....
#KP152238_KWARA179/NG/2014           ..........  .....  ..........  .........  .  ...S.....
#KP152239_KWARA180/NG/2014           ..........  .....  ..........  .........  .  ...S.....
#KP152240_KWARA181/NG/2014           ..........  .....  ..........  .........  .  ...S.....
#KP152241_KWARA182/NG/2014           ..........  .....  ..........  .........  .  ...S.....
#KP152242_KWARA184/NG/2014           ..........  .....  ..........  .........  .  ...S.....
#KP152243_UYO185/NG/2014             ........T.  .....  ..........  .........  .  ...S.....
#KP152244_UYO187/NG/2014             ..........  .....  ..........  .........  .  ...S.....
#KP152245_UYO188/NG/2014             ........T.  .....  ..........  .........  .  ...S.....
#KP152246_UYO189/NG/2014             ..........  .....  ..........  .........  .  ...S.....
#KP152247_UYO190/NG/2014             ........T.  .....  ..........  .........  .  ...S.....
#KP152248_UYO191/NG/2014             ..........  .....  ..........  .........  .  ...S.....
#KP152249_UYO192/NG/2014             ..........  .....  ..........  .........  .  ...S.....
#KP152250_UYO193/NG/2014             ..........  .....  ..........  .........  .  ...S.....
#KP152251_UYO194/NG/2014             ..........  .....  ..........  .........  .  ...S.....
#KP152252_UYO195/NG/2014             ..........  .....  ..........  .........  .  ...S.....
#KP152253_UYO196/NG/2014             ..........  .....  ..........  .........  .  ...S.....
#KP152254_UYO197/NG/2014             ..........  .....  ..........  .........  .  ...S.....
#KP152255_UYO198/NG/2014             ..........  .....  ..........  .........  .  ...S.....
#KP152256_UYO199/NG/2014             ..........  .....  ..........  .........  .  ...S.....
#KP152257_UYO200/NG/2014             ..........  .....  ..........  .........  .  ...S.....
#KP152258_UYO201/NG/2014             ..........  .....  ..........  .........  .  ...S.....
#KP152259_UYO202/NG/2014             ..........  .....  ..........  .........  .  ...S.....
#KP152260_UYO203/NG/2014             ..........  .....  ..........  .........  .  ...S.....
#KP152261_UYO204/NG/2014             ..........  .....  ..........  .........  .  ...S.....
#KP152322_UYO205/NG/2014             ..........  .....  ..........  .........  .  ...S.....
#KP152231_UYO206/NG/2014             ..........  .....  ..........  .........  .  ...S.....
#KP152232_KWARA171/NG/2014           ..........  .....  ..........  .........  .  ...S.....
                                      Major peak A                                 Minor peak 1
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                                   260        270        280        290        300
#vvIBDV_UK_661                       TIYLIGFDGT AVITRAVAAD NGLTAGTDNL MPFNIVIPTS EITQPITSIK
#KP152296_NIG20/NG/2009              .......... .......... .......... .......... A.........
#KP152233_KWARA174/NG/2014           .......... .......... .......... .......... A.........
#KP152293_PLATEAU43/NG/2012          .........S .......... .......... .......... Q.........
#KP152321_NIG79/NG/2009              .......... .......... .......... .......... A.........
#KP152320_UYO167/NG/2014             .......... .......... .......... .......... A.........
#KP152319_UYO166/NG/2014             .......... .......... .......... .......... A.........
#KP1522318_PLATEAU164/NG/2014        .......... .......... .......... .......... A.........
#KP152317_PLATEAU163/NG/2014         .......... .......... .......... .......... A.........
#KP152316_PLATEAU162/NG/2014         .......... .......... .......... .......... A.........
#KP152315_PLATEAU161/NG/2014         .......... .......... .......... .......... A.........
#KP152314_PLATEAU160/NG/2014         .........S .......... .......... .......... Q.........
#KP152313_PLATEAU159/NG/2014         .......... .......... .......... .......... A.........
#KP152312_PLATEAU158/NG/2014         .......... .......... .......... .......... A.........
#KP152311_PLATEAU157/NG/2014         .......... .......... .......... .......... A.........
#KP152310_PLATEAU156/NG/2014         .........S .......... .......... .......... Q.........
#KP152309_PLATEAU155/NG/2014         .......... .......... .......... .......... A.........
#KP152308_PLATEAU154/NG/2014         .......... .......... .......... .......... A.........
#KP152306_PLATEAU16/NG/2009          .......... .......... .......... .......... A.........
#KP152292_PLATEAU4/NG/2009           .......... .......... .......... .......... A.........
#KP152304_KWARA152/NG/2014           .......... .......... .......... .......... A.........
#KP152303_KWARA151NG/NG/2014         .......... .......... .......... .......... A.........
#KP152302_KWARA150/NG/2014           .......... .......... .......... .......... A.........
#KP152301_KWARA149/NG/2014           .......... .......... .......... .......... A.........
#KP152300_KWARA148/NG/2014           .......... ..T......N .......... .......... ..........
#KP152299_KWARA147/NG/2014           .......... .......... .......... .......... A.........
#KP152298_KWARA145/NG/2014           .......... .......... .......... .......... A.........
#KP152297_BENIN47/NG/2014            .......... .......... .......... .......... A.........
#KP266329_BAUCHI37/NG/2012           .......... .......... .......... .......... A.........
#KP152268_BAUCHI36/NG/2009           .......... .......... .......... .......... A.........
#KP266322_PLATEAU19/NG/2009          .......... .......... .......... .......... A.........
#KP266323_KADUNA61/NG/2009           .......... .......... .......... .......... A.........
#KP266334_PLATEAU39/NG/2012          .......... .......... .......... .......... A.........
#KP266324_BAUCHI58/NG/2012           .........S .......... .......... .......... Q.........
#KP266325_PLATEAU67/NG/2011          .......... .......... .......... .......... A.........
#KP266326_PLATEAU53/NG/2010          .......... .......... .......... .......... A.........
#KP266327_ANAMBRA54/NG/2012          .........S .......... .......... .......... Q.........
#KP152305_PLATEAU7/NG/2009           .......... .......... .......... .......... A.........
#KP266328_BENIN35/NG/2012            .......... .......... .......... .......... A.........
#KP152291_BAUCHI37/NG/2009           .......... .......... .......... .......... A.........
#KP152262_BAUCHI124/NG/2014          .......... .......... .......... .......... A.........
#KP152263_BAUCHI126/NG/2014          .......... .......... .......... .......... A.........
#KP152264_BAUCHI127/NG/2014          .......... .......... .......... .......... A.........
#KP152265_KADUNA136/NG/2014          .........S .......... .......... .......... Q.........
#KP152266_PLATEAU133/NG/2014         .........S .......... .......... .......... Q.........
#KP152267_PLATEAU135/NG/2014         .........S .......... .......... .......... Q.........
#KP266330_NASSARAWA77/NG/no2/_2013   .......... .........N ....T..... ....L....N ..........
#KP266331_NASSARAWA77/NG/2013        .........S .......... .......... .......... Q.........
#KP266332_PLATEAU78/NG/2011/2        .........S .......... .......... .......... Q.........
#KP266333_PLATEAU78/NG/2011/1        .........S .......... .......... .......... Q.........
#KP152270_BENIN142E/NG/2013          .......... ..T......N .......... .......... ..........
#KP152269_BENIN87/NG/2013            .......... .......... .......... .......... A.........
#KP152271_ABUJA93/NG/2013            .......... .......... .......... .......... A.........
#KP152272_BAUCHI128/NG/2014          .......... .......... .......... .......... A.........
#KP152273_PLATEAU99/NG/2011          .......... .......... .......... .......... A.........
#KP152274_PLATEAU92/NG/2013          .T........ .......... .......... .......... A.........
#KP152275_PLATEAU83/NG/2013          .......... .......... .......... .......... A.........
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#KP152277_PLATEAU95/NG/2013          .......... .......... .......... .......... A.........
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#KP152278_PLATEAU96/NG/2013          .......... .......... .......... .......... A.........
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#KP152284_PLATEAU104/NG/2010         .......... .......... .......... .......... A.........
#KP152285_BAUCHI121/NG/2013          .......... .......... .......... .......... A.........
#KP152286_KWARA139/NG/2014           .......... .......... .......... .......... A.........
#KP152287_PLATEAU40/NG/2012          .......... ..T.......N......... .......... ..........
#KP152288_PLATEAU119/NG/2013         .........S .......... .......... .......... Q.........
#KP152289_KWARA141/NG/2014           .......... ..T.......N......... .......... ..........
#KP152279_PLATEAU120/NG/2013         .........S .......... .......... .......... Q.........
#KP152280_PLATEAU132/NG/2014         .........S .......... .......... .......... Q.........
#KP152290_PLATEAU82/NG/2013          .........S .......... .......... .......... Q.........
#KP152307_PLATEAU75/NG/2011          .......... .......... .......... .......... A.........
#KP152295_ANAMBRA50/NG/2012          .........S .......... .......... .......... Q.........
#KP152294_BENIN34/NG/2012            .......... .......... .......... .......... A.........
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#Variant_DelE                        .......... .........N ......I... ...L....N ..........
#Variant_T1                          .......... .........N ......I... ...L....N ..........
#Classic_STC                         ...F...... T......... .......... ...L....N ..........
#KP152234_KWARA175/NG/2014           .......... .......... .......... .......... A.........
#KP152235_KWARA176/NG/2014           .......... .......... .......... .......... A.........
#KP152236_KWARA177/NG/2014           .......... .......... .......... .......... A.........
#KP152237_KWARA178/NG/2014           .......... .......... .......... .......... A.........
#KP152238_KWARA179/NG/2014           .......... .......... .......... .......... A.........
#KP152239_KWARA180/NG/2014           .......... .......... .......... .......... A.........
#KP152240_KWARA181/NG/2014           .......... .......... .......... .......... A.........
#KP152241_KWARA182/NG/2014           .......... .......... .......... .......... A.........
#KP152242_KWARA184/NG/2014           .......... .......... .......... .......... A.........
#KP152243_UYO185/NG/2014             .........S .......... .......... .......... Q.........
#KP152244_UYO187/NG/2014             .......... .......... .......... .......... A.........
#KP152245_UYO188/NG/2014             .........S .......... .......... .......... Q.........
#KP152246_UYO189/NG/2014             .......... .......... .......... .......... A.........
#KP152247_UYO190/NG/2014             .........S .......... .......... .......... Q.........
#KP152248_UYO191/NG/2014             .......... .......... .......... .......... A.........
#KP152249_UYO192/NG/2014             .......... .......... .......... .......... A.........
#KP152250_UYO193/NG/2014             .......... .......... .......... .......... A.........
#KP152251_UYO194/NG/2014             .......... .......... .......... .......... A.........
#KP152252_UYO195/NG/2014             .......... .......... .......... .......... A.........
#KP152253_UYO196/NG/2014             .......... .......... .......... .......... A.........
#KP152254_UYO197/NG/2014             .......... .......... .......... .......... A.........
#KP152255_UYO198/NG/2014             .......... .......... .......... .......... A.........
#KP152256_UYO199/NG/2014             .......... .......... .......... .......... A.........
#KP152257_UYO200/NG/2014             .......... .......... .......... .......... A.........
#KP152258_UYO201/NG/2014             .......... .......... .......... .......... A.........
#KP152259_UYO202/NG/2014             .......... .......... .......... .......... A.........
#KP152260_UYO203/NG/2014             .......... .......... .......... .......... A.........
#KP152261_UYO204/NG/2014             .......... .......... .......... .......... A.........
#KP152322_UYO205/NG/2014             .......... .......... .......... .......... A.........
#KP152231_UYO206/NG/2014             .......... .......... .......... .......... A.........
#KP152232_KWARA171/NG/2014           .......... .......... .......... .......... A.........
                                                          Minor peak 2          Minor peak 3
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                                   310        320        330        340        350
#vvIBDV_UK_661                      LEIVTSKSGG QAGDQMSWSA SGSLAVTIHG GNYPGALRPV TLVAYERVAT
#KP152296_NIG20/NG/2009             .......... .......... .......... .......... ..........
#KP152233_KWARA174/NG/2014          .......... .......... .......... .......... ..........
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#KP152321_NIG79/NG/2009             .......... .......... .......... .......... ..........
#KP152320_UYO167/NG/2014            .......... .......... .......... .......... ..........
#KP152319_UYO166/NG/2014            .......... .......... .......... .......... ..........
#KP1522318_PLATEAU164/NG/2014       .......... .......... .......... .......... ..........
#KP152317_PLATEAU163/NG/2014        .......... .......... .......... .......... ..........
#KP152316_PLATEAU162/NG/2014        .......... .......... .......... .......... ..........
#KP152315_PLATEAU161/NG/2014        .......... .......... .......... .......... ..........
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#KP152313_PLATEAU159/NG/2014        .......... .......... .......... .......... ..........
#KP152312_PLATEAU158/NG/2014        .......... .......... .......... .......... ..........
#KP152311_PLATEAU157/NG/2014        .......... .......... .......... .......... ..........
#KP152310_PLATEAU156/NG/2014        .......... .......... .......... .......... ..........
#KP152309_PLATEAU155/NG/2014        .......... .......... .......... .......... ..........
#KP152308_PLATEAU154/NG/2014        .......... .......... .......... .......... ..........
#KP152306_PLATEAU16/NG/2009         .......... .......... .......... .......... ..........
#KP152292_PLATEAU4/NG/2009          .......... .......... .......... .......... ..........
#KP152304_KWARA152/NG/2014          .......... .......... .......... .......... ..........
#KP152303_KWARA151NG/NG/2014        .......... .......... .......... .......... ..........
#KP152302_KWARA150/NG/2014          .......... .......... .......... .......... ..........
#KP152301_KWARA149/NG/2014          .......... .......... .......... .......... ..........
#KP152300_KWARA148/NG/2014          .......... .......... .......... .......... ..........
#KP152299_KWARA147/NG/2014          .......... .......... .......... .......... ..........
#KP152298_KWARA145/NG/2014          .......... .......... .......... .......... ..........
#KP152297_BENIN47/NG/2014           .......... .......... .......... .......... ..........
#KP266329_BAUCHI37/NG/2012          .......... .......... .......... .......... ..........
#KP152268_BAUCHI36/NG/2009          .......... .......... .......... .......... ..........
#KP266322_PLATEAU19/NG/2009         .......... .......... .......... .......... ..........
#KP266323_KADUNA61/NG/2009          .......... .......... .......... .......... ..........
#KP266334_PLATEAU39/NG/2012         .......... .......... .......... .......... ..........
#KP266324_BAUCHI58/NG/2012          .......... .......... .......... .......... ..........
#KP266325_PLATEAU67/NG/2011         .......... .......... .......... .......... ..........
#KP266326_PLATEAU53/NG/2010         .......... .......... .......... .......... ..........
#KP266327_ANAMBRA54/NG/2012         .......... .......... .......... .......... ..........
#KP152305_PLATEAU7/NG/2009          .......... .......... .......... .......... ..........
#KP266328_BENIN35/NG/2012           .......... .......... .......... .......... ..........
#KP152291_BAUCHI37/NG/2009          .......... .......... .......... .......... ..........
#KP152262_BAUCHI124/NG/2014         .......... .......... .......... .......... ..........
#KP152263_BAUCHI126/NG/2014         .......... .......... .......... .......... ..........
#KP152264_BAUCHI127/NG/2014         .......... .......... .......... .......... ..........
#KP152265_KADUNA136/NG/2014         .......... .......... .......... .......... ..........
#KP152266_PLATEAU133/NG/2014        .......... .......... .......... .......... ..........
#KP152267_PLATEAU135/NG/2014        .......... .......... .......... .......... ..........
#KP266330_NASSARAWA77/NG/no2/_2013  .......... .......... .......... .......... ..........
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#KP266332_PLATEAU78/NG/2011/2       .......... .......... R......... .......... ..........
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#KP152270_BENIN142E/NG/2013         .......... .......... .......... .......... ..........
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#KP152271_ABUJA93/NG/2013           .......... .......... .......... .......... ..........
#KP152272_BAUCHI128/NG/2014         .......... .......... .......... .......... ..........
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#KP152276_PLATEAU90/NG/2013         .......... .......... .......... .......... ..........
#KP152277_PLATEAU95/NG/2013         .......... .......... .......... .......... ..........
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#KP152278_PLATEAU96/NG/2013          ..........  ..........  ..........  ..........  ..........
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#KP152285_BAUCHI121/NG/2013          ..........  ..........  ..........  ..........  ..........
#KP152286_KWARA139/NG/2014           ..........  ..........  ..........  ..........  ..........
#KP152287_PLATEAU40/NG/2012          ..........  ..........  ..........  ..........  ..........
#KP152288_PLATEAU119/NG/2013         ..........  ..........  ..........  ..........  ..........
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#KP152279_PLATEAU120/NG/2013         ..........  ..........  ..........  ..........  ..........
#KP152280_PLATEAU132/NG/2014         ..........  ..........  ..........  ..........  ..........
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#KP152295_ANAMBRA50/NG/2012          ..........  ..........  ..........  ..........  ..........
#KP152294_BENIN34/NG/2012            ..........  ..........  ..........  ..........  ..........
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#Variant_DelE                        .........D.  ...E......  ..........  ..........  ..........
#Variant_T1                          .........N.  ..E.......  ..........  ..........  ..........
#Classic_STC                         ..V.......  ..........  ..........  ..........  ..........
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#KP152236_KWARA177/NG/2014           ..........  ..........  ..........  ..........  ..........
#KP152237_KWARA178/NG/2014           .....T....  ..........  ..........  ..........  ..........
#KP152238_KWARA179/NG/2014           ..........  ..........  ..........  ..........  ..........
#KP152239_KWARA180/NG/2014           ..........  ..........  ..........  ..........  ..........
#KP152240_KWARA181/NG/2014           ..........  ..........  ..........  ..........  ..........
#KP152241_KWARA182/NG/2014           ..........  ..........  ..........  ..........  ..........
#KP152242_KWARA184/NG/2014           ..........  ..........  ..........  ..........  ..........
#KP152243_UYO185/NG/2014             ..........  ..........  ..........  ..........  ..........
#KP152244_UYO187/NG/2014             ..........  ..........  ..........  ..........  ..........
#KP152245_UYO188/NG/2014             ..........  ..........  ..........  ..........  ..........
#KP152246_UYO189/NG/2014             ..........  ..........  ..........  ..........  ..........
#KP152247_UYO190/NG/2014             ..........  ..........  ..........  ..........  ..........
#KP152248_UYO191/NG/2014             ..........  ..........  ..........  ..........  ..........
#KP152249_UYO192/NG/2014             ..........  ..........  ..........  .......s..  ..........
#KP152250_UYO193/NG/2014             ..........  ..........  ..........  ..........  ..........
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#KP152252_UYO195/NG/2014             ..........  ..........  ..........  ..........  ..........
#KP152253_UYO196/NG/2014             ..........  ..........  ..........  ..........  ..........
#KP152254_UYO197/NG/2014             ..........  ..........  ..........  ..........  ..........
#KP152255_UYO198/NG/2014             ..........  ..........  ..........  ..........  ..........
#KP152256_UYO199/NG/2014             ..........  ..........  ..........  ..........  ..........
#KP152257_UYO200/NG/2014             ..........  ..........  ..........  ..........  ..........
#KP152258_UYO201/NG/2014             ..........  ..........  ..........  ..........  ..........
#KP152259_UYO202/NG/2014             ..........  ..........  ..........  ..........  ..........
#KP152260_UYO203/NG/2014             ..........  ..........  ..........  ..........  ..........
#KP152261_UYO204/NG/2014             ..........  ..........  ..........  ..........  ..........
#KP152322_UYO205/NG/2014             ..........  ..........  ..........  ..........  ..........
#KP152231_UYO206/NG/2014             ..........  ..........  ..........  ..........  ..........
#KP152232_KWARA171/NG/2014           ..........  ..........  ..........  ..........  ..........
                                        Major peak B  Serine-rich
                                                      Heptapeptide
                                                      region
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                                    360        370        380        390        400
#vvIBDV_UK_661                      GSVVTVAGVS NFELIPNPEL AKNLVTEYGR FDPGAMNYTK LILSERDRLG
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#KP152319_UYO166/NG/2014            .......... .......... .......... .......... ..........
#KP1522318_PLATEAU164/NG/2014       .......... .......... .......... .......... ..........
#KP152317_PLATEAU163/NG/2014        .......... .......... .......... .......... ..........
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#KP152313_PLATEAU159/NG/2014        .......... .......... .......... .......... ..........
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#KP152311_PLATEAU157/NG/2014        .......... .......... .......... .......... ..........
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#KP152302_KWARA150/NG/2014          .......... .......... .......... .......... ..........
#KP152301_KWARA149/NG/2014          .......... .......... .......... .......... ..........
#KP152300_KWARA148/NG/2014          .......... .......... .......... .......... ..........
#KP152299_KWARA147/NG/2014          .......... .......... .......... .......... ..........
#KP152298_KWARA145/NG/2014          .......... .......... .......... .......... ..........
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#KP266329_BAUCHI37/NG/2012          .......... .......... .......... .......... ..........
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#KP266323_KADUNA61/NG/2009          .......... .......... .......... .......... ..........
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#KP266324_BAUCHI58/NG/2012          .......... .......... .......... .......... ..........
#KP266325_PLATEAU67/NG/2011         .......... .......... .......... .......... ..........
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#KP152266_PLATEAU133/NG/2014        .......... .......... .......... .......... ..........
#KP152267_PLATEAU135/NG/2014        .......... .......... .......... .......... ..........
#KP266330_NASSARAWA77/NG/no2/_2013  .......... .......... .......... .......... ..........
#KP266331_NASSARAWA77/NG/2013       .......... .......... .......... .......... ..........
#KP266332_PLATEAU78/NG/2011/2       .......... .......... .......... .......... ..........
#KP266333_PLATEAU78/NG/2011/1       .......... .......... .......... .......... ..........
#KP152270_BENIN142E/NG/2013         .......... .......... .......... .......... ..........
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#KP152275_PLATEAU83/NG/2013         .......... .......... .......... .......... ..........
#KP152276_PLATEAU90/NG/2013         .......... .......... .......... .......... ..........
#KP152277_PLATEAU95/NG/2013         .......... .......... .......... .......... ..........
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#KP152278_PLATEAU96/NG/2013          .......... .......... .......... .......... ..........
#KP152281_PLATEAU131/NG/2014         .......... .......... .......... .......... ..........
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#KP152283_PLATEAU110/NG/2010         .......... .......... .......... .......... ..........
#KP152284_PLATEAU104/NG/2010         .......... .......... .......... .......... ..........
#KP152285_BAUCHI121/NG/2013          .......... .......... .......... .......... ..........
#KP152286_KWARA139/NG/2014           .......... .......... .......... .......... ..........
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#KP152289_KWARA141/NG/2014           .......... .......... .......... .......... ..........
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#KP152295_ANAMBRA50/NG/2012          .......... .......... .......... .......... ..........
#KP152294_BENIN34/NG/2012            .......... .......... .......... .......... ..........
#vvIBDV_OKYM                         .......... .......... .......... .......... ..........
#Variant_DelE                        .......... .......... .......... .......... ..........
#Variant_T1                          .......... .......... .......... .......... ..........
#Classic_STC                         .......... .......... .......... .......... ..........
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#KP152239_KWARA180/NG/2014           .......... .......... .......... .......... ..........
#KP152240_KWARA181/NG/2014           .......... .......... .......... .......... ..........
#KP152241_KWARA182/NG/2014           .......... .......... .......... .......... ..........
#KP152242_KWARA184/NG/2014           .......... .......... .......... .......... ..........
#KP152243_UYO185/NG/2014             .......... .......... .......... .......... ..........
#KP152244_UYO187/NG/2014             .......... .......... .......... .......... ..........
#KP152245_UYO188/NG/2014             .......... .......... .......... .......... ..........
#KP152246_UYO189/NG/2014             .......... .......... .......... .......... ..........
#KP152247_UYO190/NG/2014             .......... .......... .......... .......... ..........
#KP152248_UYO191/NG/2014             .......... .......... .......... .......... ..........
#KP152249_UYO192/NG/2014             .......... .......... .......... .......... ..........
#KP152250_UYO193/NG/2014             .......... .......... .......... .......... ..........
#KP152251_UYO194/NG/2014             .......... .......... .......... .......... ..........
#KP152252_UYO195/NG/2014             .......... .......... .......... .......... ..........
#KP152253_UYO196/NG/2014             .......... .......... .......... .......... ..........
#KP152254_UYO197/NG/2014             .......... .......... .......... .......... ..........
#KP152255_UYO198/NG/2014             .......... .......... .......... .......... ..........
#KP152256_UYO199/NG/2014             .......... .......... .......... .......... ..........
#KP152257_UYO200/NG/2014             .......... .......... .......... .......... ..........
#KP152258_UYO201/NG/2014             .......... .......... .......... .......... ..........
#KP152259_UYO202/NG/2014             .......... .......... .......... .......... ..........
#KP152260_UYO203/NG/2014             .......... .......... .......... .......... ..........
#KP152261_UYO204/NG/2014             .......... .......... .......... .......... ..........
#KP152322_UYO205/NG/2014             .......... .......... .......... .......... ..........
#KP152231_UYO206/NG/2014             .......... .......... .......... .......... ..........
#KP152232_KWARA171/NG/2014           .......... .......... .......... .......... ..........
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                                    410        420        430        440
#vvIBDV_UK_661                       IKTVWPTREY TDFREYFMEV ADLNSPLKIA GAFG
#KP152296_NIG20/NG/2009              .......... .......... .......... ....
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#KP152293_PLATEAU43/NG/2012          ......K... .......... .......... ....
#KP152321_NIG79/NG/2009              .......... .......... .......... ....
#KP152320_UYO167/NG/2014             .......... .......... .......... ....
#KP152319_UYO166/NG/2014             .......... .......... .......... ....
#KP1522318_PLATEAU164/NG/2014        .......... .......... .......... ....
#KP152317_PLATEAU163/NG/2014         .......... .......... .......... ....
#KP152316_PLATEAU162/NG/2014         .......... .......... .......... ....
#KP152315_PLATEAU161/NG/2014         .......... .......... .......... ....
#KP152314_PLATEAU160/NG/2014         .......... .......... .......... ....
#KP152313_PLATEAU159/NG/2014         .......... .......... .......... ....
#KP152312_PLATEAU158/NG/2014         .......... .......... .......... ....
#KP152311_PLATEAU157/NG/2014         .......... .......... .......... ....
#KP152310_PLATEAU156/NG/2014         ........... ..V....K.. .......... ....
#KP152309_PLATEAU155/NG/2014         .......... .......... .......... ....
#KP152308_PLATEAU154/NG/2014         .......... .......... .......... ....
#KP152306_PLATEAU16/NG/2009          .......... .......... .......... ....
#KP152292_PLATEAU4/NG/2009           .......... .......... .......... ....
#KP152304_KWARA152/NG/2014           .......... .......... .......... ....
#KP152303_KWARA151NG/NG/2014         .......... .......... .......... ....
#KP152302_KWARA150/NG/2014           .......... .......... ........... .P..
#KP152301_KWARA149/NG/2014           .......... .......... .......... ....
#KP152300_KWARA148/NG/2014           .......... .......... .......... ....
#KP152299_KWARA147/NG/2014           .......... .......... .......... ....
#KP152298_KWARA145/NG/2014           .......... .......... .......... ....
#KP152297_BENIN47/NG/2014            .......... .......... .......... ....
#KP266329_BAUCHI37/NG/2012           .......... .......... .......... ....
#KP152268_BAUCHI36/NG/2009           .......... .......... .......... ....
#KP266322_PLATEAU19/NG/2009          .......... .......... .......... ....
#KP266323_KADUNA61/NG/2009           .......... .......... .......... ....
#KP266334_PLATEAU39/NG/2012          .......... .......... .......... ....
#KP266324_BAUCHI58/NG/2012           .......... .......... .......... ....
#KP266325_PLATEAU67/NG/2011          .......... .......... .......... ....
#KP266326_PLATEAU53/NG/2010          .......... .......... .......... ....
#KP266327_ANAMBRA54/NG/2012          .......... .......... .......... ....
#KP152305_PLATEAU7/NG/2009           .......... .......... .......... ....
#KP266328_BENIN35/NG/2012            .......... .......... .......... ....
#KP152291_BAUCHI37/NG/2009           .......... .......... .......... ....
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#KP152263_BAUCHI126/NG/2014          .......... .......... .......... ....
#KP152264_BAUCHI127/NG/2014          .......... .......... .......... ....
#KP152265_KADUNA136/NG/2014          .......... .......... .......... ....
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#KP152267_PLATEAU135/NG/2014         .......... .......... .......... ....
#KP266330_NASSARAWA77/NG/no2/_2013   .......... .......... .......... ....
#KP266331_NASSARAWA77/NG/2013        .......... .......... .......... ....
#KP266332_PLATEAU78/NG/2011/2        .......... .......... .......... ....
#KP266333_PLATEAU78/NG/2011/1        .......... .......... .......... ....
#KP152270_BENIN142E/NG/2013          .......... .......... .......... ....
#KP152269_BENIN87/NG/2013            .......... .......... .......... ....
#KP152271_ABUJA93/NG/2013            .......... .......... .......... ....
#KP152272_BAUCHI128/NG/2014          .......... .......... .......... ....
#KP152273_PLATEAU99/NG/2011          .......... .......... .......... ....
#KP152274_PLATEAU92/NG/2013          .......... .......... .......... ....
#KP152275_PLATEAU83/NG/2013          .......... .......... .......... ....
#KP152276_PLATEAU90/NG/2013          .......... .......... .......... ....
#KP152277_PLATEAU95/NG/2013          .......... .......... .......... ....
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#KP152278_PLATEAU96/NG/2013          .......... .......... .......... ....
#KP152281_PLATEAU131/NG/2014         .......... .......... .......... ....
#KP152282_PLATEAU134/NG/2014         .......... .......... .......... ....
#KP152283_PLATEAU110/NG/2010         .......... .......... .......... ....
#KP152284_PLATEAU104/NG/2010         .......... .......... .......... ....
#KP152285_BAUCHI121/NG/2013          .......... .......... .......... ....
#KP152286_KWARA139/NG/2014           .......... .......... .......... ....
#KP152287_PLATEAU40/NG/2012          .......... .......... .......... ....
#KP152288_PLATEAU119/NG/2013         .......... .......... .......... ....
#KP152289_KWARA141/NG/2014           .......... .......... .......... ....
#KP152279_PLATEAU120/NG/2013         .......... .......... .......... ....
#KP152280_PLATEAU132/NG/2014         .......... .......... .......... ....
#KP152290_PLATEAU82/NG/2013          .......... .......... .......... ....
#KP152307_PLATEAU75/NG/2011          .......... .......... .......... ....
#KP152295_ANAMBRA50/NG/2012          T......... .......... .......... ....
#KP152294_BENIN34/NG/2012            .......... .......... .......... ....
#vvIBDV_OKYM                         .......... .......... .......... ....
#Variant_DelE                        .......... .......... .......... ....
#Variant_T1                          .......... .......... .......... ....
#Classic_STC                         .......... .......... .......... ....
#KP152234_KWARA175/NG/2014           .......... .......... .......... ....
#KP152235_KWARA176/NG/2014           .......... .......... .......... ....
#KP152236_KWARA177/NG/2014           .......... .......... .......... ....
#KP152237_KWARA178/NG/2014           .......... .......... .......... ....
#KP152238_KWARA179/NG/2014           .......... .......... .......... ....
#KP152239_KWARA180/NG/2014           .......... .......... .......... ....
#KP152240_KWARA181/NG/2014           .......... .......... .......... ....
#KP152241_KWARA182/NG/2014           .......... .......... .......... ....
#KP152242_KWARA184/NG/2014           .......... .......... .......... ....
#KP152243_UYO185/NG/2014             .......... .......... .......... ....
#KP152244_UYO187/NG/2014             .......... .......... .......... ....
#KP152245_UYO188/NG/2014             .......... .......... .......... ....
#KP152246_UYO189/NG/2014             .......... .......... .......... ....
#KP152247_UYO190/NG/2014             .......... .......... .......... ....
#KP152248_UYO191/NG/2014             .......... .......... .......... ....
#KP152249_UYO192/NG/2014             .......... .......... .......... ....
#KP152250_UYO193/NG/2014             .......... .......... .......... ....
#KP152251_UYO194/NG/2014             .......... .......... .......... ....
#KP152252_UYO195/NG/2014             .......... .......... .......... ....
#KP152253_UYO196/NG/2014             .......... .......... .......... ....
#KP152254_UYO197/NG/2014             .......... .......... .......... ....
#KP152255_UYO198/NG/2014             .......... .......... .......... ....
#KP152256_UYO199/NG/2014             .......... .......... .......... ....
#KP152257_UYO200/NG/2014             .......... .......... .......... ....
#KP152258_UYO201/NG/2014             .......... .......... .......... ....
#KP152259_UYO202/NG/2014             .......... .......... .......... ....
#KP152260_UYO203/NG/2014             .......... .......... .......... ....
#KP152261_UYO204/NG/2014             .......... .......... .......... ....
#KP152322_UYO205/NG/2014             .......... .......... .......... ....
#KP152231_UYO206/NG/2014             .......... .......... .......... ....
#KP152232_KWARA171/NG/2014           .......... .......... .......... ....
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- Major hydrophilic peak A, minor hydrophilic peak 1, 2, 3, major hydrophilic peak B, heptapeptide region.

Appendix 9: Amino acid sequence data for Nigerian IBDV viruses

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                                        211        221        231        241        251
#vvIBDV_UK_661                            ADDYQFSSQY QAGGVTITLF SANIDAITSL SIGGELVFQT SVQGLILGAT
#Variant_DelE                             ..N....... .T........ .......... .V......K. ...S.V....
#AJ586964.1_Vom_Vaccine                   .......... .P........ .......... .V........ ..H..V....
#AJ586959.1_IBDV/Oyo.NIE/00/042/c         .......... .......... .......... .......... ..........
#AJ586958.1_IBDV/Osun.NIE/00/046/c        .......... .......... .......... .......... ...S......
#AJ586957.1_IBDV/Oyo.NIE/99/054/c         .......... .......... .......... .......... ..........
#AJ586956.1_IBDV/Oyo.NIE/99/050/c         .......... .......... .......... .......... ..........
#AJ586955.1_IBDV/Oyo.NIE/99/015/c         .......... .......... .......... .......... ...S......
#AJ586954.1_IBDV/Osun.NIE/99/030/c        .......... .......... .......... .......... ..........
#AJ586953.1_IBDV/Ogun.NIE/99/034/c        .......... .......... .......... .......... ..........
#AJ586952.1_IBDV/Oyo.NIE/98/009/t         .......... .......... .......... .......... ..........
#AJ586950.1_IBDV/Oyo.NIE/98/059/c         .......... .......... .......... .......... ...S......
#AJ586949.1_IBDV/Oyo.NIE/98/227/c         .......... .......... .......... .......... ..........
#AJ586948.1_IBDV/Oyo.NIE/98/027/c         .......... .......... .......... .......... ..........
#AJ586946.1_IBDV/Osun.NIE/98/062c         .......... .T........ .......... .......... ..........
#AJ5869345.1_IBDV/Ogun.NIE/98/085/c       .......... .......... .......... .......... ...S......
#AJ586944.1_IBDV/Ogun.NIE/98/120/c        .......... .......... .......... .......... ..........
#AJ586943.1_IBDV/Oyo.NIE/97/012/t         .......... .......... .......... .......... ..........
#AJ586942.1_IBDV/Oyo.NIE/97/004/t         .......... .......... .......... .......... ...S......
#AJ586940.1_IBDV/Oyo.NIE/97/222/c         .......... .......... .......... .......... ..........
#AJ586935.1_IBDV/Osun.NIE/97/092/c        .......... .......... .......... .......... ..........
#AJ586934.1_IBDV/Ogun.NIE/97/082/c        .......... .......... .......... .......... ..........
#AJ586933.1_IBDV/Ogun.NIE/97/078/c        .......... .......... .......... .......... ...S......
#AJ586932.1_IBDV/Ogun.NIE/97/014/c        .......... .......... .......... .......... ..........
#AJ586930.1_IBDV/Lagos.NIE/97/011/c       .......... .......... .......... .......... ..........
#AJ586929.1_IBDV/Oyo.NIE/96/033/c         .......... .......... .......... .......... ..........
#AJ586928.1_IBDV/Oyo.NIE/96/077/c         .......... .......... .......... .......... ..........
#AJ586925.1_IBDV/Osun.NIE/96/036/c        .......... .......... .......... .......... ..........
#AJ586924.1_IBDV/Osun.NIE/96/076/c        .......... .......... .......... .......... ..........
#AJ586923.1_IBDV/Ogun.NIE/96/080/c        .......... .......... .......... .......... ..........
#AJ586922.1_IBDV/Ogun.NIE/96/086/c        .......... .......... .......... .......... ...S......
#AJ586920.1_IBDV/Oyo.NIE/95/016/c         .......... .......... .......... .......... ...S......
#AJ586917.1_IBDV/Ogun.NIE/95/007/c        .......... .......... .......... .......... ..........
#AF159215.1_N14                           .......... .......... .......... .......... ..........
#AF159213.1_N11                           .......... .......... .......... .......... ..........
#AF159209.1_N7                            .......... .......... .......... .......... ..........
#EF363552.1_G3                            .......... .......... .......... .......... ...S......
#EF363551.1_BK                            PN..H..... .......... .......... .......... ...S......
#JX424074.1_IBDV73/Kaduna.NG/2009         .......... .......... .......... .......... ...S......
#JX424073.1_IBDV71/Kaduna.NG/2010         .......... .......... .......... .......... ...S......
#JX424072.1_IBDV65/Kaduna.NG/2009         .......... .......... .......... .......... ...S......
#JX424070.1_IBDV63/Kaduna.NG2009          .......... .......... .......... .......... ...S......
#JX424068.1_IBDV59/Abuja.NG/2010           .......... .......... .......... .......... ..........
#JX424067.1_IBDV58/Kaduna.NG/2010          .......... .......... .......... .......... ...S......
#JX424064.1_IBDV43/Kaduna.NG/2011          .......... .......... .......... .......... ...S......
#JX424063.1_IBDV38/Kano.NG/2010            .......... .......... .......... .......... ...S......
#JX424062.1_IBDV37/Experimental/2009       .......... .......... .......... .......... ...S......
#JX424061.1_IBDV36/Katsina.NG/2010         .......... .......... .......... .......... ...S......
#JX424059.1_IBDV33/Abuja.NG/2011           .......... .......... .......... .......... ...S......
#JX424058.1_IBDV27/Kaduna.NG/2011          .......... .......... .......... .......... ...S......
#JX424055.1_IBDV17/Kaduna.NG/1998          .......... .......... .......... .......... ...S......
#JX424053.1_IBDV12/Katsina.NG/2010         .......... .......... .......... .......... ...S......
#JX424049.1_IBDV4/Kano.NG2011              .......... .......... .......... .......... ...S......
#Variant_T1                                ..N....... .T........ .......... .V......K. ...N.V....
#Classic_STC                               .......... .P........ .......... .V........ .....V....
#KP152296_NIG20/NG/2009                    .......... .......... .......... .......... ...S......
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#KP152321_NIG79/NG/2009                    .......... .......... .......... .......... ...S......
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#KP152320_UYO167/NG/2014                ..........  ..........  ..........  ..........  ...S......
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#KP152266_PLATEAU133/NG/2014            ........T.  ..........  ..........  ..........  ...S......
#KP152267_PLATEAU135/NG/2014            ........T.  ..........  ..........  ..........  ...S......
#KP266330_NASSARAWA77/NG/no2/_2013      ..........  ..........  ..........  .V........  ..HS.V....
#KP266331_NASSARAWA77/NG/2013           ..........  ..........  ..........  ..........  ..LS......
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#KP152290_PLATEAU82/NG/2013         ........T. .......... .......... .......... ...S......
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#KP152295_ANAMBRA50/NG/2012         ........T. .......... .......... .......... ...S......
#KP152294_BENIN34/NG/2012           .......... .......... .......... .......... ...S......
#vvIBDV_OKYM                        .......... .......... .......... .......... ..........
#KP152234_KWARA175/NG/2014          .......... .......... .......... .......... ...S......
#KP152235_KWARA176/NG/2014          .......... .......... .......... .......... ...S......
#KP152236_KWARA177/NG/2014          .......... .......... .......... .......... ...S......
#KP152237_KWARA178/NG/2014          .......... .......... .......... .......... ...S......
#KP152238_KWARA179/NG/2014          .......... .......... .......... .......... ...S......
#KP152239_KWARA180/NG/2014          .......... .......... .......... .......... ...S......
#KP152240_KWARA181/NG/2014          .......... .......... .......... .......... ...S......
#KP152241_KWARA182/NG/2014          .......... .......... .......... .......... ...S......
#KP152242_KWARA184/NG/2014          .......... .......... .......... .......... ...S......
#KP152243_UYO185/NG/2014            ........T. .......... .......... .......... ...S......
#KP152244_UYO187/NG/2014            .......... .......... .......... .......... ...S......
#KP152245_UYO188/NG/2014            ........T. .......... .......... .......... ...S......
#KP152246_UYO189/NG/2014            .......... .......... .......... .......... ...S......
#KP152247_UYO190/NG/2014            ........T. .......... .......... .......... ...S......
#KP152248_UYO191/NG/2014            .......... .......... .......... .......... ...S......
#KP152249_UYO192/NG/2014            .......... .......... .......... .......... ...S......
#KP152250_UYO193/NG/2014            .......... .......... .......... .......... ...S......
#KP152251_UYO194/NG/2014            .......... .......... .......... .......... ...S......
#KP152252_UYO195/NG/2014            .......... .......... .......... .......... ...S......
#KP152253_UYO196/NG/2014            .......... .......... .......... .......... ...S......
#KP152254_UYO197/NG/2014            .......... .......... .......... .......... ...S......
#KP152255_UYO198/NG/2014            .......... .......... .......... .......... ...S......
#KP152256_UYO199/NG/2014            .......... .......... .......... .......... ...S......
#KP152257_UYO200/NG/2014            .......... .......... .......... .......... ...S......
#KP152258_UYO201/NG/2014            .......... .......... .......... .......... ...S......
#KP152259_UYO202/NG/2014            .......... .......... .......... .......... ...S......
#KP152260_UYO203/NG/2014            .......... .......... .......... .......... ...S......
#KP152261_UYO204/NG/2014            .......... .......... .......... .......... ...S......
#KP152322_UYO205/NG/2014            .......... .......... .......... .......... ...S......
#KP152231_UYO206/NG/2014            .......... .......... .......... .......... ...S......
#KP152232_KWARA171/NG/2014          .......... .......... .......... .......... ...S......
                                   261        271        281        291        311
#vvIBDV_UK_661                      IYLIGFDGTA VITRAVAADN GLTAGTDNLM PFNIVIPTSE ITQPITSIKL
#Variant_DelE                       .......... ........N. .....I.... ...L....N. ..........
#AJ586964.1_Vom_Vaccine             .........T ........N. ...T...... ...L....N. ..........
#AJ586959.1_IBDV/Oyo.NIE/00/042/c   .......... .......... .......... .........A ..........
#AJ586958.1_IBDV/Osun.NIE/00/046/c  .......... .......... .......... .........Q ..........
#AJ586957.1_IBDV/Oyo.NIE/99/054/c   .......... .......... .......... .........A ..........
#AJ586956.1_IBDV/Oyo.NIE/99/050/c   .......... .......... .......... .........A ..........
#AJ586955.1_IBDV/Oyo.NIE/99/015/c   .......... .......... .......... .......... ..........
#AJ586954.1_IBDV/Osun.NIE/99/030/c  .......... .......... .......... .........A ..........
#AJ586953.1_IBDV/Ogun.NIE/99/034/c  .......... .......... .......... .........A ..........
#AJ586952.1_IBDV/Oyo.NIE/98/009/t   .......... .......... .......... .......... ..........
#AJ586950.1_IBDV/Oyo.NIE/98/059/c   .......... .......... .......... .........A ..........
#AJ586949.1_IBDV/Oyo.NIE/98/227/c   .......... .......... .......... .........A ..........
#AJ586948.1_IBDV/Oyo.NIE/98/027/c   .......... .......... .......... .........A ..........
#AJ586946.1_IBDV/Osun.NIE/98/062c   .......... .......... .......... .......... ..........
#AJ5869345.1_IBDV/Ogun.NIE/98/085/c .......... .......... .......... .........A ..........
#AJ586944.1_IBDV/Ogun.NIE/98/120/c  .......... .......... .......... .........A ..........
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#AJ586943.1_IBDV/Oyo.NIE/97/012/t       .......... .......... .......... .........A ..........
#AJ586942.1_IBDV/Oyo.NIE/97/004/t       .......... .......... .......... .........A ..........
#AJ586940.1_IBDV/Oyo.NIE/97/222/c       .......... .......... .......... .........A ..........
#AJ586935.1_IBDV/Osun.NIE/97/092/c      .......... .......... .......... .........A ..........
#AJ586934.1_IBDV/Ogun.NIE/97/082/c      .......... .......... .......... .........A ..........
#AJ586933.1_IBDV/Ogun.NIE/97/078/c      .......... .......... .......... .........A ..........
#AJ586932.1_IBDV/Ogun.NIE/97/014/c      .......... .......... .......... .........A ..........
#AJ586930.1_IBDV/Lagos.NIE/97/011/c     .......... .......... .......... .........A ..........
#AJ586929.1_IBDV/Oyo.NIE/96/033/c       .......... .......... .......... .........A ..........
#AJ586928.1_IBDV/Oyo.NIE/96/077/c       .......... .......... .......... .........A ..........
#AJ586925.1_IBDV/Osun.NIE/96/036/c      .......... .......... .......... .........A ..........
#AJ586924.1_IBDV/Osun.NIE/96/076/c      .......... .......... .......... .........A ..........
#AJ586923.1_IBDV/Ogun.NIE/96/080/c      .......... .......... .......... .........A ..........
#AJ586922.1_IBDV/Ogun.NIE/96/086/c      .......... .......... .......... .........A ..........
#AJ586920.1_IBDV/Oyo.NIE/95/016/c       .......... .......... .......... .........A ..........
#AJ586917.1_IBDV/Ogun.NIE/95/007/c      .......... .......... .......... .......... ..........
#AF159215.1_N14                         .......... .......... .......... .........A ..........
#AF159213.1_N11                         .......... .......... .......... .........A ..........
#AF159209.1_N7                          .......... .......... .......... .........A ..........
#EF363552.1_G3                          .......... .......... .......... .........A ..........
#EF363551.1_BK                          .......... .......... .......... .........A ..........
#JX424074.1_IBDV73/Kaduna.NG/2009       .......... .......... .......... .........A ..........
#JX424073.1_IBDV71/Kaduna.NG/2010       .......... .......... .......... .........A ..........
#JX424072.1_IBDV65/Kaduna.NG/2009       .......... .......... .......... .........A ..........
#JX424070.1_IBDV63/Kaduna.NG2009        .......... .......... .......... .........A ..........
#JX424068.1_IBDV59/Abuja.NG/2010        ........... .T......N. .......... .......... ..........
#JX424067.1_IBDV58/Kaduna.NG/2010       .......... .......... .......... .........A ..........
#JX424064.1_IBDV43/Kaduna.NG/2011       .......... .......... .......... .........A ..........
#JX424063.1_IBDV38/Kano.NG/2010         .......... .......... .......... .........A ..........
#JX424062.1_IBDV37/Experimental/2009    .......... .......... .......... .........A ..........
#JX424061.1_IBDV36/Katsina.NG/2010      .......... .......... .......... .........A ..........
#JX424059.1_IBDV33/Abuja.NG/2011        .......... .......... .......... .........A ..........
#JX424058.1_IBDV27/Kaduna.NG/2011       .......... .......... .......... .........A ..........
#JX424055.1_IBDV17/Kaduna.NG/1998       .......... .......... .......... .........A ..........
#JX424053.1_IBDV12/Katsina.NG/2010      .......... .......... .......... .........A ..........
#JX424049.1_IBDV4/Kano.NG2011           .......... .......... .......... .........A ..........
#Variant_T1                             .......... ........N. .....I.... ...L....N. ..........
#Classic_STC                            ..F......T .......... .......... ...L....N. ..........
#KP152296_NIG20/NG/2009                 .......... .......... .......... .........A ..........
#KP152233_KWARA174/NG/2014              .......... .......... .......... .........A ..........
#KP152293_PLATEAU43/NG/2012             ........S. .......... .......... .........Q ..........
#KP152321_NIG79/NG/2009                 .......... .......... .......... .........A ..........
#KP152320_UYO167/NG/2014                .......... .......... .......... .........A ..........
#KP152319_UYO166/NG/2014                .......... .......... .......... .........A ..........
#KP1522318_PLATEAU164/NG/2014           .......... .......... .......... .........A ..........
#KP152317_PLATEAU163/NG/2014            .......... .......... .......... .........A ..........
#KP152316_PLATEAU162/NG/2014            .......... .......... .......... .........A ..........
#KP152315_PLATEAU161/NG/2014            .......... .......... .......... .........A ..........
#KP152314_PLATEAU160/NG/2014            ........S. .......... .......... .........Q ..........
#KP152313_PLATEAU159/NG/2014            .......... .......... .......... .........A ..........
#KP152312_PLATEAU158/NG/2014            .......... .......... .......... .........A ..........
#KP152311_PLATEAU157/NG/2014            .......... .......... .......... .........A ..........
#KP152310_PLATEAU156/NG/2014            ........S. .......... .......... .........Q ..........
#KP152309_PLATEAU155/NG/2014            .......... .......... .......... .........A ..........
#KP152308_PLATEAU154/NG/2014            .......... .......... .......... .........A ..........
#KP152306_PLATEAU16/NG/2009             .......... .......... .......... .........A ..........
#KP152292_PLATEAU4/NG/2009              .......... .......... .......... .........A ..........
#KP152304_KWARA152/NG/2014              .......... .......... .......... .........A ..........
#KP152303_KWARA151NG/NG/2014            .......... .......... .......... .........A ..........
#KP152302_KWARA150/NG/2014              .......... .......... .......... .........A ..........
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#KP152301_KWARA149/NG/2014          .......... .......... .......... .........A ..........
#KP152300_KWARA148/NG/2014          .......... .T......N. .......... .......... ..........
#KP152299_KWARA147/NG/2014          .......... .......... .......... .........A ..........
#KP152298_KWARA145/NG/2014          .......... .......... .......... .........A ..........
#KP152297_BENIN47/NG/2014           .......... .......... .......... .........A ..........
#KP266329_BAUCHI37/NG/2012          .......... .......... .......... .........A ..........
#KP152268_BAUCHI36/NG/2009          .......... .......... .......... .........A ..........
#KP266322_PLATEAU19/NG/2009         .......... .......... .......... .........A ..........
#KP266323_KADUNA61/NG/2009          .......... .......... .......... .........A ..........
#KP266334_PLATEAU39/NG/2012         .......... .......... .......... .........A ..........
#KP266324_BAUCHI58/NG/2012          ........S. .......... .......... .........Q ..........
#KP266325_PLATEAU67/NG/2011         .......... .......... .......... .........A ..........
#KP266326_PLATEAU53/NG/2010         .......... .......... .......... .........A ..........
#KP266327_ANAMBRA54/NG/2012         ........S. .......... .......... .........Q ..........
#KP152305_PLATEAU7/NG/2009          .......... .......... .......... .........A ..........
#KP266328_BENIN35/NG/2012           .......... .......... .......... .........A ..........
#KP152291_BAUCHI37/NG/2012          .......... .......... .......... .........A ..........
#KP152262_BAUCHI124/NG/2014         .......... .......... .......... .........A ..........
#KP152263_BAUCHI126/NG/2014         .......... .......... .......... .........A ..........
#KP152264_BAUCHI127/NG/2014         .......... .......... .......... .........A ..........
#KP152265_KADUNA136/NG/2014         ........S. .......... .......... .........Q ..........
#KP152266_PLATEAU133/NG/2014        ........S. .......... .......... .........Q ..........
#KP152267_PLATEAU135/NG/2014        ........S. .......... .......... .........Q ..........
#KP266330_NASSARAWA77/NG/no2/_2013  .......... ........N. ...T...... ...L....N. ..........
#KP266331_NASSARAWA77/NG/2013       ........S. .......... .......... .........Q ..........
#KP266332_PLATEAU78/NG/2011/2       ........S. .......... .......... .........Q ..........
#KP266333_PLATEAU78/NG/2011/1       ........S. .......... .......... .........Q ..........
#KP152270_BENIN142E/NG/2013         .......... .T......N. .......... .......... ..........
#KP152269_BENIN87/NG/2013           .......... .......... .......... .........A ..........
#KP152271_ABUJA93/NG/2013           .......... .......... .......... .........A ..........
#KP152272_BAUCHI128/NG/2014         .......... .......... .......... .........A ..........
#KP152273_PLATEAU99/NG/2011         .......... .......... .......... .........A ..........
#KP152274_PLATEAU92/NG/2013         T......... .......... .......... .........A ..........
#KP152275_PLATEAU83/NG/2013         .......... .......... .......... .........A ..........
#KP152276_PLATEAU90/NG/2013         .......... .......... .......... .........A ..........
#KP152277_PLATEAU95/NG/2013         .......... .......... .......... .........A ..........
#KP152278_PLATEAU96/NG/2013         .......... .......... .......... .........A ..........
#KP152281_PLATEAU131/NG/2014        .......... .......... .......... .........A ..........
#KP152282_PLATEAU134/NG/2014        .......... .......... .......... .........A ..........
#KP152283_PLATEAU110/NG/2010        .......... .......... .......... .........A ..........
#KP152284_PLATEAU104/NG/2010        .......... .......... .......... .........A ..........
#KP152285_BAUCHI121/NG/2013         .......... .......... .......... .........A ..........
#KP152286_KWARA139/NG/2014          .......... .......... .......... .........A ..........
#KP152287_PLATEAU40/NG/2012         .......... .T......N. .......... .......... ..........
#KP152288_PLATEAU119/NG/2013        ........S. .......... .......... .........Q ..........
#KP152289_KWARA141/NG/2014          .......... .T......N. .......... .......... ..........
#KP152279_PLATEAU120/NG/2013        ........S. .......... .......... .........Q ..........
#KP152280_PLATEAU132/NG/2014        ........S. .......... .......... .........Q ..........
#KP152290_PLATEAU82/NG/2013         ........S. .......... .......... .........Q ..........
#KP152307_PLATEAU75/NG/2011         .......... .......... .......... .........A ..........
#KP152295_ANAMBRA50/NG/2012         ........S. .......... .......... .........Q ..........
#KP152294_BENIN34/NG/2012           .......... .......... .......... .........A ..........
#vvIBDV_OKYM                        .......... .......... .......... .......... ..........
#KP152234_KWARA175/NG/2014          .......... .......... .......... .........A ..........
#KP152235_KWARA176/NG/2014          .......... .......... .......... .........A ..........
#KP152236_KWARA177/NG/2014          .......... .......... .......... .........A ..........
#KP152237_KWARA178/NG/2014          .......... .......... .......... .........A ..........
#KP152238_KWARA179/NG/2014          .......... .......... .......... .........A ..........
#KP152239_KWARA180/NG/2014          .......... .......... .......... .........A ..........
#KP152240_KWARA181/NG/2014          .......... .......... .......... .........A ..........
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#KP152241_KWARA182/NG/2014          .......... .......... .......... .........A ..........
#KP152242_KWARA184/NG/2014          .......... .......... .......... .........A ..........
#KP152243_UYO185/NG/2014            ........S. .......... .......... .........Q ..........
#KP152244_UYO187/NG/2014            .......... .......... .......... .........A ..........
#KP152245_UYO188/NG/2014            ........S. .......... .......... .........Q ..........
#KP152246_UYO189/NG/2014            .......... .......... .......... .........A ..........
#KP152247_UYO190/NG/2014            ........S. .......... .......... .........Q ..........
#KP152248_UYO191/NG/2014            .......... .......... .......... .........A ..........
#KP152249_UYO192/NG/2014            .......... .......... .......... .........A ..........
#KP152250_UYO193/NG/2014            .......... .......... .......... .........A ..........
#KP152251_UYO194/NG/2014            .......... .......... .......... .........A ..........
#KP152252_UYO195/NG/2014            .......... .......... .......... .........A ..........
#KP152253_UYO196/NG/2014            .......... .......... .......... .........A ..........
#KP152254_UYO197/NG/2014            .......... .......... .......... .........A ..........
#KP152255_UYO198/NG/2014            .......... .......... .......... .........A ..........
#KP152256_UYO199/NG/2014            .......... .......... .......... .........A ..........
#KP152257_UYO200/NG/2014            .......... .......... .......... .........A ..........
#KP152258_UYO201/NG/2014            .......... .......... .......... .........A ..........
#KP152259_UYO202/NG/2014            .......... .......... .......... .........A ..........
#KP152260_UYO203/NG/2014            .......... .......... .......... .........A ..........
#KP152261_UYO204/NG/2014            .......... .......... .......... .........A ..........
#KP152322_UYO205/NG/2014            .......... .......... .......... .........A ..........
#KP152231_UYO206/NG/2014            .......... .......... .......... .........A ..........
#KP152232_KWARA171/NG/2014          .......... .......... .......... .........A ..........
                                  321        331        341        351        361
#vvIBDV_UK_661                      EIVTSKSGGQ AGDQMSWSAS GSLAVTIHGG NYPGALRPVT LVAY
#Variant_DelE                       .......D.. ..E....... .......... .......... ....
#AJ586964.1_Vom_Vaccine             .......... .........R .......... .......... ....
#AJ586959.1_IBDV/Oyo.NIE/00/042/c   .......... .......... .......... .......... ....
#AJ586958.1_IBDV/Osun.NIE/00/046/c  .......... .......... .......... .......... ....
#AJ586957.1_IBDV/Oyo.NIE/99/054/c   .......... .......... .......... .......... ....
#AJ586956.1_IBDV/Oyo.NIE/99/050/c   .......... .......... .......... .......... ....
#AJ586955.1_IBDV/Oyo.NIE/99/015/c   .......... .......... .......... .......... ....
#AJ586954.1_IBDV/Osun.NIE/99/030/c  .......... .......... .......... .......... ....
#AJ586953.1_IBDV/Ogun.NIE/99/034/c  .......... .......... .......... .......... ....
#AJ586952.1_IBDV/Oyo.NIE/98/009/t   .......... .......... .......... .......... ....
#AJ586950.1_IBDV/Oyo.NIE/98/059/c   .......... .......... .......... .......... ....
#AJ586949.1_IBDV/Oyo.NIE/98/227/c   .......... .......... .......... .......... .I..
#AJ586948.1_IBDV/Oyo.NIE/98/027/c   .......... .......... .......... .......... ....
#AJ586946.1_IBDV/Osun.NIE/98/062c   .......... .......... .......... .......... ....
#AJ5869345.1_IBDV/Ogun.NIE/98/085/c .......... .......... .......... .......... ....
#AJ586944.1_IBDV/Ogun.NIE/98/120/c  .......... .......... .......... .......... ....
#AJ586943.1_IBDV/Oyo.NIE/97/012/t   .......... .......... .......... .......... ....
#AJ586942.1_IBDV/Oyo.NIE/97/004/t   .......... .......... .......... .......... ....
#AJ586940.1_IBDV/Oyo.NIE/97/222/c   .......... .......... .......... .......... ....
#AJ586935.1_IBDV/Osun.NIE/97/092/c  .......... .......... .......... .......... ....
#AJ586934.1_IBDV/Ogun.NIE/97/082/c  .......... .......... .......... .......... ....
#AJ586933.1_IBDV/Ogun.NIE/97/078/c  .......... .......... .......... .......... ....
#AJ586932.1_IBDV/Ogun.NIE/97/014/c  .......... .......... .......... .......... ....
#AJ586930.1_IBDV/Lagos.NIE/97/011/c .......... .......... .......... .......... ....
#AJ586929.1_IBDV/Oyo.NIE/96/033/c   .......... .......... .......... .......... ....
#AJ586928.1_IBDV/Oyo.NIE/96/077/c   .......... .......... .......... .......... ....
#AJ586925.1_IBDV/Osun.NIE/96/036/c  .......... .......... .......... .......... ....
#AJ586924.1_IBDV/Osun.NIE/96/076/c  .......... .......... .......... .......... ....
#AJ586923.1_IBDV/Ogun.NIE/96/080/c  .......... .......... .......... .......... ....
#AJ586922.1_IBDV/Ogun.NIE/96/086/c  .......... .......... .......... .......... ....
#AJ586920.1_IBDV/Oyo.NIE/95/016/c   .......... .......... .......... .......... ....
#AJ586917.1_IBDV/Ogun.NIE/95/007/c  .......... .......... .......... .......... ....
#AF159215.1_N14                     .......... .......... .......... .......... ....
#AF159213.1_N11                     .......... .......... .......... .......... ....
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#AF159209.1_N7                          .......... .......... .......... .......... ....
#EF363552.1_G3                          .......... .......... .......... .......... ....
#EF363551.1_BK                          .......... .......... .......... .......... ....
#JX424074.1_IBDV73/Kaduna.NG/2009       .......... .......... .......... .......... ....
#JX424073.1_IBDV71/Kaduna.NG/2010       .......... .......... .......... .......... ....
#JX424072.1_IBDV65/Kaduna.NG/2009       .......... .......... .......... .......... ....
#JX424070.1_IBDV63/Kaduna.NG2009        .......... .......... .......... .......... ....
#JX424068.1_IBDV59/Abuja.NG/2010        .......... .......... .......... .......... ....
#JX424067.1_IBDV58/Kaduna.NG/2010       .......... .......... .......... .......... ....
#JX424064.1_IBDV43/Kaduna.NG/2011       .......... .......... .......... .......... ....
#JX424063.1_IBDV38/Kano.NG/2010         .......... .......... .......... .......... ....
#JX424062.1_IBDV37/Experimental/2009    .......... .......... .......... .......... ....
#JX424061.1_IBDV36/Katsina.NG/2010      .......... .......... .......... .......... ....
#JX424059.1_IBDV33/Abuja.NG/2011        .......... .......... .......... .......... ....
#JX424058.1_IBDV27/Kaduna.NG/2011       .......... .......... .......... .......... ....
#JX424055.1_IBDV17/Kaduna.NG/1998       .......... .......... .......... .......... ....
#JX424053.1_IBDV12/Katsina.NG/2010      .......... .......... .......... .......... ....
#JX424049.1_IBDV4/Kano.NG2011           .......... .......... .......... .......... ....
#Variant_T1                             .......N.. .E........ .......... .......... ....
#Classic_STC                            .V........ .......... .......... .......... ....
#KP152296_NIG20/NG/2009                 .......... .......... .......... .......... ....
#KP152233_KWARA174/NG/2014              .......... .......... .......... .......... ....
#KP152293_PLATEAU43/NG/2012             .......... .......... .......... .......... ....
#KP152321_NIG79/NG/2009                 .......... .......... .......... .......... ....
#KP152320_UYO167/NG/2014                .......... .......... .......... .......... ....
#KP152319_UYO166/NG/2014                .......... .......... .......... .......... ....
#KP1522318_PLATEAU164/NG/2014           .......... .......... .......... .......... ....
#KP152317_PLATEAU163/NG/2014            .......... .......... .......... .......... ....
#KP152316_PLATEAU162/NG/2014            .......... .......... .......... .......... ....
#KP152315_PLATEAU161/NG/2014            .......... .......... .......... .......... ....
#KP152314_PLATEAU160/NG/2014            .......... .......... .......... .......... ....
#KP152313_PLATEAU159/NG/2014            .......... .......... .......... .......... ....
#KP152312_PLATEAU158/NG/2014            .......... .......... .......... .......... ....
#KP152311_PLATEAU157/NG/2014            .......... .......... .......... .......... ....
#KP152310_PLATEAU156/NG/2014            .......... .......... .......... .......... ....
#KP152309_PLATEAU155/NG/2014            .......... .......... .......... .......... ....
#KP152308_PLATEAU154/NG/2014            .......... .......... .......... .......... ....
#KP152306_PLATEAU16/NG/2009             .......... .......... .......... .......... ....
#KP152292_PLATEAU4/NG/2009              .......... .......... .......... .......... ....
#KP152304_KWARA152/NG/2014              .......... .......... .......... .......... ....
#KP152303_KWARA151NG/NG/2014            .......... .......... .......... .......... ....
#KP152302_KWARA150/NG/2014              .......... .......... .......... .......... ....
#KP152301_KWARA149/NG/2014              .......... .......... .......... .......... ....
#KP152300_KWARA148/NG/2014              .......... .......... .......... .......... ....
#KP152299_KWARA147/NG/2014              .......... .......... .......... .......... ....
#KP152298_KWARA145/NG/2014              .......... .......... .......... .......... ....
#KP152297_BENIN47/NG/2014               .......... .......... .......... .......... ....
#KP266329_BAUCHI37/NG/2012              .......... .......... .......... .......... ....
#KP152268_BAUCHI36/NG/2009              .......... .......... .......... .......... ....
#KP266322_PLATEAU19/NG/2009             .......... .......... .......... .......... ....
#KP266323_KADUNA61/NG/2009              .......... .......... .......... .......... ....
#KP266334_PLATEAU39/NG/2012             .......... .......... .......... .......... ....
#KP266324_BAUCHI58/NG/2012              .......... .......... .......... .......... ....
#KP266325_PLATEAU67/NG/2011             .......... .......... .......... .......... ....
#KP266326_PLATEAU53/NG/2010             .......... .......... .......... .......... ....
#KP266327_ANAMBRA54/NG/2012             .......... .......... .......... .......... ....
#KP152305_PLATEAU7/NG/2009              .......... .......... .......... .......... ....
#KP266328_BENIN35/NG/2012               .......... .......... .......... .......... ....
#KP152291_BAUCHI37/NG/2012              .......... .......... .......... .......... ....
#KP152262_BAUCHI124/NG/2014             .......... .......... .......... .......... ....
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#KP152263_BAUCHI126/NG/2014            .......... .......... .......... .......... ....
#KP152264_BAUCHI127/NG/2014            .......... .......... .......... .......... ....
#KP152265_KADUNA136/NG/2014            .......... .......... .......... .......... ....
#KP152266_PLATEAU133/NG/2014           .......... .......... .......... .......... ....
#KP152267_PLATEAU135/NG/2014           .......... .......... .......... .......... ....
#KP266330_NASSARAWA77/NG/no2/_2013     .......... .......... .......... .......... ....
#KP266331_NASSARAWA77/NG/2013          .......... .........R .......... .......... ....
#KP266332_PLATEAU78/NG/2011/2          .......... .........R .......... .......... ....
#KP266333_PLATEAU78/NG/2011/1          .......... .......... .......... .......... ....
#KP152270_BENIN142E/NG/2013            .......... .......... .......... .......... ....
#KP152269_BENIN87/NG/2013              .......... .......... .......... .......... ....
#KP152271_ABUJA93/NG/2013              .......... .......... .......... .......... ....
#KP152272_BAUCHIi128/NG/2014           .......... .......... .......... .......... ....
#KP152273_PLATEAU99/NG/2011            .......... .......... .......... .......... ....
#KP152274_PLATEAU92/NG/2013            .......... .......... .......... .......... ....
#KP152275_PLATEAU83/NG/2013            .......... .......... .......... .......... ....
#KP152276_PLATEAU90/NG/2013            .......... .......... .......... .......... ....
#KP152277_PLATEAU95/NG/2013            .......... .......... .......... .......... ....
#KP152278_PLATEAU96/NG/2013            .......... .......... .......... .......... ....
#KP152281_PLATEAU131/NG/2014           .......... .......... .......... .......... ....
#KP152282_PLATEAU134/NG/2014           .......... .......... .......... .......... ....
#KP152283_PLATEAU110/NG/2010           .......... .......... .......... .......... ....
#KP152284_PLATEAU104/NG/2010           .......... .......... .......... .......... ....
#KP152285_BAUCHI121/NG/2013            .......... .......... .......... .......... ....
#KP152286_KWARA139/NG/2014             .......... .......... .......... .......... ....
#KP152287_PLATEAU40/NG/2012            .......... .......... .......... .......... ....
#KP152288_PLATEAU119/NG/2013           .......... .......... .......... .......... ....
#KP152289_KWARA141/NG/2014             .......... .......... .......... .......... ....
#KP152279_PLATEAU120/NG/2013           .......... .......... .......... .......... ....
#KP152280_PLATEAU132/NG/2014           .......... .......... .......... .......... ....
#KP152290_PLATEAU82/NG/2013            .......... .......... .......... .......... ....
#KP152307_PLATEAU75/NG/2011            .......... .......... .......... .......... ....
#KP152295_ANAMBRA50/NG/2012            .......... .......... .......... .......... ....
#KP152294_BENIN34/NG/2012              .......... .......... .......... .......... ....
#vvIBDV_OKYM                           .......... .......... .......... .......... ....
#KP152234_KWARA175/NG/2014             .......... .......... .......... .......... ....
#KP152235_KWARA176/NG/2014             ....T..... .......... .......... .......... ....
#KP152236_KWARA177/NG/2014             .......... .......... .......... .......... ....
#KP152237_KWARA178/NG/2014             ....T..... .......... .......... .......... ....
#KP152238_KWARA179/NG/2014             .......... .......... .......... .......... ....
#KP152239_KWARA180/NG/2014             .......... .......... .......... .......... ....
#KP152240_KWARA181/NG/2014             .......... .......... .......... .......... ....
#KP152241_KWARA182/NG/2014             .......... .......... .......... .......... ....
#KP152242_KWARA184/NG/2014             .......... .......... .......... .......... ....
#KP152243_UYO185/NG/2014               .......... .......... .......... .......... ....
#KP152244_UYO187/NG/2014               .......... .......... .......... .......... ....
#KP152245_UYO188/NG/2014               .......... .......... .......... .......... ....
#KP152246_UYO189/NG/2014               .......... .......... .......... .......... ....
#KP152247_UYO190/NG/2014               .......... .......... .......... .......... ....
#KP152248_UYO191/NG/2014               .......... .......... .......... .......... ....
#KP152249_UYO192/NG/2014               .......... .......... .......... .......... ....
#KP152250_UYO193/NG/2014               .......... .......... .......... .......... ....
#KP152251_UYO194/NG/2014               .......... .......... .......... .......... ....
#KP152252_UYO195/NG/2014               .......... .......... .......... .......... ....
#KP152253_UYO196/NG/2014               .......... .......... .......... .......... ....
#KP152254_UYO197/NG/2014               .......... .......... .......... .......... ....
#KP152255_UYO198/NG/2014               .......... .......... .......... .......... ....
#KP152256_UYO199/NG/2014               .......... .......... .......... .......... ....
#KP152257_UYO200/NG/2014               .......... .......... .......... .......... ....
#KP152258_UYO201/NG/2014               .......... .......... .......... .......... ....
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#KP152259_UYO202/NG/2014                ..........  ..........  ..........  ..........  ....
#KP152260_UYO203/NG/2014                ..........  ..........  ..........  ..........  ....
#KP152261_UYO204/NG/2014                ..........  ..........  ..........  ..........  ....
#KP152322_UYO205/NG/2014                ..........  ..........  ..........  ..........  ....
#KP152231_UYO206/NG/2014                ..........  ..........  ..........  ..........  ....
#KP152232_KWARA171/NG/2014              ..........  ..........  ..........  ..........  ....
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Appendix 10: Amino acid sequence data of Nigerian viruses used in this study and previously published Nigerian IBDV strains from the GenBank

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                                      220        230        240        250        260
#vvIBDV_UK_661                         YQAGGVTITL FSANIDAITS LSIGGELVFQ TSVQGLILGA TIYLIGFDGT
#AB306716.1_KTP-13                     .......... .......... .......... .......... ..........
#AB306715.1_KTG-8                      ..P....... .......... ..V......H ....S.V... .........S
#ABI97816.1 South_Africa_97_SA6        .......... .......... .......... .......... ..........
#ABI97814.1 South_Africa_97_SA3        .......... .......... .......... .......... ..........
#ABI97806.1 South_Africa_05_SA807      .......... .......... .......... ....S..... ..........
#ABI97804.1 South_Africa_05_SA10       .......... .......... .........K ....S.V... ..........
#AJ878887_99006/1552_SENEGAL           ..P....... .......... ..V....... ......V... ...F......
#AJ878888_88035.628_IVORY_COAST        ..Q....... .......... .......... .......... ..........
#AJ878889_88035.693_IVORY_COAST        ..Q....... .......... .......... .......... ..........
#AJ878890_88035.737_IVORY_COAST        ..Q....... .......... .......... .......... ..........
#Variant_DelE                          ..T....... .......... ..V......K ....S.V... ..........
#AJ583500_99323_EGYPT                  F......... .......... .......... ....S..... ..........
#KP152296_NIG20/NG/2009                .......... .......... .......... ....S..... ..........
#KP152233_KWARA174/NG/2014             .......... .......... .......... ....S..... ..........
#KP152293_PLATEAU43/NG/2012            .......... .......... .......... ....S..... .........S
#KP152321_NIG79/NG/2009                .......... .......... .......... ....S..... ..........
#KP152320_UYO167/NG/2014               .......... .......... .......... ....S..... ..........
#KP152319_UYO166/NG/2014               .......... .......... .......... ....S..... ..........
#KP1522318_PLATEAU164/NG/2014          .......... .......... .......... ....S..... ..........
#KP152317_PLATEAU163/NG/2014           .......... .......... .......... ....S..... ..........
#KP152316_PLATEAU162/NG/2014           .......... .......... .......... ....S..... ..........
#KP152315_PLATEAU161/NG/2014           .......... .......... .......... ....S..... ..........
#KP152314_PLATEAU160/NG/2014           .......... .......... .......... ....S..... .........S
#KP152313_PLATEAU159/NG/2014           .......... .......... .......... ....S..... ..........
#KP152312_PLATEAU158/NG/2014           .......... .......... .......... ....S..... ..........
#KP152311_PLATEAU157/NG/2014           .......... .......... .......... ....S..... ..........
#KP152310_PLATEAU156/NG/2014           .......... .......... .......... ....S..... .........S
#KP152309_PLATEAU155/NG/2014           .......... .......... .......... ....S..... ..........
#KP152308_PLATEAU154/NG/2014           .......... .......... .......... ....S..... ..........
#KP152306_PLATEAU16/NG/2009            .......... .......... .......... ....S..... ..........
#KP152292_PLATEAU4/NG/2009             .......... .......... .......... ....S..... ..........
#KP152304_KWARA152/NG/2014             .......... .......... .......... ....S..... ..........
#KP152303_KWARA151NG/NG/2014           .......... .......... .......... ....S..... ..........
#KP152302_KWARA150/NG/2014             .......... .......... .......... ....S..... ..........
#KP152301_KWARA149/NG/2014             .......... .......... .......... ....S..... ..........
#KP152300_KWARA148/NG/2014             .......... .......... .......... .......... ..........
#KP152299_KWARA147/NG/2014             .......... .......... .......... ....S..... ..........
#KP152298_KWARA145/NG/2014             .......... .......... .......... ....S..... ..........
#KP152297_BENIN47/NG/2014              .......... .......... .......... ....S..... ..........
#KP266329_BAUCHI37/NG/2012             ..T....... .......... .......... ....S..... ..........
#KP152268_BAUCHI36/NG/2009             .......... .......... .......... ....S..... ..........
#KP266322_PLATEAU19/NG/2009            .......... .......... .......... ....S..... ..........
#KP266323_Kaduna61/NG/2009             .......... .......... .......... ....S..... ..........
#KP266334_PLATEAU39/NG/2012            .......... .......... .......... ....S..... ..........
#KP266324_BAUCHI58/NG/2012             .......... .......... .......... ....S..... .........S
#KP266325_PLATEAU67/NG/2011            .......... .......... .......... ....S..... ..........
#KP266326_PLATEAU53/NG/2010            .......... .......... .......... ....S..... ..........
#KP266327_ANAMBRA54/NG/2012            .......... .......... .......... ....S..... .........S
#KP152305_Plateau7/NG/2009             .......... .......... .......... ....S..... ..........
#KP266328_BENIN35/NG/2012              .......... .......... .......... ....S..... ..........
#KP152291_BAUCHI37/NG/2009             ..T....... .......... .......... ....S..... ..........
#KP152262_BAUCHI124/NG/2014            .......... .......... .......... ....S..... ..........
#KP152263_BAUCHI126/NG/2014            .......... .......... .......... ....S..... ..........
#KP152264_BAUCHI127/NG/2014            .......... .......... .......... ....S..... ..........
#KP152265_KADUNA136/NG/2014            .......... .......... .......... ....S..... .........S
#KP152266_PLATEAU133/NG/2014           .......... .......... .......... ....S..... .........S
#KP152267_PLATEAU135/NG/2014           .......... .......... .......... ....S..... .........S
#KP266330_NASSARAWA77/NG/no2/_2013     .......... .......... ..V....... ...HS.V... ..........
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#KP266331_NASSARAWA77/NG/2013          .......... .......... .......... ...LS..... .........S
#KP266332_PLATEAU78/NG/2011/2          .......... .......... ..V....... ....S..... .........S
#KP266333_PLATEAU78/NG/2011/1          .......... .......... .......... ...LS..... .........S
#KP152270_BENIN142E/NG/2013            .......... .......... .......... .......... ..........
#KP152269_BENIN87/NG/2013              .......... .......... .......... ....S..... ..........
#KP152271_ABUJA93/NG/2013              .......... .......... .......... ....S..... ..........
#KP152272_BAUCHI128/NG/2014            .......... .......... .......... ....S..... ..........
#KP152273_PLATEAU99/NG/2011            .......... .......... .......... ....S..... ..........
#KP152274_PLATEAU92/NG/2013            .......... .......... .......... ....S..... .T........
#KP152275_PLATEAU83/NG/2013            .......... .......... .......... ....S..... ..........
#KP152276_PLATEAU90/NG/2013            .......... .......... .......... ....S..... ..........
#KP152277_PLATEAU95/NG/2013            .......... .......... .......... ....S..... ..........
#KP152278_PLATEAU96/NG/2013            .......... .......... .......... ....S..... ..........
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#KP152282_PLATEAU134/NG/2014           .......... .......... .......... ....S..... ..........
#KP152283_PLATEAU110/NG/2010           .......... .......... .......... ....S..... ..........
#KP152284_PLATEAU104/NG/2010           .......... .......... .......... ....S..... ..........
#KP152285_BAUCHI121/NG/2013            .......... .......... .......... ....S..... ..........
#KP152286_KWARA139/NG/2014             .......... .......... .......... ....S..... ..........
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#KP152288_PLATEAU119/NG/2013           .......... .......... .......... ....S..... .........S
#KP152289_KWARA141/NG/2014             .......... .......... .......... .......... ..........
#KP152279_PLATEAU120/NG/2013           .......... .......... .......... ....S..... .........S
#KP152280_PLATEAU132/NG/2014           .......... .......... .......... ....S..... .........S
#KP152290_PLATEAU82/NG/2013            .......... .......... .......... ....S..... .........S
#KP152307_PLATEAU75/NG/2011            .......... .......... .......... ....S..... ..........
#KP152295_ANAMBRA50/NG/2012            .......... .......... .......... ....S..... .........S
#KP152294_BENIN34/NG/2012              .......... .......... .......... ....S..... ..........
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#KP152238_KWARA179/NG/2014             .......... .......... .......... ....S..... ..........
#KP152239_KWARA180/NG/2014             .......... .......... .......... ....S..... ..........
#KP152240_KWARA181/NG/2014             .......... .......... .......... ....S..... ..........
#KP152241_KWARA182/NG/2014             .......... .......... .......... ....S..... ..........
#KP152242_KWARA184/NG/2014             .......... .......... .......... ....S..... ..........
#KP152243_UYO185/NG/2014               .......... .......... .......... ....S..... .........S
#KP152244_UYO187/NG/2014               .......... .......... .......... ....S..... ..........
#KP152245_UYO188/NG/2014               .......... .......... .......... ....S..... .........S
#KP152246_UYO189/NG/2014               .......... .......... .......... ....S..... ..........
#KP152247_UYO190/NG/2014               .......... .......... .......... ....S..... .........S
#KP152248_UYO191/NG/2014               .......... .......... .......... ....S..... ..........
#KP152249_UYO192/NG/2014               .......... .......... .......... ....S..... ..........
#KP152250_UYO193/NG/2014               .......... .......... .......... ....S..... ..........
#KP152251_UYO194/NG/2014               .......... .......... .......... ....S..... ..........
#KP152252_UYO195/NG/2014               .......... .......... .......... ....S..... ..........
#HQ231797.1_86-2006Eth                 .......... .......... .......... ....S..... ..........
#JF826453.1_72Eth                      .......... .......... .......... ....S..... ..........
#JF826451.1_57Eth                      .......... .......... .......... ....S..... ..........
#JF826457.1_85Eth                      .......... .......... .......... ....S..... ..........
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#KP152254_UYO197/NG/2014               .......... .......... .......... ....S..... ..........
#KP152255_UYO198/NG/2014               .......... .......... .......... ....S..... ..........
#KP152256_UYO199/NG/2014               .......... .......... .......... ....S..... ..........
#KP152257_UYO200/NG/2014               .......... .......... .......... ....S..... ..........
#KP152258_UYO201/NG/2014               .......... .......... .......... ....S..... ..........
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#KP152260_UYO203/NG/2014               .......... .......... .......... ....S..... ..........
#KP152261_UYO204/NG/2014               .......... .......... .......... ....S..... ..........
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#KP152322_UYO205/NG/2014              .......... .......... .......... ....S..... ..........
#KP152231_UYO206/NG/2014              .......... .......... .......... ....S..... ..........
#KP152232_KWARA171/NG/2014            .......... .......... .......... ....S..... ..........
#ABI97803.1 South_Africa_04_D74       .......... .......... .......... ....S..... ....T.....
#ABI97801.1 South_Africa_04_A2        .......... .......... .......... ....S..... ..........
#ACU45373.1IBDV_strain_D1054/85      .......... .......... ..V......K ....N.V... ..........
#ACU45360.1 IBDV_strain_D430/5        .......... .......... ..V......K ....S.V... ..........
#ACU45359.1 IBDV_strain_D430/4        .......... .......... ..V......K ....S.V... ..........
#ACU45369.1 IBDV_strain_D892/92       .......... .......... .........K ....S.V... ..........
#ACU45365.1 IBDV_strain_D862/20       .......... .......... .........K ....S.V... ..........
#ACU45363.1 IBDV_strain_D836/14       .......... .......... .........K ....S.V... ..........

                                     270        280        290        300        310
#vvIBDV_UK_661                        AVITRAVAAD NGLTAGTDNL MPFNIVIPTS EITQPITSIK LEIVTSKSGG
#AB306716.1_KTP-13                    .......... .......G.. .......... .......... ..........
#AB306715.1_KTG-8                     T.......T. .......... ....L....N .......... ..........
#ABI97816.1 South_Africa_97_SA6       .......... .......... .......... .......... ..........
#ABI97814.1 South_Africa_97_SA3       .......... .......... .......... .......... ..........
#ABI97806.1 South_Africa_05_SA807     .......... .......... .......... Q......... ..........
#ABI978041 South_Africa_05_SA10       .........N ......I... ....L....N D......... ........D.
#AJ878887_99006/1552_SENEGAL          T......... .......... ....L....N .......... ..V.......
#AJ878888_88035.628_IVORY_COAST       .......... .......... ....L....N Q......... ..........
#AJ878889_88035.693_IVORY_COAST       .......... .......... ....L....N Q......... ..........
#AJ878890_88035.737_IVORY_COAST       .......... .......... ....L....N Q......... ..........
#Variant_DelE                         .........N ......I... ....L....N .......... ........D.
#AJ583500_99323_EGYPT                 .......... .......... .......... .......... ..........
#KP152296_NIG20/NG/2009               .......... .......... .......... A......... ..........
#KP152233_KWARA174/NG/2014            .......... .......... .......... A......... ..........
#KP152293_PLATEAU43/NG/2012           .......... .......... .......... Q......... ..........
#KP152321_NIG79/NG/2009               .......... .......... .......... A......... ..........
#KP152320_UYO167/NG/2014              .......... .......... .......... A......... ..........
#KP152319_UYO166/NG/2014              .......... .......... .......... A......... ..........
#KP1522318_PLATEAU164/NG/2014         .......... .......... .......... A......... ..........
#KP152317_PLATEAU163/NG/2014          .......... .......... .......... A......... ..........
#KP152316_PLATEAU162/NG/2014          .......... .......... .......... A......... ..........
#KP152315_PLATEAU161/NG/2014          .......... .......... .......... A......... ..........
#KP152314_PLATEAU160/NG/2014          .......... .......... .......... Q......... ..........
#KP152313_PLATEAU159/NG/2014          .......... .......... .......... A......... ..........
#KP152312_PLATEAU158/NG/2014          .......... .......... .......... A......... ..........
#KP152311_PLATEAU157/NG/2014          .......... .......... .......... A......... ..........
#KP152310_PLATEAU156/NG/2014          .......... .......... .......... Q......... ..........
#KP152309_PLATEAU155/NG/2014          .......... .......... .......... A......... ..........
#KP152308_PLATEAU154/NG/2014          .......... .......... .......... A......... ..........
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#KP152304_KWARA152/NG/2014            .......... .......... .......... A......... ..........
#KP152303_KWARA151NG/NG/2014          .......... .......... .......... A......... ..........
#KP152302_KWARA150/NG/2014            .......... .......... .......... A......... ..........
#KP152301_KWARA149/NG/2014            .......... .......... .......... A......... ..........
#KP152300_KWARA148/NG/2014            ..T......N .......... .......... .......... ..........
#KP152299_KWARA147/NG/2014            .......... .......... .......... A......... ..........
#KP152298_KWARA145/NG/2014            .......... .......... .......... A......... ..........
#KP152297_BENIN47/NG/2014             .......... .......... .......... A......... ..........
#KP266329_BAUCHI37/NG/2012            .......... .......... .......... A......... ..........
#KP152268_BAUCHI36/NG/2009            .......... .......... .......... A......... ..........
#KP266322_PLATEAU19/NG/2009           .......... .......... .......... A......... ..........
#KP266323_Kaduna61/NG/2009            .......... .......... .......... A......... ..........
#KP266334_PLATEAU39/NG/2012           .......... .......... .......... A......... ..........
#KP266324_BAUCHI58/NG/2012            .......... .......... .......... Q......... ..........
#KP266325_PLATEAU67/NG/2011           .......... .......... .......... A......... ..........
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#KP266326_PLATEAU53/NG/2010          .......... .......... .......... A......... ..........
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#KP266328_BENIN35/NG/2012            .......... .......... .......... A......... ..........
#KP152291_BAUCHI37/NG/2009           .......... .......... .......... A......... ..........
#KP152262_BAUCHI124/NG/2014          .......... .......... .......... A......... ..........
#KP152263_BAUCHI126/NG/2014          .......... .......... .......... A......... ..........
#KP152264_BAUCHI127/NG/2014          .......... .......... .......... A......... ..........
#KP152265_KADUNA136/NG/2014          .......... .......... .......... Q......... ..........
#KP152266_PLATEAU133/NG/2014         .......... .......... .......... Q......... ..........
#KP152267_PLATEAU135/NG/2014         .......... .......... .......... Q......... ..........
#KP266330_NASSARAWA77/NG/no2/_2013   .........N ....T..... ....L....N .......... ..........
#KP266331_NASSARAWA77/NG/2013        .......... .......... .......... Q......... ..........
#KP266332_PLATEAU78/NG/2011/2        .......... .......... .......... Q......... ..........
#KP266333_PLATEAU78/NG/2011/1        .......... .......... .......... Q......... ..........
#KP152270_BENIN142E/NG/2013          ..T......N .......... .......... .......... ..........
#KP152269_BENIN87/NG/2013            .......... .......... .......... A......... ..........
#KP152271_ABUJA93/NG/2013            .......... .......... .......... A......... ..........
#KP152272_BAUCHI128/NG/2014          .......... .......... .......... A......... ..........
#KP152273_PLATEAU99/NG/2011          .......... .......... .......... A......... ..........
#KP152274_PLATEAU92/NG/2013          .......... .......... .......... A......... ..........
#KP152275_PLATEAU83/NG/2013          .......... .......... .......... A......... ..........
#KP152276_PLATEAU90/NG/2013          .......... .......... .......... A......... ..........
#KP152277_PLATEAU95/NG/2013          .......... .......... .......... A......... ..........
#KP152278_PLATEAU96/NG/2013          .......... .......... .......... A......... ..........
#KP152281_PLATEAU131/NG/2014         .......... .......... .......... A......... ..........
#KP152282_PLATEAU134/NG/2014         .......... .......... .......... A......... ..........
#KP152283_PLATEAU110/NG/2010         .......... .......... .......... A......... ..........
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#KP152285_BAUCHI121/NG/2013          .......... .......... .......... A......... ..........
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#KP152288_PLATEAU119/NG/2013         .......... .......... .......... Q......... ..........
#KP152289_KWARA141/NG/2014           ..T......N .......... .......... .......... ..........
#KP152279_PLATEAU120/NG/2013         .......... .......... .......... Q......... ..........
#KP152280_PLATEAU132/NG/2014         .......... .......... .......... Q......... ..........
#KP152290_PLATEAU82/NG/2013          .......... .......... .......... Q......... ..........
#KP152307_PLATEAU75/NG/2011          .......... .......... .......... A......... ..........
#KP152295_ANAMBRA50/NG/2012          .......... .......... .......... Q......... ..........
#KP152294_BENIN34/NG/2012            .......... .......... .......... A......... ..........
#KP152234_KWARA175/NG/2014           .......... .......... .......... A......... ..........
#KP152235_KWARA176/NG/2014           .......... .......... .......... A......... .....T....
#KP152236_KWARA177/NG/2014           .......... .......... .......... A......... ..........
#KP152237_KWARA178/NG/2014           .......... .......... .......... A......... .....T....
#KP152238_KWARA179/NG/2014           .......... .......... .......... A......... ..........
#KP152239_KWARA180/NG/2014           .......... .......... .......... A......... ..........
#KP152240_KWARA181/NG/2014           .......... .......... .......... A......... ..........
#KP152241_KWARA182/NG/2014           .......... .......... .......... A......... ..........
#KP152242_KWARA184/NG/2014           .......... .......... .......... A......... ..........
#KP152243_UYO185/NG/2014             .......... .......... .......... Q......... ..........
#KP152244_UYO187/NG/2014             .......... .......... .......... A......... ..........
#KP152245_UYO188/NG/2014             .......... .......... .......... Q......... ..........
#KP152246_UYO189/NG/2014             .......... .......... .......... A......... ..........
#KP152247_UYO190/NG/2014             .......... .......... .......... Q......... ..........
#KP152248_UYO191/NG/2014             .......... .......... .......... A......... ..........
#KP152249_UYO192/NG/2014             .......... .......... .......... A......... ..........
#KP152250_UYO193/NG/2014             .......... .......... .......... A......... ..........
#KP152251_UYO194/NG/2014             .......... .......... .......... A......... ..........
#KP152252_UYO195/NG/2014             .......... .......... .......... A......... ..........
#HQ231797.1_86-2006Ethipia           .......... .......... .......... Q......... ..........
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#JF826453.1_72Ethiopia                 .......... .......... .......... Q......... ..........
#JF826451.1_57Ethiopia                 .......... .......... .......... Q......... ..........
#JF826457.1_85Ethiopia                 .......... .......... .......... Q......... ..........
#KP152253_UYO196/NG/2014               .......... .......... .......... A......... ..........
#KP152254_UYO197/NG/2014               .......... .......... .......... A......... ..........
#KP152255_UYO198/NG/2014               .......... .......... .......... A......... ..........
#KP152256_UYO199/NG/2014               .......... .......... .......... A......... ..........
#KP152257_UYO200/NG/2014               .......... .......... .......... A......... ..........
#KP152258_UYO201/NG/2014               .......... .......... .......... A......... ..........
#KP152259_UYO202/NG/2014               .......... .......... .......... A......... ..........
#KP152260_UYO203/NG/2014               .......... .......... .......... A......... ..........
#KP152261_UYO204/NG/2014               .......... .......... .......... A......... ..........
#KP152322_UYO205/NG/2014               .......... .......... .......... A......... ..........
#KP152231_UYO206/NG/2014               .......... .......... .......... A......... ..........
#KP152232_KWARA171/NG/2014             .......... .......... .......... A......... ..........
#ABI97803.1 South_Africa_04_D74        .......... .......... .......... .......... ..........
#ABI97801.1 South_Africa_04_A2         .......... .......... .......... .......... ..........
#ACU45373.1IBDV_strain_D1054/85       .........N ......I... ....L....N .......... ........D.
#ACU45360.1 IBDV_strain_D430/5         .........N ......I... ....L....N D......... ........D.
#ACU45359.1 IBDV_strain_D430/4         .........N ......I... ....L....N D......... ........D.
#ACU45369.1 IBDV_strain_D892/92        .........N ......I... ....L....N D......... ........D.
#ACU45365.1 IBDV_strain_D862/20        .........N ......I... ....L....N D......... ........D.
#ACU45363.1 IBDV_strain_D836/14        .........N ......I... ....L....N D......... ........D.

                                      320        330        340
#vvIBDV_UK_661                         QAGDQMSWSA SGSLAVTIHG GNYPG
#AB306716.1_KTP-13                     .T........ .......... .....
#AB306715.1_KTG-8                      .......... .E........ .....
#ABI97816.1 South_Africa_97_SA6        .......... .......... .....
#ABI97814.1 South_Africa_97_SA3        .......... .......... .....
#ABI97806.1 South_Africa_05_SA807      .......... .......... .....
#ABI97804.1 South_Africa_05_SA10       T.......L. .......... .....
#AJ878887_99006/1552_SENEGAL           .......... .......... .....
#AJ878888_88035.628_IVORY_COAST        .......... ....V..... .....
#AJ878889_88035.693_IVORY_COAST        .......... ....V..... .....
#AJ878890_88035.737_IVORY_COAST        .......... ....V..... .....
#Variant_DelE                          ...E...... .......... .....
#AJ583500_99323_EGYPT                  .T........ .......... .....
#KP152296_NIG20/NG/2009                .......... .......... .....
#KP152233_KWARA174/NG/2014             .......... .......... .....
#KP152293_PLATEAU43/NG/2012            .......... .......... .....
#KP152321_NIG79/NG/2009                .......... .......... .....
#KP152320_UYO167/NG/2014               .......... .......... .....
#KP152319_UYO166/NG/2014               .......... .......... .....
#KP1522318_PLATEAU164/NG/2014          .......... .......... .....
#KP152317_PLATEAU163/NG/2014           .......... .......... .....
#KP152316_PLATEAU162/NG/2014           .......... .......... .....
#KP152315_PLATEAU161/NG/2014           .......... .......... .....
#KP152314_PLATEAU160/NG/2014           .......... .......... .....
#KP152313_PLATEAU159/NG/2014           .......... .......... .....
#KP152312_PLATEAU158/NG/2014           .......... .......... .....
#KP152311_PLATEAU157/NG/2014           .......... .......... .....
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#KP152309_PLATEAU155/NG/2014           .......... .......... .....
#KP152308_PLATEAU154/NG/2014           .......... .......... .....
#KP152306_PLATEAU16/NG/2009            .......... .......... .....
#KP152292_PLATEAU4/NG/2009             .......... .......... .....
#KP152304_KWARA152/NG/2014             .......... .......... .....
#KP152303_KWARA151NG/NG/2014           .......... .......... .....
#KP152302_KWARA150/NG/2014             .......... .......... .....
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#KP152300_KWARA148/NG/2014            .......... .......... .....
#KP152299_KWARA147/NG/2014            .......... .......... .....
#KP152298_KWARA145/NG/2014            .......... .......... .....
#KP152297_BENIN47/NG/2014             .......... .......... .....
#KP266329_BAUCHI37/NG/2012            .......... .......... .....
#KP152268_BAUCHI36/NG/2009            .......... .......... .....
#KP266322_PLATEAU19/NG/2009           .......... .......... .....
#KP266323_Kaduna61/NG/2009            .......... .......... .....
#KP266334_PLATEAU39/NG/2012           .......... .......... .....
#KP266324_BAUCHI58/NG/2012            .......... .......... .....
#KP266325_PLATEAU67/NG/2011           .......... .......... .....
#KP266326_PLATEAU53/NG/2010           .......... .......... .....
#KP266327_ANAMBRA54/NG/2012           .......... .......... .....
#KP152305_Plateau7/NG/2009            .......... .......... .....
#KP266328_BENIN35/NG/2012             .......... .......... .....
#KP152291_BAUCHI37/NG/2009            .......... .......... .....
#KP152262_BAUCHI124/NG/2014           .......... .......... .....
#KP152263_BAUCHI126/NG/2014           .......... .......... .....
#KP152264_BAUCHI127/NG/2014           .......... .......... .....
#KP152265_KADUNA136/NG/2014           .......... .......... .....
#KP152266_PLATEAU133/NG/2014          .......... .......... .....
#KP152267_PLATEAU135/NG/2014          .......... .......... .....
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#KP266332_PLATEAU78/NG/2011/2         .......... R......... .....
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#KP152269_BENIN87/NG/2013             .......... .......... .....
#KP152271_ABUJA93/NG/2013             .......... .......... .....
#KP152272_BAUCHI128/NG/2014           .......... .......... .....
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#KP152274_PLATEAU92/NG/2013           .......... .......... .....
#KP152275_PLATEAU83/NG/2013           .......... .......... .....
#KP152276_PLATEAU90/NG/2013           .......... .......... .....
#KP152277_PLATEAU95/NG/2013           .......... .......... .....
#KP152278_PLATEAU96/NG/2013           .......... .......... .....
#KP152281_PLATEAU131/NG/2014          .......... .......... .....
#KP152282_PLATEAU134/NG/2014          .......... .......... .....
#KP152283_PLATEAU110/NG/2010          .......... .......... .....
#KP152284_PLATEAU104/NG/2010          .......... .......... .....
#KP152285_BAUCHI121/NG/2013           .......... .......... .....
#KP152286_KWARA139/NG/2014            .......... .......... .....
#KP152287_PLATEAU40/NG/2012           .......... .......... .....
#KP152288_PLATEAU119/NG/2013          .......... .......... .....
#KP152289_KWARA141/NG/2014            .......... .......... .....
#KP152279_PLATEAU120/NG/2013          .......... .......... .....
#KP152280_PLATEAU132/NG/2014          .......... .......... .....
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#KP152237_KWARA178/NG/2014            .......... .......... .....
#KP152238_KWARA179/NG/2014            .......... .......... .....
#KP152239_KWARA180/NG/2014            .......... .......... .....
#KP152240_KWARA181/NG/2014            .......... .......... .....
#KP152241_KWARA182/NG/2014            .......... .......... .....
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#KP152242_KWARA184/NG/2014            .......... .......... .....
#KP152243_UYO185/NG/2014              .......... .......... .....
#KP152244_UYO187/NG/2014              .......... .......... .....
#KP152245_UYO188/NG/2014              .......... .......... .....
#KP152246_UYO189/NG/2014              .......... .......... .....
#KP152247_UYO190/NG/2014              .......... .......... .....
#KP152248_UYO191/NG/2014              .......... .......... .....
#KP152249_UYO192/NG/2014              .......... .......... .....
#KP152250_UYO193/NG/2014              .......... .......... .....
#KP152251_UYO194/NG/2014              .......... .......... .....
#KP152252_UYO195/NG/2014              .......... .......... .....
#HQ231797.1_86-2006Eth                .......... .......... .....
#JF826453.1_72Eth                     .......... .......... .....
#JF826451.1_57Eth                     .......... .......... .....
#JF826457.1_85Eth                     .......... .......... .....
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#KP152254_UYO197/NG/2014              .......... .......... .....
#KP152255_UYO198/NG/2014              .......... .......... .....
#KP152256_UYO199/NG/2014              .......... .......... .....
#KP152257_UYO200/NG/2014              .......... .......... .....
#KP152258_UYO201/NG/2014              .......... .......... .....
#KP152259_UYO202/NG/2014              .......... .......... .....
#KP152260_UYO203/NG/2014              .......... .......... .....
#KP152261_UYO204/NG/2014              .......... .......... .....
#KP152322_UYO205/NG/2014              .......... .......... .....
#KP152231_UYO206/NG/2014              .......... .......... .....
#KP152232_KWARA171/NG/2014            .......... .......... .....
#ABI97803.1 South_Africa_04_D74       .......... .......... .....
#ABI97801.1 South_Africa_04_A2        .......... .......... .....
#ACU45373.1IBDV_strain_D1054/85      T......... .......... .....
#ACU45360.1 IBDV_strain_D430/5        T.......L. .......... .....
#ACU45359.1 IBDV_strain_D430/4        T.....L.L. .......... .....
#ACU45369.1 IBDV_strain_D892/92       T.......L. .......... .....
#ACU45365.1 IBDV_strain_D862/20       T.......L. .......... .....
#ACU45363.1 IBDV_strain_D836/14       T.......L. .......... .....
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Appendix 11: Amino acid sequence data for Nigerian IBDV viruses and IBDV strains from other African countries

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                                    211        221        231        241        251        261        271        281
#vvIBDV_UK_661                       ADDYQFSSQY QAGGVTITLF SANIDAITSL SIGGELVFQT SVQGLILGAT IYLIGFDGTA VITRAVAADN GLTAGTDNLM
#AJ878900_HK46                       .......... .......... .......... .......... .......... .......... .......... ..........
#Variant_DelE                        ..N....... .T........ .......... .V......K. ...S.V.... .......... ........N. .....I....
#Variant_T1                          ..N....... .T........ .......... .V......K. ...N.V.... .......... ........N. .....I....
#Classic_STC                         .......... .P........ .......... .V........ .....V.... ..F......T .......... ..........
#vvIBDV_OKYM                         .......... .......... .......... .......... .......... .......... .......... ..........
#AJ878909_02015.1                    .......... .......... .......... .......... .......... .......... .......... ..........
#AJ878908_002-73                     .......... .P........ .......... .V........ .....L.N.. V..V...AT .T......G. ..........
#AJ878907_Gx                         .......... .......... .......... .......... .......... .......... .......... ..........
#AJ878901_HENAN                      .......... .......... .......... .......... .......... .......... .......... ..........
#AJ878886_CU-1WT                     .......... .P........ .......... .......... .....V.... .......... .......... ..........
#AJ878911_02015.3                    .......... .......... .......... .......... .......... .......... .......... ..........
#AJ878883_06/95                      .......... .P........ .......... .V........ .....V.N.. ...V.....T .T......G. ..........
#AJ878884_N1/99                      .......... .P........ .......... .V........ .....V.N.N ...V....AT .T......GT ..........
#AJ878885_05/5                       .NG....... .......... .......... .V........ ...S.A.D.. .........T .T........ ..........
#AJ508759_93/95                      .......... .......... .......... .......... .......... .......... .......... ..........
#AJ508758_00/40                      .......... .......... .......... .......... .......... .......... .......... ..........
#AJ508761_78GSz                      .......... .S........ .......... .V..G..... .....V.... .........T .T......N. ........PI
#AJ508762_80GA                       .......... .S........ .......... .V..G..... ...D.V.... .........T .T......N. ........PI
#Y14961_CT4/4                        .......... .P........ .......... .V........ ..H..V.... .........T ........N. ...T......
#Y14956_89163                        .......... .......... .......... .......... .......... .......... .......... ..........
#AJ001946_95072.2                    .......... .......... .......... .......... .......... .......... .......... ..........
#AJ001948_96108                      ..N....... .......... .......... .......... .......... .......... .......... ..........
#AJ001943_91184                      ..N....... .......... .......... .......... .......... .......... .......... ..........
#AJ878902_99009                      .......... .......... .......... .......... .......... .......... .......... ..........
#AJ878903_BD3                        .......... .......... .......... .......... .......... .......... .......... ..........
#AJ878904_99141                      .......... .......... ...V...... .......... .......... .......... .......... ..........
#AJ878906_Variant_GLS5               .......... .T........ .......... .V......K. ...S.V.... ........S. ........N. ..........
#Y14957_91168                        .......... .......... .......... .......... .......... .......... .......... ..........
#AJ878910_02015.2                    .......... .......... .......... .......... .......... .......... .......... ..........
#AJ878899_DV86                       .......... .......... .......... .......... .......... .......... .......... ..........
#AJ878897_91233                      .......... .......... .......... .......... .......... .......... .......... ..........
#AJ878896_89299                      .......... .......... .......... .......... .......... .......... .......... ..........
#HARBIN                              .......... .......... .......... .......... .......... .......... .......... ..........
#F52/70                              .......... .P........ .......... .......... .....V.... .......... .......... ..........
#KP152296_NIG20/NG/2009              .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152233_KWARA174/NG/2014           .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152293_PLATEAU43/NG/2012          .......... .......... .......... .......... ...S...... ........S. .......... ..........
#KP152321_NIG79/NG/2009              .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152320_UYO167/NG/2014             .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152319_UYO166/NG/2014             .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP1522318_PLATEAU164/NG/2014        .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152317_PLATEAU163/NG/2014         .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152316_PLATEAU162/NG/2014         .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152315_PLATEAU161/NG/2014         .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152314_PLATEAU160/NG/2014         ........T. .......... .......... .......... ...S...... ........S. .......... ..........
#KP152313_PLATEAU159/NG/2014         .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152312_PLATEAU158/NG/2014         .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152311_PLATEAU157/NG/2014         .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152310_PLATEAU156/NG/2014         ........T. .......... .......... .......... ...S...... ........S. .......... ..........
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#KP152304_KWARA152/NG/2014           .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152303_KWARA151NG/NG/2014         .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152302_KWARA150/NG/2014           .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152301_KWARA149/NG/2014           .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152300_KWARA148/NG/2014           .......... .......... .......... .......... .......... .......... .T......N. ..........
#KP152299_KWARA147/NG/2014           .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152298_KWARA145/NG/2014           .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152297_BENIN47/NG/2014            .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP266329_BAUCHI37/NG/2012           .......... .T........ .......... .......... ...S...... .......... .......... ..........
#KP152268_BAUCHI36/NG/2009           .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP266322_PLATEAU19/NG/2009          .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP266323_Kaduna61/NG/2009           .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP266334_PLATEAU39/NG/2012          .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP266324_BAUCHI58/NG/2012           ........T. .......... .......... .......... ...S...... ........S. .......... ..........
#KP266325_PLATEAU67/NG/2011          .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP266326_PLATEAU53/NG/2010          .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP266327_ANAMBRA54/NG/2012          ........T. .......... .......... .......... ...S...... ........S. .......... ..........
#KP152305_Plateau7/NG/2009           .......... .......... .......... .......... ...S...... .......... .......... ..........
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#KP152262_BAUCHI124/NG/2014          .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152263_BAUCHI126/NG/2014          .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152264_BAUCHI127/NG/2014          .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152265_KADUNA136/NG/2014          ........T. .......... .......... .......... ...S...... ........S. .......... ..........
#KP152266_PLATEAU133/NG/2014         ........T. .......... .......... .......... ...S...... ........S. .......... ..........
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#KP152267_PLATEAU135/NG/2014          ........T. .......... .......... .......... ...S...... ........S. .......... ..........
#KP266330_NASSARAWA77/NG/no2/_2013    .......... .......... .......... .V........ ..HS.V.... .......... ........N. ...T......
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#KP266332_PLATEAU78/NG/2011/2         ........P. .......... .......... .V........ ...S...... ........S. .......... ..........
#KP266333_PLATEAU78/NG/2011/1         ........T. .......... .......... .......... ..LS...... ........S. .......... ..........
#KP152270_BENIN142E/NG/2013           .......... .......... .......... .......... .......... .......... .T......N. ..........
#KP152269_BENIN87/NG/2013             .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152271_ABUJA93/NG/2013             .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152272_BAUCHI128/NG/2014           .......... .......... .......... .......... ...S...... .......... .......... ..........
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#KP152274_PLATEAU92/NG/2013           .......... .......... .......... .......... ...S...... T......... .......... ..........
#KP152275_PLATEAU83/NG/2013           .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152276_PLATEAU90/NG/2013           .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152277_PLATEAU95/NG/2013           .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152278_PLATEAU96/NG/2013           .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152281_PLATEAU131/NG/2014          .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152282_PLATEAU134/NG/2014          .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152283_PLATEAU110/NG/2010          .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152284_PLATEAU104/NG/2010          .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152285_BAUCHI121/NG/2013           .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152286_KWARA139/NG/2014            .......... .......... .......... .......... ...S...... .......... .......... ..........
#KP152287_PLATEAU40/NG/2012           .......... .......... .......... .......... ...S...... .......... .T......N. ..........
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#KP152289_KWARA141/NG/2014            .......... .......... .......... .......... .......... .......... .T......N. ..........
#KP152279_PLATEAU120/NG/2013          ........T. .......... .......... .......... ...S...... ........S. .......... ..........
#KP152280_PLATEAU132/NG/2014          ........T. .......... .......... .......... ...S...... ........S. .......... ..........
#KP152290_PLATEAU82/NG/2013           ........T. .......... .......... .......... ...S...... ........S. .......... ..........
#KP152307_PLATEAU75/NG/2011           .......... .......... .......... .......... ...S...... .......... .......... ..........
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#KP152238_KWARA179/NG/2014            .......... .......... .......... .......... ...S...... .......... .......... ..........
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#KP152243_UYO185/NG/2014              ........T. .......... .......... .......... ...S...... ........S. .......... ..........
#KP152244_UYO187/NG/2014              .......... .......... .......... .......... ...S...... .......... .......... ..........
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#KP152246_UYO189/NG/2014              .......... .......... .......... .......... ...S...... .......... .......... ..........
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#VARIANT_A                            .......... .Q........ .......... .V......K. ..HS.V.... .......... ........N. ...T.I....
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#CAA65132.1_849VB                     .......... .......... ........K. .V........ R......... .......... .......... ..........
                                      291        301        311        321        331        341
#vvIBDV_UK_661                         PFNIVIPTSE ITQPITSIKL EIVTSKSGGQ AGDQMSWSAS GSLAVTIHGG NYPGALRPVT
#AJ878900_HK46                         .......... .......... .......... ...L...... .......... ..........
#Variant_DelE                          ...L....N. .......... .......D.. ..E....... .......... ..........
#Variant_T1                            ...L....N. .......... .......N.. .E........ .......... ..........
#Classic_STC                           ...L....N. ........... .V........ .......... .......... ..........
#vvIBDV_OKYM                           .......... .......... .......... .......... .......... ..........
#AJ878909_02015.1                      .......... .......... .......... .......... .......... ..........
#AJ878908_002-73                       ...L...... ....V..... .......... .......... .N........ ..........
#AJ878907_Gx                           .......... .......... .......... .......... .......... ..........
#AJ878901_HENAN                        .......... .......... .......... .......... .......... ..........
#AJ878886_CU-1WT                       ...L....N. .......... .......... .......... .......... ..........
#AJ878911_02015.3                      .......... .......... .......... .......... .......... ..........
#AJ878883_06/95                        ...L...... ....V..... .......... .......L.. .N........ ....T.....
#AJ878884_N1/99                        ...L...... ....V..... .......... ........T. .N........ ..........
#AJ878885_05/5                         ...L...... .......... ......R... T..E...LV. .N.V...... ..........
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#AJ508759_93/95                    .......... .......... .......... ...L...... .......... ..........
#AJ508758_00/40                    .......... .......... .......... .......... .......... ..........
#AJ508761_78GSz                    ...L.F..N. .......... .......... .......... .......... ..........
#AJ508762_80GA                     ...L.F..N. .......... .......... .......... .......... ..........
#Y14961_CT4/4                      ...L....N. .......... .......... .........R .......... ..........
#Y14956_89163                      .......... .......... .......... .......... .......... ..........
#AJ001946_95072.2                  .......... .......... .......... .......... .......... ..........
#AJ001948_96108                    .......... .......... .......... .......... .......... ..........
#AJ001943_91184                    .......... .......... .......... .......... .......... ..........
#AJ878902_99009                    .......... .......... .......... .......... .......... ..........
#AJ878903_BD3                      .........A .......... .......... .......... .......... ..........
#AJ878904_99141                    .........A .......... .......... .......... .......... ..........
#AJ878906_Variant_GLS5             ...L....N. .......... .......... E......... .......... ..........
#Y14957_91168                      .......... .......... .......... ...L...... .......... ..........
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#AJ878899_DV86                     .......... .......... .......... .......... .......... ..........
#AJ878897_91233                    .......... .......... .......... .......... .......... ..........
#AJ878896_89299                    .......... .......... .......... .......... .......... ..........
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#F52/70                            ...L....N. .......... .......... .......... .......... ..........
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#KP152262_BAUCHI124/NG/2014        .........A .......... .......... .......... .......... ..........
#KP152263_BAUCHI126/NG/2014        .........A .......... .......... .......... .......... ..........
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#KP152246_UYO189/NG/2014              .........A .......... .......... .......... .......... ..........
#KP152247_UYO190/NG/2014              .........Q .......... .......... .......... .......... ..........
#KP152248_UYO191/NG/2014              .........A .......... .......... .......... .......... ..........
#KP152249_UYO192/NG/2014              .........A .......... .......... .......... .......... ..........
#KP152250_UYO193/NG/2014              .........A .......... .......... .......... .......... ..........
#KP152251_UYO194/NG/2014              .........A .......... .......... .......... .......... ..........
#KP152252_UYO195/NG/2014              .........A .......... .......... .......... .......... ..........
#KP152253_UYO196/NG/2014              .........A .......... .......... .......... .......... ..........
#KP152254_UYO197/NG/2014              .........A .......... .......... .......... .......... ..........
#KP152255_UYO198/NG/2014              .........A .......... .......... .......... .......... ..........
#KP152256_UYO199/NG/2014              .........A .......... .......... .......... .......... ..........
#KP152257_UYO200/NG/2014              .........A .......... .......... .......... .......... ..........
#KP152258_UYO201/NG/2014              .........A .......... .......... .......... .......... ..........
#KP152259_UYO202/NG/2014              .........A .......... .......... .......... .......... ..........
#KP152260_UYO203/NG/2014              .........A .......... .......... .......... .......... ..........
#KP152261_UYO204/NG/2014              .........A .......... .......... .......... .......... ..........
#KP152322_UYO205/NG/2014              .........A .......... .......... .......... .......... ..........
#KP152231_UYO206/NG/2014              .........A .......... .......... .......... .......... ..........
#KP152232_KWARA171/NG/2014            .........A .......... .......... .......... .......... ..........
#VARIANT_A                            ...L....N. .......... .....R.... .......... .......... ..........
#USA_GEORGIA                          ...L....N. .......... .......... .........R .......... ..........
#CAJ44475.1_France_94432              .......... .......... .......... V......... .......... ..........
#CAA65132.1_849VB                     ........N. .......... .......... .......... .......... ..........
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Appendix 12: Amino acid sequence data of Nigerian IBDV viruses and IBDV strains from other countries of the world

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                                    211        221        231        241        251
#NVRI_VOM_Vaccine                    ADDYQFSSQY QPGGVTITLF SANIDAITSL SVGGELVFQT SVHGLVLGAT
#KP152296_NIG20/NG/2009              .......... .A........ .......... .I........ ..QS.I....
#KP152233_KWARA174/NG/2014           .......... .A........ .......... .I........ ..QS.I....
#KP152293_PLATEAU43/NG/2012          .......... .A........ .......... .I........ ..QS.I....
#KP152321_NIG79/NG/2009              .......... .A........ .......... .I........ ..QS.I....
#KP152320_UYO167/NG/2014             .......... .A........ .......... .I........ ..QS.I....
#KP152319_UYO166/NG/2014             .......... .A........ .......... .I........ ..QS.I....
#KP1522318_PLATEAU164/NG/2014        .......... .A........ .......... .I........ ..QS.I....
#KP152317_PLATEAU163/NG/2014         .......... .A........ .......... .I........ ..QS.I....
#KP152316_PLATEAU162/NG/2014         .......... .A........ .......... .I........ ..QS.I....
#KP152315_PLATEAU161/NG/2014         .......... .A........ .......... .I........ ..QS.I....
#KP152314_PLATEAU160/NG/2014         ........T. .A........ .......... .I........ ..QS.I....
#KP152313_PLATEAU159/NG/2014         .......... .A........ .......... .I........ ..QS.I....
#KP152312_PLATEAU158/NG/2014         .......... .A........ .......... .I........ ..QS.I....
#KP152311_PLATEAU157/NG/2014         .......... .A........ .......... .I........ ..QS.I....
#KP152310_PLATEAU156/NG/2014         ........T. .A........ .......... .I........ ..QS.I....
#KP152309_PLATEAU155/NG/2014         .......... .A........ .......... .I........ ..QS.I....
#KP152308_PLATEAU154/NG/2014         .......... .A........ .......... .I........ ..QS.I....
#KP152306_PLATEAU16/NG/2009          .......... .A........ .......... .I........ ..QS.I....
#KP152292_PLATEAU4/NG/2009           .......... .A........ .......... .I........ ..QS.I....
#KP152304_KWARA152/NG/2014           .......... .A........ .......... .I........ ..QS.I....
#KP152303_KWARA151NG/NG/2014         .......... .A........ .......... .I........ ..QS.I....
#KP152302_KWARA150/NG/2014           .......... .A........ .......... .I........ ..QS.I....
#KP152301_KWARA149/NG/2014           .......... .A........ .......... .I........ ..QS.I....
#KP152300_KWARA148/NG/2014           .......... .A........ .......... .I........ ..Q..I....
#KP152299_KWARA147/NG/2014           .......... .A........ .......... .I........ ..QS.I....
#KP152298_KWARA145/NG/2014           .......... .A........ .......... .I........ ..QS.I....
#KP152297_BENIN47/NG/2014            .......... .A........ .......... .I........ ..QS.I....
#KP266329_BAUCHI37/NG/2012           .......... .T........ .......... .I........ ..QS.I....
#KP152268_BAUCHI36/NG/2009           .......... .A........ .......... .I........ ..QS.I....
#KP266322_PLATEAU19/NG/2009          .......... .A........ .......... .I........ ..QS.I....
#KP266323_KADUNA61/NG/2009           .......... .A........ .......... .I........ ..QS.I....
#KP266334_PLATEAU39/NG/2012          .......... .A........ .......... .I........ ..QS.I....
#KP266324_BAUCHI58/NG/2012           ........T. .A........ .......... .I........ ..QS.I....
#KP266325_PLATEAU67/NG/2011          .......... .A........ .......... .I........ ..QS.I....
#KP266326_PLATEAU53/NG/2010          .......... .A........ .......... .I........ ..QS.I....
#KP266327_ANAMBRA54/NG/2012          ........T. .A........ .......... .I........ ..QS.I....
#KP152305_PLATEAU7/NG/2009           .......... .A........ .......... .I........ ..QS.I....
#KP266328_BENIN35/NG/2012            .......... .A........ .......... .I........ ..QS.I....
#KP152291_BAUCHI37/NG/2009           .......... .T........ .......... .I........ ..QS.I....
#KP152262_BAUCHI124/NG/2014          .......... .A........ .......... .I........ ..QS.I....
#KP152263_BAUCHI126/NG/2014          .......... .A........ .......... .I........ ..QS.I....
#KP152264_BAUCHI127/NG/2014          .......... .A........ .......... .I........ ..QS.I....
#KP152265_KADUNA136/NG/2014          ........T. .A........ .......... .I........ ..QS.I....
#KP152266_PLATEAU133/NG/2014         ........T. .A........ .......... .I........ ..QS.I....
#KP152267_PLATEAU135/NG/2014         ........T. .A........ .......... .I........ ..QS.I....
#KP266330_NASSARAWA77/NG/no2/_2013   .......... .A........ .......... .......... ...S......
#KP266331_NASSARAWA77/NG/2013        .......... .A........ .......... .I........ ..LS.I....
#KP266332_PLATEAU78/NG/2011/2        ........P. .A........ .......... .......... ..QS.I....
#KP266333_PLATEAU78/NG/2011/1        ........T. .A........ .......... .I........ ..LS.I....
#KP152270_BENIN142E/NG/2013          .......... .A........ .......... .I........ ..Q..I....
#KP152269_BENIN87/NG/2013            .......... .A........ .......... .I........ ..QS.I....
#KP152271_ABUJA93/NG/2013            .......... .A........ .......... .I........ ..QS.I....
#KP152272_BAUCHI128/NG/2014          .......... .A........ .......... .I........ ..QS.I....
#KP152273_PLATEAU99/NG/2011          .......... .A........ .......... .I........ ..QS.I....
#KP152274_PLATEAU92/NG/2013          .......... .A........ .......... .I........ ..QS.I....
#KP152275_PLATEAU83/NG/2013          .......... .A........ .......... .I........ ..QS.I....
#KP152276_PLATEAU90/NG/2013          .......... .A........ .......... .I........ ..QS.I....
#KP152277_PLATEAU95/NG/2013          .......... .A........ .......... .I........ ..QS.I....
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#KP152278_PLATEAU96/NG/2013          .......... .A........ .......... .I........ ..QS.I....
#KP152281_PLATEAU131/NG/2014         .......... .A........ .......... .I........ ..QS.I....
#KP152282_PLATEAU134/NG/2014         .......... .A........ .......... .I........ ..QS.I....
#KP152283_PLATEAU110/NG/2010         .......... .A........ .......... .I........ ..QS.I....
#KP152284_PLATEAU104/NG/2010         .......... .A........ .......... .I........ ..QS.I....
#KP152285_BAUCHI121/NG/2013          .......... .A........ .......... .I........ ..QS.I....
#KP152286_KWARA139/NG/2014           .......... .A........ .......... .I........ ..QS.I....
#KP152287_PLATEAU40/NG/2012          .......... .A........ .......... .I........ ..Q..I....
#KP152288_PLATEAU119/NG/2013         ........T. .A........ .......... .I........ ..QS.I....
#KP152289_KWARA141/NG/2014           .......... .A........ .......... .I........ ..Q..I....
#KP152279_PLATEAU120/NG/2013         ........T. .A........ .......... .I........ ..QS.I....
#KP152280_PLATEAU132/NG/2014         ........T. .A........ .......... .I........ ..QS.I....
#KP152290_PLATEAU82/NG/2013          ........T. .A........ .......... .I........ ..QS.I....
#KP152307_PLATEAU75/NG/2011          .......... .A........ .......... .I........ ..QS.I....
#KP152295_ANAMBRA50/NG/2012          ........T. .A........ .......... .I........ ..QS.I....
#KP152294_BENIN34/NG/2012            .......... .A........ .......... .I........ ..QS.I....
#KP152234_KWARA175/NG/2014           .......... .A........ .......... .I........ ..QS.I....
#KP152235_KWARA176/NG/2014           .......... .A........ .......... .I........ ..QS.I....
#KP152236_KWARA177/NG/2014           .......... .A........ .......... .I........ ..QS.I....
#KP152237_KWARA178/NG/2014           .......... .A........ .......... .I........ ..QS.I....
#KP152238_KWARA179/NG/2014           .......... .A........ .......... .I........ ..QS.I....
#KP152239_KWARA180/NG/2014           .......... .A........ .......... .I........ ..QS.I....
#KP152240_KWARA181/NG/2014           .......... .A........ .......... .I........ ..QS.I....
#KP152241_KWARA182/NG/2014           .......... .A........ .......... .I........ ..QS.I....
#KP152242_KWARA184/NG/2014           .......... .A........ .......... .I........ ..QS.I....
#KP152243_UYO185/NG/2014             ........T. .A........ .......... .I........ ..QS.I....
#KP152244_UYO187/NG/2014             .......... .A........ .......... .I........ ..QS.I....
#KP152245_UYO188/NG/2014             ........T. .A........ .......... .I........ ..QS.I....
#KP152246_UYO189/NG/2014             .......... .A........ .......... .I........ ..QS.I....
#KP152247_UYO190/NG/2014             ........T. .A........ .......... .I........ ..QS.I....
#KP152248_UYO191/NG/2014             .......... .A........ .......... .I........ ..QS.I....
#KP152249_UYO192/NG/2014             .......... .A........ .......... .I........ ..QS.I....
#KP152250_UYO193/NG/2014             .......... .A........ .......... .I........ ..QS.I....
#KP152251_UYO194/NG/2014             .......... .A........ .......... .I........ ..QS.I....
#KP152252_UYO195/NG/2014             .......... .A........ .......... .I........ ..QS.I....
#KP152253_UYO196/NG/2014             .......... .A........ .......... .I........ ..QS.I....
#KP152254_UYO197/NG/2014             .......... .A........ .......... .I........ ..QS.I....
#KP152255_UYO198/NG/2014             .......... .A........ .......... .I........ ..QS.I....
#KP152256_UYO199/NG/2014             .......... .A........ .......... .I........ ..QS.I....
#KP152257_UYO200/NG/2014             .......... .A........ .......... .I........ ..QS.I....
#KP152258_UYO201/NG/2014             .......... .A........ .......... .I........ ..QS.I....
#KP152259_UYO202/NG/2014             .......... .A........ .......... .I........ ..QS.I....
#KP152260_UYO203/NG/2014             .......... .A........ .......... .I........ ..QS.I....
#KP152261_UYO204/NG/2014             .......... .A........ .......... .I........ ..QS.I....
#KP152322_UYO205/NG/2014             .......... .A........ .......... .I........ ..QS.I....
#KP152231_UYO206/NG/2014             .......... .A........ .......... .I........ ..QS.I....
#KP152232_KWARA171/NG/2014           .......... .A........ .......... .I........ ..QS.I....
#MB_Vaccine                          .......... .A........ .......... .I........ ..Q..I....
#IBA                                 .......... .......... .......... .......... ..........
#BURS706                             .......... .......... .......... .......... ..........
#ABIC                                .......... .A........ .......... .I........ ..Q..I....
#BURSAVAC                            .......... .......... .......... .......... ..Q....N..
#BURSINE_PLUS                        .......... .L........ .......... .I......H. ..Q..A.N..
#VENTRI                              .......... .S........ .......... .I......H. ..Q..A.N..
#BURSINE_2                           .......... .S........ .......... .I......H. .....A.D..
#USA_GEORGIA                         .......... .......... .......... .......... ..........
#Bursaplex_Vaccine                   ......L... .......... .......... .......... ..Q.......
#Nobilis_Gumboro_vaccine_228E        .......... .S........ .......... .......... ..Q..I....
#D78_Vaccine                         .......... .......... .......... .......... ..........
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                                    261        271        281        291        301
#NVRI_VOM_Vaccine                    IYLIGFDGTT VITRAVAANN GLTTGTDNLM PFNLVIPTNE ITQPITSIKL
#KP152296_NIG20/NG/2009              .........A ........D. ...A...... ...I....SA ..........
#KP152233_KWARA174/NG/2014           .........A ........D. ...A...... ...I....SA ..........
#KP152293_PLATEAU43/NG/2012          ........SA ........D. ...A...... ...I....SQ ..........
#KP152321_NIG79/NG/2009              .........A ........D. ...A...... ...I....SA ..........
#KP152320_UYO167/NG/2014             .........A ........D. ...A...... ...I....SA ..........
#KP152319_UYO166/NG/2014             .........A ........D. ...A...... ...I....SA ..........
#KP1522318_PLATEAU164/NG/2014        .........A ........D. ...A...... ...I....SA ..........
#KP152317_PLATEAU163/NG/2014         .........A ........D. ...A...... ...I....SA ..........
#KP152316_PLATEAU162/NG/2014         .........A ........D. ...A...... ...I....SA ..........
#KP152315_PLATEAU161/NG/2014         .........A ........D. ...A...... ...I....SA ..........
#KP152314_PLATEAU160/NG/2014         ........SA ........D. ...A...... ...I....SQ ..........
#KP152313_PLATEAU159/NG/2014         .........A ........D. ...A...... ...I....SA ..........
#KP152312_PLATEAU158/NG/2014         .........A ........D. ...A...... ...I....SA ..........
#KP152311_PLATEAU157/NG/2014         .........A ........D. ...A...... ...I....SA ..........
#KP152310_PLATEAU156/NG/2014         ........SA ........D. ...A...... ...I....SQ ..........
#KP152309_PLATEAU155/NG/2014         .........A ........D. ...A...... ...I....SA ..........
#KP152308_PLATEAU154/NG/2014         .........A ........D. ...A...... ...I....SA ..........
#KP152306_PLATEAU16/NG/2009          .........A ........D. ...A...... ...I....SA ..........
#KP152292_PLATEAU4/NG/2009           .........A ........D. ...A...... ...I....SA ..........
#KP152304_KWARA152/NG/2014           .........A ........D. ...A...... ...I....SA ..........
#KP152303_KWARA151NG/NG/2014         .........A ........D. ...A...... ...I....SA ..........
#KP152302_KWARA150/NG/2014           .........A ........D. ...A...... ...I....SA ..........
#KP152301_KWARA149/NG/2014           .........A ........D. ...A...... ...I....SA ..........
#KP152300_KWARA148/NG/2014           .........A .T........ ...A...... ...I....S. ..........
#KP152299_KWARA147/NG/2014           .........A ........D. ...A...... ...I....SA ..........
#KP152298_KWARA145/NG/2014           .........A ........D. ...A...... ...I....SA ..........
#KP152297_BENIN47/NG/2014            .........A ........D. ...A...... ...I....SA ..........
#KP266329_BAUCHI37/NG/2012           .........A ........D. ...A...... ...I....SA ..........
#KP152268_BAUCHI36/NG/2009           .........A ........D. ...A...... ...I....SA ..........
#KP266322_PLATEAU19/NG/2009          .........A ........D. ...A...... ...I....SA ..........
#KP266323_KADUNA61/NG/2009           .........A ........D. ...A...... ...I....SA ..........
#KP266334_PLATEAU39/NG/2012          .........A ........D. ...A...... ...I....SA ..........
#KP266324_BAUCHI58/NG/2012           ........SA ........D. ...A...... ...I....SQ ..........
#KP266325_PLATEAU67/NG/2011          .........A ........D. ...A...... ...I....SA ..........
#KP266326_PLATEAU53/NG/2010          .........A ........D. ...A...... ...I....SA ..........
#KP266327_ANAMBRA54/NG/2012          ........SA ........D. ...A...... ...I....SQ ..........
#KP152305_PLATEAU7/NG/2009           .........A ........D. ...A...... ...I....SA ..........
#KP266328_BENIN35/NG/2012            .........A ........D. ...A...... ...I....SA ..........
#KP152291_BAUCHI37/NG/2009           .........A ........D. ...A...... ...I....SA ..........
#KP152262_BAUCHI124/NG/2014          .........A ........D. ...A...... ...I....SA ..........
#KP152263_BAUCHI126/NG/2014          .........A ........D. ...A...... ...I....SA ..........
#KP152264_BAUCHI127/NG/2014          .........A ........D. ...A...... ...I....SA ..........
#KP152265_KADUNA136/NG/2014          ........SA ........D. ...A...... ...I....SQ ..........
#KP152266_PLATEAU133/NG/2014         ........SA ........D. ...A...... ...I....SQ ..........
#KP152267_PLATEAU135/NG/2014         ........SA ........D. ...A...... ...I....SQ ..........
#KP266330_NASSARAWA77/NG/no2/_2013   .........A .......... .......... .......... ..........
#KP266331_NASSARAWA77/NG/2013        ........SA ........D. ...A...... ...I....SQ ..........
#KP266332_PLATEAU78/NG/2011/2        ........SA ........D. ...A...... ...I....SQ ..........
#KP266333_PLATEAU78/NG/2011/1        ........SA ........D. ...A...... ...I....SQ ..........
#KP152270_BENIN142E/NG/2013          .........A .T........ ...A...... ...I....S. ..........
#KP152269_BENIN87/NG/2013            .........A ........D. ...A...... ...I....SA ..........
#KP152271_ABUJA93/NG/2013            .........A ........D. ...A...... ...I....SA ..........
#KP152272_BAUCHI128/NG/2014          .........A ........D. ...A...... ...I....SA ..........
#KP152273_PLATEAU99/NG/2011          .........A ........D. ...A...... ...I....SA ..........
#KP152274_PLATEAU92/NG/2013          T........A ........D. ...A...... ...I....SA ..........
#KP152275_PLATEAU83/NG/2013          .........A ........D. ...A...... ...I....SA ..........
#KP152276_PLATEAU90/NG/2013          .........A ........D. ...A...... ...I....SA ..........
#KP152277_PLATEAU95/NG/2013          .........A ........D. ...A...... ...I....SA ..........
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#KP152278_PLATEAU96/NG/2013          .........A ........D. ...A...... ...I....SA ..........
#KP152281_PLATEAU131/NG/2014         .........A ........D. ...A...... ...I....SA ..........
#KP152282_PLATEAU134/NG/2014         .........A ........D. ...A...... ...I....SA ..........
#KP152283_PLATEAU110/NG/2010         .........A ........D. ...A...... ...I....SA ..........
#KP152284_PLATEAU104/NG/2010         .........A ........D. ...A...... ...I....SA ..........
#KP152285_BAUCHI121/NG/2013          .........A ........D. ...A...... ...I....SA ..........
#KP152286_KWARA139/NG/2014           .........A ........D. ...A...... ...I....SA ..........
#KP152287_PLATEAU40/NG/2012          .........A .T........ ...A...... ...I....S. ..........
#KP152288_PLATEAU119/NG/2013         ........SA ........D. ...A...... ...I....SQ ..........
#KP152289_KWARA141/NG/2014           .........A .T........ ...A...... ...I....S. ..........
#KP152279_PLATEAU120/NG/2013         ........SA ........D. ...A...... ...I....SQ ..........
#KP152280_PLATEAU132/NG/2014         ........SA ........D. ...A...... ...I....SQ ..........
#KP152290_PLATEAU82/NG/2013          ........SA ........D. ...A...... ...I....SQ ..........
#KP152307_PLATEAU75/NG/2011          .........A ........D. ...A...... ...I....SA ..........
#KP152295_ANAMBRA50/NG/2012          ........SA ........D. ...A...... ...I....SQ ..........
#KP152294_BENIN34/NG/2012            .........A ........D. ...A...... ...I....SA ..........
#KP152234_KWARA175/NG/2014           .........A ........D. ...A...... ...I....SA ..........
#KP152235_KWARA176/NG/2014           .........A ........D. ...A...... ...I....SA ..........
#KP152236_KWARA177/NG/2014           .........A ........D. ...A...... ...I....SA ..........
#KP152237_KWARA178/NG/2014           .........A ........D. ...A...... ...I....SA ..........
#KP152238_KWARA179/NG/2014           .........A ........D. ...A...... ...I....SA ..........
#KP152239_KWARA180/NG/2014           .........A ........D. ...A...... ...I....SA ..........
#KP152240_KWARA181/NG/2014           .........A ........D. ...A...... ...I....SA ..........
#KP152241_KWARA182/NG/2014           .........A ........D. ...A...... ...I....SA ..........
#KP152242_KWARA184/NG/2014           .........A ........D. ...A...... ...I....SA ..........
#KP152243_UYO185/NG/2014             ........SA ........D. ...A...... ...I....SQ ..........
#KP152244_UYO187/NG/2014             .........A ........D. ...A...... ...I....SA ..........
#KP152245_UYO188/NG/2014             ........SA ........D. ...A...... ...I....SQ ..........
#KP152246_UYO189/NG/2014             .........A ........D. ...A...... ...I....SA ..........
#KP152247_UYO190/NG/2014             ........SA ........D. ...A...... ...I....SQ ..........
#KP152248_UYO191/NG/2014             .........A ........D. ...A...... ...I....SA ..........
#KP152249_UYO192/NG/2014             .........A ........D. ...A...... ...I....SA ..........
#KP152250_UYO193/NG/2014             .........A ........D. ...A...... ...I....SA ..........
#KP152251_UYO194/NG/2014             .........A ........D. ...A...... ...I....SA ..........
#KP152252_UYO195/NG/2014             .........A ........D. ...A...... ...I....SA ..........
#KP152253_UYO196/NG/2014             .........A ........D. ...A...... ...I....SA ..........
#KP152254_UYO197/NG/2014             .........A ........D. ...A...... ...I....SA ..........
#KP152255_UYO198/NG/2014             .........A ........D. ...A...... ...I....SA ..........
#KP152256_UYO199/NG/2014             .........A ........D. ...A...... ...I....SA ..........
#KP152257_UYO200/NG/2014             .........A ........D. ...A...... ...I....SA ..........
#KP152258_UYO201/NG/2014             .........A ........D. ...A...... ...I....SA ..........
#KP152259_UYO202/NG/2014             .........A ........D. ...A...... ...I....SA ..........
#KP152260_UYO203/NG/2014             .........A ........D. ...A...... ...I....SA ..........
#KP152261_UYO204/NG/2014             .........A ........D. ...A...... ...I....SA ..........
#KP152322_UYO205/NG/2014             .........A ........D. ...A...... ...I....SA ..........
#KP152231_UYO206/NG/2014             .........A ........D. ...A...... ...I....SA ..........
#KP152232_KWARA171/NG/2014           .........A ........D. ...A...... ...I....SA ..........
#MB_Vaccine                          .........A .T........ ...A...... ...I....S. ..........
#IBA                                 .......... .......... .......... .......... ..........
#BURS706                             .......... .......... .......... .......... ..........
#ABIC                                .........A .T........ ...A...... ...I....S. ..........
#BURSAVAC                            ...V...... .T......G. ...A...... ........S. ....V.....
#BURSINE_PLUS                        .......... .......SD. .....I.... .......... ..........
#VENTRI                              .......... .......SD. .....I.... .......... ..........
#BURSINE_2                           .......... .......SD. .....I.... .......... ..........
#USA_GEORGIA                         .......... .......... .......... .......... ..........
#Bursaplex_Vaccine                   .......... ........D. ...A...... ...I...... ..........
#Nobilis_Gumboro_vaccine_228E        .......... .......... ...A...... .......... ..........
#D78_Vaccine                         .......... .......... .......... .......... ..........
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                                    311        321        331
#NVRI_VOM_Vaccine                    EIVTSKSGGQ AGDQMSWSAR GSLAVTIHGG
#KP152296_NIG20/NG/2009              .......... .........S ..........
#KP152233_KWARA174/NG/2014           .......... .........S ..........
#KP152293_PLATEAU43/NG/2012          .......... .........S ..........
#KP152321_NIG79/NG/2009              .......... .........S ..........
#KP152320_UYO167/NG/2014             .......... .........S ..........
#KP152319_UYO166/NG/2014             .......... .........S ..........
#KP1522318_PLATEAU164/NG/2014        .......... .........S ..........
#KP152317_PLATEAU163/NG/2014         .......... .........S ..........
#KP152316_PLATEAU162/NG/2014         .......... .........S ..........
#KP152315_PLATEAU161/NG/2014         .......... .........S ..........
#KP152314_PLATEAU160/NG/2014         .......... .........S ..........
#KP152313_PLATEAU159/NG/2014         .......... .........S ..........
#KP152312_PLATEAU158/NG/2014         .......... .........S ..........
#KP152311_PLATEAU157/NG/2014         .......... .........S ..........
#KP152310_PLATEAU156/NG/2014         .......... .........S ..........
#KP152309_PLATEAU155/NG/2014         .......... .........S ..........
#KP152308_PLATEAU154/NG/2014         .......... .........S ..........
#KP152306_PLATEAU16/NG/2009          .......... .........S ..........
#KP152292_PLATEAU4/NG/2009           .......... .........S ..........
#KP152304_KWARA152/NG/2014           .......... .........S ..........
#KP152303_KWARA151NG/NG/2014         .......... .........S ..........
#KP152302_KWARA150/NG/2014           .......... .........S ..........
#KP152301_KWARA149/NG/2014           .......... .........S ..........
#KP152300_KWARA148/NG/2014           .......... .........S ..........
#KP152299_KWARA147/NG/2014           .......... .........S ..........
#KP152298_KWARA145/NG/2014           .......... .........S ..........
#KP152297_BENIN47/NG/2014            .......... .........S ..........
#KP266329_BAUCHI37/NG/2012           .......... .........S ..........
#KP152268_BAUCHI36/NG/2009           .......... .........S ..........
#KP266322_PLATEAU19/NG/2009          .......... .........S ..........
#KP266323_KADUNA61/NG/2009           .......... .........S ..........
#KP266334_PLATEAU39/NG/2012          .......... .........S ..........
#KP266324_BAUCHI58/NG/2012           .......... .........S ..........
#KP266325_PLATEAU67/NG/2011          .......... .........S ..........
#KP266326_PLATEAU53/NG/2010          .......... .........S ..........
#KP266327_ANAMBRA54/NG/2012          .......... .........S ..........
#KP152305_PLATEAU7/NG/2009           .......... .........S ..........
#KP266328_BENIN35/NG/2012            .......... .........S ..........
#KP152291_BAUCHI37/NG/2009           .......... .........S ..........
#KP152262_BAUCHI124/NG/2014          .......... .........S ..........
#KP152263_BAUCHI126/NG/2014          .......... .........S ..........
#KP152264_BAUCHI127/NG/2014          .......... .........S ..........
#KP152265_KADUNA136/NG/2014          .......... .........S ..........
#KP152266_PLATEAU133/NG/2014         .......... .........S ..........
#KP152267_PLATEAU135/NG/2014         .......... .........S ..........
#KP266330_NASSARAWA77/NG/no2/_2013   .......... .........S ..........
#KP266331_NASSARAWA77/NG/2013        .......... .......... ..........
#KP266332_PLATEAU78/NG/2011/2        .......... .......... ..........
#KP266333_PLATEAU78/NG/2011/1        .......... .........S ..........
#KP152270_BENIN142E/NG/2013          .......... .........S ..........
#KP152269_BENIN87/NG/2013            .......... .........S ..........
#KP152271_ABUJA93/NG/2013            .......... .........S ..........
#KP152272_BAUCHI128/NG/2014          .......... .........S ..........
#KP152273_PLATEAU99/NG/2011          .......... .........S ..........
#KP152274_PLATEAU92/NG/2013          .......... .........S ..........
#KP152275_PLATEAU83/NG/2013          .......... .........S ..........
#KP152276_PLATEAU90/NG/2013          .......... .........S ..........
#KP152277_PLATEAU95/NG/2013          .......... .........S ..........
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#KP152278_PLATEAU96/NG/2013          .......... .........S ..........
#KP152281_PLATEAU131/NG/2014         .......... .........S ..........
#KP152282_PLATEAU134/NG/2014         .......... .........S ..........
#KP152283_PLATEAU110/NG/2010         .......... .........S ..........
#KP152284_PLATEAU104/NG/2010         .......... .........S ..........
#KP152285_BAUCHI121/NG/2013          .......... .........S ..........
#KP152286_KWARA139/NG/2014           .......... .........S ..........
#KP152287_PLATEAU40/NG/2012          .......... .........S ..........
#KP152288_PLATEAU119/NG/2013         .......... .........S ..........
#KP152289_KWARA141/NG/2014           .......... .........S ..........
#KP152279_PLATEAU120/NG/2013         .......... .........S ..........
#KP152280_PLATEAU132/NG/2014         .......... .........S ..........
#KP152290_PLATEAU82/NG/2013          .......... .........S ..........
#KP152307_PLATEAU75/NG/2011          .......... .........S ..........
#KP152295_ANAMBRA50/NG/2012          .......... .........S ..........
#KP152294_BENIN34/NG/2012            .......... .........S ..........
#KP152234_KWARA175/NG/2014           .......... .........S ..........
#KP152235_KWARA176/NG/2014           ....T..... .........S ..........
#KP152236_KWARA177/NG/2014           .......... .........S ..........
#KP152237_KWARA178/NG/2014           ....T..... .........S ..........
#KP152238_KWARA179/NG/2014           .......... .........S ..........
#KP152239_KWARA180/NG/2014           .......... .........S ..........
#KP152240_KWARA181/NG/2014           .......... .........S ..........
#KP152241_KWARA182/NG/2014           .......... .........S ..........
#KP152242_KWARA184/NG/2014           .......... .........S ..........
#KP152243_UYO185/NG/2014             .......... .........S ..........
#KP152244_UYO187/NG/2014             .......... .........S ..........
#KP152245_UYO188/NG/2014             .......... .........S ..........
#KP152246_UYO189/NG/2014             .......... .........S ..........
#KP152247_UYO190/NG/2014             .......... .........S ..........
#KP152248_UYO191/NG/2014             .......... .........S ..........
#KP152249_UYO192/NG/2014             .......... .........S ..........
#KP152250_UYO193/NG/2014             .......... .........S ..........
#KP152251_UYO194/NG/2014             .......... .........S ..........
#KP152252_UYO195/NG/2014             .......... .........S ..........
#KP152253_UYO196/NG/2014             .......... .........S ..........
#KP152254_UYO197/NG/2014             .......... .........S ..........
#KP152255_UYO198/NG/2014             .......... .........S ..........
#KP152256_UYO199/NG/2014             .......... .........S ..........
#KP152257_UYO200/NG/2014             .......... .........S ..........
#KP152258_UYO201/NG/2014             .......... .........S ..........
#KP152259_UYO202/NG/2014             .......... .........S ..........
#KP152260_UYO203/NG/2014             .......... .........S ..........
#KP152261_UYO204/NG/2014             .......... .........S ..........
#KP152322_UYO205/NG/2014             .......... .........S ..........
#KP152231_UYO206/NG/2014             .......... .........S ..........
#KP152232_KWARA171/NG/2014           .......... .........S ..........
#MB_Vaccine                          .......... .........S ..........
#IBA                                 .......... .......... ..........
#BURS706                             .......... .......... ..........
#ABIC                                .......... .........S ..........
#BURSAVAC                            .......... .......L.S .N........
#BURSINE_PLUS                        ......N... .........S ..........
#VENTRI                              .......... .........S ..........
#BURSINE_2                           .......... .........S ..........
#USA_GEORGIA                         .......... .......... ..........
#Bursaplex_Vaccine                   .......... .........S ..........
#Nobilis_Gumboro_vaccine_228E        .......... ........VS ..........
#D78_Vaccine                         .......... .......... ..........
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Appendix 13: Amino acid sequence data of Nigeria IBDV viruses and some IBDV vaccine strains

312	371
#AJ878668_HK46	CCT GAG AAT GAG GAG TAT GAG ACC GAT CAA ATA CTC CCT GAC CTA GCT TGG ATG AGG CAG
#AJ878666_UK661	... ...
#D49707_OKYM	... ..T ... .A ... ...
#AJ878676_DEL_E	..C ... ...C ... ...A ... T.. ..A ... ... C.A ...
#AJ878671_HENAN	..C ... ...C ..G ... .A ... .A ... ... C.A ...
#AY099457_T09	... ...
#KP172084_ABUJA93/NG/2013	..C ..A ... ...C ..G ... .A ... ...
#KP266335_PLATEAU19/NG/2009	..C ..A ... ...C ..G ... .A ... ...
#KP266336_KADUNA61/NG/2012	..C ..A ... ...C ..G ... .A ... ...
#KP266337_BAUCHI58/NG/2012	..C ... ...C ..G ..T ... ..A ... ...
#KP266338_PLATEAU53/NG/2010	..C ..A ... ...C ..G ... .A ... ...
#KP266339_PLATEAU7/NG/2009	..C ..A ... ...C ..G ... .A ... ...
#KP266340_BAUCHI37/NG/2012	..C ..A ... ...C ..G ... .A ... ...
#KP172085_BAUCHI121/NG/2013	..C ..A ... ...C ..G ... .A ... ...
#KP172086_BAUCHI124/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172087_BAUCHI126/NG/2014	... ..A ... ...C ..G ... .A ... ...
#KP172088_KWARA171/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172089_KWARA175/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172090_KWARA177/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172091_KWARA178/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172092_KWARA180/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172093_KWARA181/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172094_UYO187/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172095_UYO189/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172096_UYO190/NG/2014	..C ... ...C ..G ..T ... ..A ... ...
#KP172097_UYO191/NG/2014	..C ... ...C ..G ..T ... ..A ... ...
#KP172098_UYO192/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172099_UYO193/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172100_UYO194/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172101_UYO195/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172102_UYO196/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172103_UYO197/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172104_UYO198/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172105_UYO200/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172106_UYO202/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172107_UYO203/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172108_UYO207/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172109_KWARA184/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172110_UYO188/NG/2014	..C ... ...C ..G ..T ... ..A ... ...
#KP172111_BAUCHI128/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172112_BENIN87/NG/2013	..C ..A ... ...C ..G ... .A ... ...
#KP172113_KADUNA136/NG/2014	..C ... ...C ..G ..T ... ..A ... ...
#KP172114_KWARA141/NG/2014	... ...
#KP172115_PLATEAU134/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172116_PLATEAU40/NG/2012	... ...
#KP172117_PLATEAU82/NG/2013	..C ... ...C ..G ..T ... ..A ... ...
#KP172118_PLATEAU83/NG/2013	..C ... ...C ..G ..T ... ..A ... ...
#KP172119_PLATEAU90/NG/2013	..C ..A ... ...C ..G ... .A ... ...
#KP172120_PLATEAU92/NG/2013	..C ..A ... ...C ..G ... .A ... ...
#KP172121_PLATEAU95/NG/2013	..C ... ...C ..G ..T ... ..A ... ...
#KP172122_PLATAEU96/NG/2013	..C ... ...C ..G ..T ... ..A ... ...
#KP172123_PLATEAU104/NG/2010	..C ..A ... ...C ..G ... .A ... ...
#KP172124_PLATEAU110/NG/2010	..C ..A ... ...C ..G ... .A ... ...
#KP172125_PLATEAU119/NG/2013	..C ... ...C ..G ..T ... ..A ... ...
#KP172126_PLATEAU154/NG/2014	..C ..A ... ...C ..G ... .A ... ...A ...
#KP172127_PLATEAU120/NG/2013	..C ... ...C ..G ..T ... ..A ... ...
#KP172128_PLATEAU131/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172129_PLATEAU132/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172130_PLATEAU133/NG/2014	..C ... ...C ..G ..T ... ..A ... ...
#KP172131_PLATEAU135/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172132_KWARA148/NG/2014	... ...
#KP172133_PLATEAU156/NG/2014	..C ... ...C ..G ..T ... ..A ... ...
#KP172134_PLATEAU163/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172135_PLATEAU164/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172136_PLATEAU39/NG/2012	..C ..A ... ...C ..G ... .A ... ...
#KP172137_PLATEAU43/NG/2012	..C ... ...C ..G ..T ... ..A ... ...
#KP172138_PLATEAU157/NG/2014	..C ..A ... ...C ..G ... .A ... ...
#KP172139_PLATEAU160/NG/2014	..C ... ...C ..G ..T ... ..A ... ...

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#KP172140_UYO167/NG/2014        ..C ..A ... ... ... ... ... ... ..C ..G ... ... ..A ... ... ... ... ... ... ...
#KP172141_PLATEAU161/NG/2014    ..C ..A ... ... ... ... ... ... ..C ..G ... ... ..A ... ... ... ... ... ... ...
#KP172142_PLATEAU162/NG/2014    ..C ..A ... ... ... ... ... ... ..C ..G ... ... ..A ... ... ... ... ... ... ...
#KP172143_ANAMBRA50/NG/2012     ..C ... ... ... ... ... ... ... ..C ..G ..T ... ..A ... ... ... ... ... ... ...
#KP172144_PLATEAU158/NG/2014    ..C ..A ... ... ... ... ... ... ..C ..G ... ... ..A ... ... ... ... ... ... ...
#AJ878656_BURS_706              ..C ... ... ... ... ... ... ... ..C ... ... ... ..A ... T.. ..A ... ... C.A ...

                            372                                                                                 431
#AJ878668_HK46                  ATA GAG GGA GCT GTT TTA AAA CCA ACT CTA TCA CTC CCC ATT GGA GAC CAG GAG TAC TTC
#AJ878666_UK661                 ... ... ... ... ... ... ... ... ..C ... ..T ... ... ... ... ... ... ... ... ...
#D49707_OKYM                    ... ... ..G ... ... ... ... ... ..C ... ..T ... ... ... ... ... ... ... ... ...
#AJ878676_DEL_E                 ... ..A ..G ... ..C ..G ..G ..T ... ... ..T ... ... ... ... ... ... ... ... ...
#AJ878671_HENAN                 ... ..A ..G ..A ... ... ..G ... ... ... ..T ... ... ... ..G ... ..A ... ... ...
#AY099457_T09                   ... ... ... ..G ... ... ... ... ..C ... ..T ... ... ... ..G ... ... ... ... ...
#KP172084_ABUJA93/NG/2013       ... ..A ..G ... ..A C.. ..G ..T ... ... ..T ... ... ... ... ... ... ... ... ...
#KP266335_PLATEAU19/NG/2009     ... ..A ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
#KP266336_KADUNA61/NG/2012      ... ... ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
#KP266337_BAUCHI58/NG/2012      ... ..A ..G ... ..A C.. ..G ..T ... ... ..T ... ... ... ..G ... ..A ... ... ...
#KP266338_PLATEAU53/NG/2010     ... ... ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
#KP266339_PLATEAU7/NG/2009      ... ..A ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
#KP266340_BAUCHI37/NG/2012      ... ... ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
#KP172085_BAUCHI121/NG/2013     ... ... ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
#KP172086_BAUCHI124/NG/2014     ... ..A ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
#KP172087_BAUCHI126/NG/2014     ... ... ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
#KP172088_KWARA171/NG/2014      ... ... ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
#KP172089_KWARA175/NG/2014      ... ... ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
#KP172090_KWARA177/NG/2014      ... ... ..G ..C ..G C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
#KP172091_KWARA178/NG/2014      ... ... ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
#KP172092_KWARA180/NG/2014      ... ... ..G ... ..A C.. ..G ..C ... ... ..T ..T ... ... ... ... ... ... ... ...
#KP172093_KWARA181/NG/2014      ... ... ..G ... ..A C.. ..G ..C ... ... ..T ..T ... ... ... ... ... ... ... ...
#KP172094_UYO187/NG/2014        ... ..A ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
#KP172095_UYO189/NG/2014        ... ..A ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
#KP172096_UYO190/NG/2014        ... ..A ..G ... ..A C.. ..G ..T ... T.. ..T ... ... ... ... ... ..A ... ... ...
#KP172097_UYO191/NG/2014        ... ..A ..G ... ..A C.. ..G ..T ... T.. ..T ... ... ... ... ... ..A ... ... ...
#KP172098_UYO192/NG/2014        ... ... ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
#KP172099_UYO193/NG/2014        ... ..A ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
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#KP172104_UYO198/NG/2014        ... ..A ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
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#KP172108_UYO207/NG/2014        ... ..A ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
#KP172109_KWARA184/NG/2014      ... ... ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
#KP172110_UYO188/NG/2014        ... ..A ..G ... ..A C.. ..G ..T ... T.. ..T ... ... ... ... ... ..A ... ... ...
#KP172111_BAUCHI128/NG/2014     ... ..A ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
#KP172112_BENIN87/NG/2013       ... ... ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
#KP172113_KADUNA136/NG/2014     ... ..A ..G ... ..A C.. ..G ..T ... ... ..T ... ... ... ..G ... ..A ... ... ...
#KP172114_KWARA141/NG/2014      ... ... ... ... ... ... ... ... .AC ... ..T ... ... ... ... ... ... ... ... ...
#KP172115_PLATEAU134/NG/2014    ... ... ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
#KP172116_PLATEAU40/NG/2012     ... ... ... ... ... ... ... ... .AC ... ..T ... ... ... ... ... ... ... ... ...
#KP172117_PLATEAU82/NG/2013     ... ..A ..G ... ..A C.. ..G ..T ... ... ..T ... ... ... ..G ... ..A ... ... ...
#KP172118_PLATEAU83/NG/2013     ... ..A ..G ... ..A C.. ..G ..T ... ... ..T ... ... ... ..G ... ..A ... ... ...
#KP172119_PLATEAU90/NG/2013     ... ..A ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
#KP172120_PLATEAU92/NG/2013     ... ... ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
#KP172121_PLATEAU95/NG/2013     ... ..A ..G ... ..A C.. ..G ..T ... ... ..T ... ... ... ..G ... ..A ... ... ...
#KP172122_PLATAEU96/NG/2013     ... ..A ..G ... ..A C.. ..G ..T ... ... ..T ... ... ... ..G ... ..A ... ... ...
#KP172123_PLATEAU104/NG/2010    ... ..A ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
#KP172124_PLATEAU110/NG/2010    ... ..A ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
#KP172125_PLATEAU119/NG/2013    ... ..A ..G ... ..A C.. ..G ..T ... ... ..T ... ... ... ..G ... ..A ... ... ...
#KP172126_PLATEAU154/NG/2014    ... ... ..G ... ..A C.. ..G ..T ... ... ..T ... ... ... ... ... ... ... ... ...
#KP172127_PLATEAU120/NG/2013    ... ..A ..G ... ..A C.. ..G ..T ... ... ..T ... ... ... ..G ... ..A ... ... ...
#KP172128_PLATEAU131/NG/2014    ... ... ..G ... ..A C.. ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
#KP172129_PLATEAU132/NG/2014    ... ..A ..G ... ..A C.G ..G ..C ... ... ..T ... ... ... ... ... ... ... ... ...
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#KP172118_PLATEAU83/NG/2013     ...
#KP172119_PLATEAU90/NG/2013     ...
#KP172120_PLATEAU92/NG/2013     ...
#KP172121_PLATEAU95/NG/2013     ...
#KP172122_PLATAEU96/NG/2013     ...
#KP172123_PLATEAU104/NG/2010    ...
#KP172124_PLATEAU110/NG/2010    ...
#KP172125_PLATEAU119/NG/2013    ...
#KP172126_PLATEAU154/NG/2014    ...
#KP172127_PLATEAU120/NG/2013    ...
#KP172128_PLATEAU131/NG/2014    ...
#KP172129_PLATEAU132/NG/2014    ...
#KP172130_PLATEAU133/NG/2014    ...
#KP172131_PLATEAU135/NG/2014    ...
#KP172132_KWARA148/NG/2014      ...
#KP172133_PLATEAU156/NG/2014    ...
#KP172134_PLATEAU163/NG/2014    ...
#KP172135_PLATEAU164/NG/2014    ...
#KP172136_PLATEAU39/NG/2012     ...
#KP172137_PLATEAU43/NG/2012     ...
#KP172138_PLATEAU157/NG/2014    ...
#KP172139_PLATEAU160/NG/2014    ...
#KP172140_UYO167/NG/2014        ...
#KP172141_PLATEAU161/NG/2014    ...
#KP172142_PLATEAU162/NG/2014    ...
#KP172143_ANAMBRA50/NG/2012     ...
#KP172144_PLATEAU158/NG/2014    ...
#AJ878656_BURS_706              ...
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Appendix 14: Nucleotide sequence data of Nigerian IBDV segment B and some reference IBDV segment B

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                                68         78         88         98         108
#AJ878668_HK46                   PENEEYETDQ ILPDLAWMRQ IEGAVLKPTL SLPIGDQEYF PKYYPTHRPS
#D49707 OKYM                     .D........ .......... .......... .......... ..........
#KP266335_PLATEAU19/NG/2009      .......... .......... .......... .......... ..........
#KP172132_KWARA148/NG/2014       .......... .......... ........N. .......... ..........
#KP172133_PLATEAU156/NG/2014     .......... .......... .......... .......... ..........
#KP172114_PLATEAU161/NG/2014     .......... .......... .......... .......... ..........
#KP172134_PLATEAU163/NG/2014     .......... .......... .......... .......... ..........
#KP172135_PLATEAU164/NG/2014     .......... .......... .......... .......... ..........
#KP172126_PLATEAU154/NG/2014     .......... .......... .......... .......... ..........
#KP172140_UYO167/NG/2014         .......... .......... .......... .......... ..........
#KP172136_PLATEAU39/NG/2012      .......... .......... .......... .......... ..........
#KP172137_PLATEAU43/NG/2012      .......... .......... .......... .......... ..........
#KP172138_PLATEAU157/NG/2014     .......... .......... .......... .......... ..........
#KP172139_PLATEAU160/NG/2014     .......... .......... .......... .......... ..........
#KP172142_PLATEAU162/NG/2014     .......... .......... .......... .......... ..........
#KP172143_ANAMBRA50/NG/2012      .......... .......... .......... .......... ..........
#KP172144_PLATEAU158/NG/2014     .......... .......... .......... T......... ..........
#KP172084_ABUJA93/NG/2013        .......... .......... .......... .......... ..........
#KP172085_BAUCHI121/NG/2013      .......... .......... .......... .......... ..........
#KP172089_KWARA175/NG/2014       .......... .......... .......... .......... ..........
#KP172090_KWARA177/NG/2014       .......... .......... .......... .......... ..........
#KP172091_KWARA178/NG/2014       .......... .......... .......... .......... ..........
#KP172092_KWARA180/NG/2014       .......... .......... .......... .......... ..........
#KP172093_KWARA181/NG/2014       .......... .......... .......... .......... ..........
#KP172094_UYO187/NG/2014         .......... .......... .......... .......... ..........
#KP172095_UYO189/NG/2014         .......... .......... .......... .......... ..........
#KP172096_UYO190/NG/2014         .......... .......... .......... .......... ..........
#KP172097_UYO191/NG/2014         .......... .......... .......... .......... ..........
#KP192098_UYO192/NG/2014         .......... .......... .......... .......... ..........
#KP172099_UYO193/NG/2014         .......... .......... .......... .......... ..........
#KP172100_UYO194/NG/2014         .......... .......... .......... .......... ..........
#KP172101_UYO195/NG/2014         .......... .......... .......... .......... ..........
#KP172102_UYO196/NG/2014         .......... .......... .......... .......... ..........
#KP172103_UYO197/NG/2014         .......... .......... .......... .......... ..........
#KP172104_UYO198/NG/2014         .......... .......... .......... .......... ..........
#KP172105_UYO200/NG/2014         .......... .......... .......... .......... ..........
#KP172106_UYO202/NG/2014         .......... .......... .......... .......... ..........
#KP172107_UYO203/NG/2014         .......... .......... .......... .......... ..........
#KP172108_UYO207/NG/2014         .......... .......... .......... .......... ..........
#KP172109_KWARA184/NG/2014       .......... .......... .......... .......... ..........
#KP172110_UYO188/NG/2014         .......... .......... .......... .......... ..........
#KP172088_KWARA171/NG/2014       .......... .......... .......... .......... ..........
#KP172086_BAUCHI124/NG/2014      .......... .......... .......... .......... ..........
#KP172087_BAUCHI126/NG/2014      .......... .......... .......... .......... ..........
#KP172111_BAUCHI128/NG/2014      .......... .......... .......... .......... ..........
#KP172112_BENIN87/NG/2013        .......... .......... .......... .......... ..........
#KP172113_KADUNA136/NG/2014      .......... .......... .......... .......... ..........
#KP172114_KWARA141/NG/2014       .......... .......... ........N. .......... ..........
#KP172122_PLATAEU96/NG/2013      .......... .......... .......... .......... ..........
#KP172116_PLATEAU40/NG/2012      .......... .......... ........N. .......... ..........
#KP172117_PLATEAU82/NG/2013      .......... .......... .......... .......... ..........
#KP172118_PLATEAU83/NG/2013      .......... .......... .......... .......... ..........
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#KP172121_PLATEAU95/NG/2013      .......... .......... .......... .......... ..........
#KP172123_PLATEAU104/NG/2010     .......... .......... .......... .......... ..........
#KP172124_PLATEAU110/NG/2010     .......... .......... .......... .......... ..........
#KP172125_PLATEAU119/NG/2013     .......... .......... .......... .......... ..........
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#KP172127_PLATEAU120/NG/2013    .......... .......... .......... .......... ..........
#KP172128_PLATEAU131/NG/2014    .......... .......... .......... .......... ..........
#KP172129_PLATEAU132/NG/2014    .......... .......... .......... .......... ..........
#KP172130_PLATEAU133/NG/2014    .......... .......... .......... .......... ..........
#KP172115_PLATEAU134/NG/2014    .......... .......... .......... .......... ..........
#KP172131_PLATEAU135/NG/2014    .......... .......... .......... .......... ..........
#AJ878666_UK661                 .......... .......... .......... .......... ..........
#AJ878676_DEL_E                 .......... .......... .......... .......... ..........
#KP266336_KADUNA61/NG/2012      .......... .......... .......... .......... ..........
#KP266337_BAUCHI58/NG/2012      .......... .......... .......... .......... ..........
#KP266338_PLATEAU53/NG/2010     .......... .......... .......... .......... ..........
#KP266339_PLATEAU7/NG/2009      .......... .......... .......... .......... ..........
#KP266340_BAUCHI37/NG/2012      .......... .......... .......... .......... ..........
#AJ878671_HENAN                 .......... .......... .......... .......... ..........
#AY099457_T09                   .......... .......... .......... .......... ..........
#BURS706                        .......... .......... .......... .......... ..........

                               118        128        138        148        158
#AJ878668_HK46                  KEKPNAYPPD IALLKQMIYL FLQVPEATDN LKDEVTLLTQ NIRDKAYGSG
#OKYM                           .......S.. .......... .......... .......... ..........
#KP266335_PLATEAU19/NG/2009     .D........ .......... ...I...HEG ..E....... .....V....
#KP172132_KWARA148/NG/2014      .......... .......... .......... .......... ...A......
#KP172133_PLATEAU156/NG/2014    .......... .......... ...I...QEG P.E....... ..........
#KP172114_PLATEAU161/NG/2014    .D........ .......... ...I...QEG ..E....... .....V....
#KP172134_PLATEAU163/NG/2014    .D........ .......... ...I...QEG ..E....... .....V....
#KP172135_PLATEAU164/NG/2014    .D........ .......... ...I...QEG ..E....... .....V....
#KP172126_PLATEAU154/NG/2014    .D........ .......... ...I...QEG ..E....... .....V....
#KP172140_UYO167/NG/2014        .D........ .......... ...I...HEG ..E....... .....V....
#KP172136_PLATEAU39/NG/2012     .......... .......... ...I...QEG ..E....... .....V....
#KP172137_PLATEAU43/NG/2012     .......... .......... ...I...QEG P.E....... ..........
#KP172138_PLATEAU157/NG/2014    .D........ .......... ...I...Q.G ..E...I... .....V....
#KP172139_PLATEAU160/NG/2014    .......... .......... ...I...QEG P.E....... ..........
#KP172142_PLATEAU162/NG/2014    .D........ .......... ...I...QEG ..E....... .....V....
#KP172143_ANAMBRA50/NG/2012     .......... .......... ...I...QEG P.E....... ..........
#KP172144_PLATEAU158/NG/2014    .D........ .......... ...I...QEG ..E....... .....V....
#KP172084_ABUJA93/NG/2013       .D........ .......... ...I...QEG .......... .....V....
#KP172085_BAUCHI121/NG/2013     .D........ .......... ...I...QEG ..E....... .....V....
#KP172089_KWARA175/NG/2014      .D........ .......... ...I...QEG ..E....... .....V....
#KP172090_KWARA177/NG/2014      .D........ .......... ...I...QEG ..E....... .....V....
#KP172091_KWARA178/NG/2014      .D........ .......... ...I...QEG ..E....... .....V....
#KP172092_KWARA180/NG/2014      .D........ .......... ...I...QEG ..E....... .....V....
#KP172093_KWARA181/NG/2014      .D........ .......... ...I...QEG ..E....... .....V....
#KP172094_UYO187/NG/2014        .D........ .......... ...I...QEG ..E....... .....V....
#KP172095_UYO189/NG/2014        .D........ .......... ...I...QEG ..E....... .....V....
#KP172096_UYO190/NG/2014        .....T.... .......... ...T...QEG P.E....... .....V....
#KP172097_UYO191/NG/2014        .....T.... .......... ...T...QEG P.E....... .....V....
#KP192098_UYO192/NG/2014        .D........ .......... ...I...QEG ..E....... .....V....
#KP172099_UYO193/NG/2014        .D........ .......... ...I...HEG ..E....... .....V....
#KP172100_UYO194/NG/2014        .D........ .......... ...I...HEG ..E....... .....V....
#KP172101_UYO195/NG/2014        .D........ .......... ...I...HEG ..E....... .....V....
#KP172102_UYO196/NG/2014        .D........ .......... ...I...HEG ..E....... .....V....
#KP172103_UYO197/NG/2014        .D........ .......... ...I...HEG ..E....... .....V....
#KP172104_UYO198/NG/2014        .D........ .......... ...I...HEG ..E....... .....V....
#KP172105_UYO200/NG/2014        .D........ .......... ...I...HEG ..E....... .....V....
#KP172106_UYO202/NG/2014        .D........ .......... ...I...HEG ..E....... .....V....
#KP172107_UYO203/NG/2014        .D........ .......... ...I...HEG ..E....... .....V....
#KP172108_UYO207/NG/2014        .D........ .......... ...I...HEG ..E....... .....V....
#KP172109_KWARA184/NG/2014      .D........ .......... ...I...QEG ..E....... .....V....
#KP172110_UYO188/NG/2014        .....T.... .......... ...T...QEG P.E....... .....V....
#KP172088_KWARA171/NG/2014      .D........ .......... ...I...QEG ..E....... .....V....
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#KP172086_BAUCHI124/NG/2014     .D........ .......... ...I...QEG .......... .....V....
#KP172087_BAUCHI126/NG/2014     .......... .......... ...I...QEG ..E..A.... .....V....
#KP172111_BAUCHI128/NG/2014     .D........ .......... ...I...QEG .......... .....V....
#KP172112_BENIN87/NG/2013       .D........ .......... ...I...QEG ..E....... .....V....
#KP172113_KADUNA136/NG/2014     .......... .......... ...I...QEG P.E....... ..........
#KP172114_KWARA141/NG/2014      .......... .......... .......... .......... ...A......
#KP172122_PLATAEU96/NG/2013     .......... .......... ...I...QEG P.E....... ..........
#KP172116_PLATEAU40/NG/2012     .......... .......... .......... .......... ...A......
#KP172117_PLATEAU82/NG/2013     .......... .......... ...I...QEG P.E....... ..........
#KP172118_PLATEAU83/NG/2013     .......... .......... ...I...QEG P.E....... ..........
#KP172119_PLATEAU90/NG/2013     .D........ .......... ...I...QEG ..E...I... .....V....
#KP172120_PLATEAU92/NG/2013     .D........ .......... ...I...QEG ..E....... .....V....
#KP172121_PLATEAU95/NG/2013     .......... .......... ...I...QEG P.E....... ..........
#KP172123_PLATEAU104/NG/2010    .D........ .......... ...I...QEG ..E....... .....V....
#KP172124_PLATEAU110/NG/2010    .D........ .......... ...I...QEG ..E....... .....V....
#KP172125_PLATEAU119/NG/2013    .......... .......... ...I...QEG P.E....... ..........
#KP172127_PLATEAU120/NG/2013    .......... .......... ...I...QEG P.E....... ..........
#KP172128_PLATEAU131/NG/2014    .D........ .......... ...I...QEG ..E....... .....V....
#KP172129_PLATEAU132/NG/2014    .D........ .......... ...I...QEG ..E...I... .....V....
#KP172130_PLATEAU133/NG/2014    .......... .......... ...I...QEG P.E....... ..........
#KP172115_PLATEAU134/NG/2014    .D........ .......... ...I...QEG ..E....... .....V....
#KP172131_PLATEAU135/NG/2014    .D........ .......... ...I...QEG ..E...I... .....V....
#AJ878666_UK661                 .......... .......... .......... .......... ..........
#AJ878676_DEL_E                 .......... .......... .......NED .......... ..........
#KP266336_KADUNA61/NG/2012      .D........ .......... ...I...QEG ..E....... .....V....
#KP266337_BAUCHI58/NG/2012      .......... .......... ...I...QEG P.E....... ..........
#KP266338_PLATEAU53/NG/2010     .D........ .......... ...I...QEG ..E....... .....V....
#KP266339_PLATEAU7/NG/2009      .D........ .......... ...I...H.G ..E....... .....V....
#KP266340_BAUCHI37/NG/2012      .D........ .......... ...I...QEG ..E....... .....V....
#AJ878671_HENAN                 .......... .......... ........EG .......... ..........
#AY099457_T09                   .......... .......... .......... .......... ..........
#BURS706                        .......... .......... .......NEG .......... ..........

                               168        178        188        198        208
#AJ878668_HK46                  TYMGQATRLV AMKEVATGRN PNKDPLKLGY TFESIAQLLD ITLPVGPPGE
#OKYM                           .......... .......... .......... .......... ..........
#KP266335_PLATEAU19/NG/2009     .......... .......... .......... .......... ..........
#KP172132_KWARA148/NG/2014      .......... .......... .......... .......... ..........
#KP172133_PLATEAU156/NG/2014    .......... .......... .......... .......... ..........
#KP172114_PLATEAU161/NG/2014    .......... .......... .......... .......... ..........
#KP172134_PLATEAU163/NG/2014    .......... .......... .......... .......... ..........
#KP172135_PLATEAU164/NG/2014    .......... .......... .......... .......... ..........
#KP172126_PLATEAU154/NG/2014    .......... .......... .......... .......... ..........
#KP172140_UYO167/NG/2014        .......... .......... .......... .......... ..........
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#KP172142_PLATEAU162/NG/2014    .......... .......... .......... .......... ..........
#KP172143_ANAMBRA50/NG/2012     .......... .......... .......... ........... .P........
#KP172144_PLATEAU158/NG/2014    .......... .......... .......... .......... ..........
#KP172084_ABUJA93/NG/2013       ..T....... .......... .......... .......... ..........
#KP172085_BAUCHI121/NG/2013     .......... .......... .......... .......... ..........
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#KP172093_KWARA181/NG/2014      .......... .......... .......... .......... ..........
#KP172094_UYO187/NG/2014        .......... .......... .......... .......... ..........
#KP172095_UYO189/NG/2014        .......... .......... .......... .......... ..........
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#KP172096_UYO190/NG/2014        .......... .......... .......... .......... ..........
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#KP172108_UYO207/NG/2014        .......... .......... .......... .......... ..........
#KP172109_KWARA184/NG/2014      .......... .......... .......... .......... ..........
#KP172110_UYO188/NG/2014        .......... .......... .......... .......... ..........
#KP172088_KWARA171/NG/2014      .......... .......... .......... .......... ..........
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#KP172087_BAUCHI126/NG/2014     .......... .......... .......... .......... ..........
#KP172111_BAUCHI128/NG/2014     .......... .......... .......... .......... ..........
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#KP172114_KWARA141/NG/2014      .......... .......... .......... .......... ..........
#KP172122_PLATAEU96/NG/2013     .......... .......... .......... .......... ..........
#KP172116_PLATEAU40/NG/2012     .......... .......... .......... .......... ..........
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#KP172125_PLATEAU119/NG/2013    ......A... .......... .......... .......... ..........
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#KP172115_PLATEAU134/NG/2014    .......... .......... .......... .......... ..........
#KP172131_PLATEAU135/NG/2014    .......... .......... .......... .......... ..........
#AJ878666_UK661                 .......... .......... .......... .......... ..........
#AJ878676_DEL_E                 .......... .......... .......... .......... ..........
#KP266336_KADUNA61/NG/2012      ......S... .......... .......... .......... ..........
#KP266337_BAUCHI58/NG/2012      .......... .......... .......... .......... ..........
#KP266338_PLATEAU53/NG/2010     .......... .......... .......... .......... ..........
#KP266339_PLATEAU7/NG/2009      .......... .......... .......... .......... ..........
#KP266340_BAUCHI37/NG/2012      .......... .......... .......... .......... ..........
#AJ878671_HENAN                 .......... .......... .......... .......... ..........
#AY099457_T09                   .......... .......... .......... .......... ..........
#BURS706                        .......... .......... .......... .......... ..........
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                                 218        228        238        248
#AJ878668_HK46                    DDKPWVPLTR VPSRMLVLTG DVDGEFEVED Y
#OKYM                             .......... .......... .......... .
#KP266335_PLATEAU19/NG/2009       .E........ .......... ....D..... .
#KP172132_KWARA148/NG/2014        .......... .......... .......... .
#KP172133_PLATEAU156/NG/2014      .E........ .......... ....D..... .
#KP172114_PLATEAU161/NG/2014      .E........ .......... ....D..... .
#KP172134_PLATEAU163/NG/2014      .E........ .......... ....D..... .
#KP172135_PLATEAU164/NG/2014      .E........ .......... ....D..... .
#KP172126_PLATEAU154/NG/2014      .E........ .......... ....D..... .
#KP172140_UYO167/NG/2014          .E........ .......... ....D..... .
#KP172136_PLATEAU39/NG/2012       .E........ .......... ....D..... .
#KP172137_PLATEAU43/NG/2012       .E........ .......... ....D..... .
#KP172138_PLATEAU157/NG/2014      .E........ .......... ....D..... .
#KP172139_PLATEAU160/NG/2014      .E........ .......... ....D..... .
#KP172142_PLATEAU162/NG/2014      .E........ .......... ..Y.D..... .
#KP172143_ANAMBRA50/NG/2012       .E........ .......... ....D..... .
#KP172144_PLATEAU158/NG/2014      .E........ .......... ....D..... .
#KP172084_ABUJA93/NG/2013         .E........ .......... ....D..... .
#KP172085_BAUCHI121/NG/2013       .E........ .......... ....D..... .
#KP172089_KWARA175/NG/2014        .E........ .......... ....D..... .
#KP172090_KWARA177/NG/2014        .E........ ......M... ....D..... .
#KP172091_KWARA178/NG/2014        .E........ .......... ....D..... .
#KP172092_KWARA180/NG/2014        .E........ .......... ....D..... .
#KP172093_KWARA181/NG/2014        .E........ .......... ....D..... .
#KP172094_UYO187/NG/2014          .E........ .......... ....D..... .
#KP172095_UYO189/NG/2014          .E........ .......... ....D..... .
#KP172096_UYO190/NG/2014          .E........ .......... ....D..... .
#KP172097_UYO191/NG/2014          .E........ .......... ....D..... .
#KP192098_UYO192/NG/2014          .E........ .......... ....D..... .
#KP172099_UYO193/NG/2014          .E........ .......... ....D..... .
#KP172100_UYO194/NG/2014          .E........ .......... ....D..... .
#KP172101_UYO195/NG/2014          .E........ .......... ....D..... .
#KP172102_UYO196/NG/2014          .E........ .......... ....D..... .
#KP172103_UYO197/NG/2014          .E........ .......... ....D..... .
#KP172104_UYO198/NG/2014          .E........ .......... ....D..... .
#KP172105_UYO200/NG/2014          .E........ .......... ....D..... .
#KP172106_UYO202/NG/2014          .E........ .......... ....D..... .
#KP172107_UYO203/NG/2014          .E........ .......... ....D..... .
#KP172108_UYO207/NG/2014          .E........ .......... ....D..... .
#KP172109_KWARA184/NG/2014        .E........ .......... ....D..... .
#KP172110_UYO188/NG/2014          .E........ .......... ....D..... .
#KP172088_KWARA171/NG/2014        .E........ .......... ....D..... .
#KP172086_BAUCHI124/NG/2014       .E........ .......... ....D..... .
#KP172087_BAUCHI126/NG/2014       .E........ .......... ....D..... .
#KP172111_BAUCHI128/NG/2014       .E........ .......... ....D..... .
#KP172112_BENIN87/NG/2013         .E........ .......... ....D..... .
#KP172113_KADUNA136/NG/2014       .E........ .......... ....D..... .
#KP172114_KWARA141/NG/2014        .......... .......... .......... .
#KP172122_PLATAEU96/NG/2013       .E........ .......... ....D..... .
#KP172116_PLATEAU40/NG/2012       .......... .......... .......... .
#KP172117_PLATEAU82/NG/2013       .E........ .......... ....D..... .
#KP172118_PLATEAU83/NG/2013       .E........ .......... ....D..... .
#KP172119_PLATEAU90/NG/2013       .E........ .......... ....D..... .
#KP172120_PLATEAU92/NG/2013       .E........ .......... ....D..... .
#KP172121_PLATEAU95/NG/2013       .E........ .......... ....D..... .
#KP172123_PLATEAU104/NG/2010      .E........ .......... ....D..... .
#KP172124_PLATEAU110/NG/2010      .E........ .......... ....D..... .
#KP172125_PLATEAU119/NG/2013      .E........ .......... ....D..... .
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#KP172127_PLATEAU120/NG/2013    .E........ .......... ....D..... .
#KP172128_PLATEAU131/NG/2014    .E........ .......... ....D..... .
#KP172129_PLATEAU132/NG/2014    .E........ .......... ....D..... .
#KP172130_PLATEAU133/NG/2014    .E........ .......... ....D..... .
#KP172115_PLATEAU134/NG/2014    .E........ .......... ....D..... .
#KP172131_PLATEAU135/NG/2014    .E........ .......... ....D..... .
#AJ878666_UK661                 .......... .......... .......... .
#AJ878676_DEL_E                 .......... .......... ....D..... .
#KP266336_KADUNA61/NG/2012      .E........ .......... ....D..... .
#KP266337_BAUCHI58/NG/2012      .E........ .......... ....D..... .
#KP266338_PLATEAU53/NG/2010     .E........ .......... ....D..... .
#KP266339_PLATEAU7/NG/2009      .E........ .......... ....D..... .
#KP266340_BAUCHI37/NG/2012      .E........ .......... ....D..... .
#AJ878671_HENAN                 .......... ........S. ....D..... .
#AY099457_T09                   .......... .......... .......... .
#BURS706                        .......... .......... ....D..... .
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Appendix 15: Amino acid sequence data of Nigerian IBDV segment B and some reference IBDV segment

```
                         186                                                        245
#AF092944_HK46           CAG GAT GTG GAA GAA CTC CTG ATC CCT AAG GTC TGG GTG CCA CCT GAG GAT CCC TTG GCC
#AJ318897_UK661          ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#FJ695139_YS07           ... ... ... ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ...
#AF362774_23/82          ..A ..C ... ... ... ... T.. ... ..C ..A ... ... ... ... ... ... ... ..G C.C ...
#EF576658_IL             ..A ..C ... .G. ... ... T.. ... ... ... ..T ... ..A ... ... ... ... ..G C.T ...
#EF517529_HARBIN-1       ... ... ... ... ... ... ... ... ..C ... ... ... ..T ..T ... ... ..C ..A ... ..A
#KC109815_HN04           ..A ..C ... ... ... ... T.. ... ... ..A ..T ... ... ... ... ... ... ..G C.T ...
#AY705393_Gx             ..A ..C ... ... ... ... T.. ... ... ... ... ... ..T ..C ... ... ... ..A C.. ..A
#D49707_OKYM             ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#AY099457_T09            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ... C.. ...
#AF133905_VARIANT_E      ..A ..C ... ... ... ... T.. ... ... ..A ..T ... ... ... ... ... ... ..G C.T ...
#JQ619640_T1             ..A ... ... ... ... ... T.. ... ... ..A ..T ... ... ... ... ... ... ..G C.T ...
#PLATEAU19/NG/2009       ... ... ... ... ... ... ... ... ... ... ..G ... ..T ... ... ... ..C ..T C.T ...
#KADUNA61/NG/2012        ... ... ... ... ... ... ... ... ... ... ..G ... ..T ... ... ... ..C ..T C.T ...
#BAUCHI58/NG/2012        ... ... ... ... ... ... ... ... ..C ... ..G ... ..T ... ... ... ..C ..T C.T ...
#PLATEAU53/NG/2010       ... ... ... .T. ... ... T.. ... ... ... ..G ... ..T ... ... ... ..C ..T C.T ...
#PLATEAU7/NG/2009        ... ... ... ... ... ... ... ... ... ... ..G ... ..T ... ... ... ..C ..T C.T ...
#BAUCHI37/NG/2012        ... ... ... ... ... ... ... ... ... ... ..G ... ..T ... ... ... ..C ..T C.T ...

                         246                                                        305
#AF092944_HK46           AGC CCT AGT CGT CTG GCC AAG TTC CTC AGG GAA AAC GGT TAC AAG ATT CTG CAG CCA CGG
#AJ318897_UK661          ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... ... ... ... ...
#FJ695139_YS07           ... ... ... ... ... ... ... ... ..T ... ... ..T ..C ... ... ... ... ... ... ...
#AF362774_23/82          ... ... ... ..A ... ..A ... ... ... ..A ..G ... ..C ..T ..A G.. T.. ... ... ...
#EF576658_IL             ... ... ... ..A ... ..A ... ..T ... ..A ..G ..T ..C ... ..A G.. T.. ... ... ...
#EF517529_HARBIN-1       ... ... ... ..G ..T ... ..A ... ... ..A ... ..T ..C ... ..A ..C ... ... ..G ...
#KC109815_HN04           ... ... ... ..A ... ..A ... ... ... ..A ..G ... ..C ... ..A G.. T.. ... ... ...
#AY705393_Gx             ... ... ... ..G ..T ... ... ... ... ..A ..G ..T ..C ... ..A ..C T.. ... ... ...
#D49707_OKYM             ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... ... ... ... ...
#AY099457_T09            ... ... ... ..A ... ... ... ... ..T ... ... ... ..C ... ... ... ... ... ... ...
#AF133905_VARIANT_E      ... ... ... ..A ... ..A ..A ..T ... ..A ..G ... ..C ... ..A G.. T.. ..A ... ...
#JQ619640_T1             ... ... ... ..A ... ..A ... ... ... ..A ..G ..T ..C ... ..A G.. T.. ... ... ...
#PLATEAU19/NG/2009       ..T ... ..C ..C ... ..A ... ..T ... ..A ... ..T ..C ... ..A ..C T.. ... ... ...
#KADUNA61/NG/2012        ..T ... ..C ..C ... ..A ... ..T ... ..A ... ..T ..C ... ..A ..C T.. ... ... ...
#BAUCHI58/NG/2012        ..T ..C ... ..C ... ..A ... ..T ... ... ... ... ..C ... ..A ..C T.. ... ... ...
#PLATEAU53/NG/2010       ..T ... ..C ..C ... ..A ... ..T ... ..A ... ..T ..C ... ..A ..C T.. ... ... ...
#PLATEAU7/NG/2009        ..T ... ..C ..C ... ..A ... ..T ... ..A ... ..T ..C ... ..A ..C T.. ... ... ...
#BAUCHI37/NG/2012        ..T ... ..C ..C ... ..A ... ..T ... ..A ... ..T ..C ... ..A ..C T.. ... ... ...

                         306                                                        365
#AF092944_HK46           TCT CTA CCT GAG AAT GAG GAA TAT GAG ACC GAT CAA ATA CTC CCT GAC CTA GCT TGG ATG
#AJ318897_UK661          ... ... ... ... ... ... ..G ... ... ... ... ... ... ... ... ... ... ... ... ...
#FJ695139_YS07           ... ... ... ... ... ... ..G ... ... ... ... ... ... ... ... ... ... ... ... ...
#AF362774_23/82          ... ..G ..C ... ... ... ..G ... ... ... ..C ... ... ... ..A ... ... ... ... ...
#EF576658_IL             ... ..G ..C ... ... ... ..G ... ... ... ..C ... ... ... ..A ... ... ..A ... ...
#EF517529_HARBIN-1       ... ..G ..C ... ... ... ..G ... ... ... ..C ..G ... ... ..A ... ... ..A ... ...
#KC109815_HN04           ... ..G ..C ... ... ... ..G ... ... ... ..C ... ... ... ..A ... T.. ..A ... ...
#AY705393_Gx             ... ... ..C ..A ... ... ..G ... ... ... ..C ... ... ... ..A ... ... ..A ... ...
#D49707_OKYM             ... ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#AY099457_T09            ... ... ... ... ... ... ..G ... ... ... ... ... ... ... ... ... ... ... ... ...
#AF133905_VARIANT_E      ... ... ..C ... ... ... ..G ... ... ... ..C ... ... ... ..A ... T.. ..A ... ...
#JQ619640_T1             ... ..G ..C ... ..C ... ..G ..C ... ... ..C ... ... ... ..A ... T.. ..A ... ...
#PLATEAU19/NG/2009       ... ... ..C ..A ... ... ..G ... ... ... ..C ..G ... ... ..A ... ... ... ... ...
#KADUNA61/NG/2012        ... ... ..C ..A ... ... ..G ... ... ... ..C ..G ... ... ..A ... ... ... ... ...
#BAUCHI58/NG/2012        ... ... ..C ... ... ... ..G ... ... ... ..C ..G ..T ... ..A ... ... ... ... ...
#PLATEAU53/NG/2010       ... ... ..C ..A ... ... ..G ... ... ... ..C ..G ... ... ..A ... ... ... ... ...
#PLATEAU7/NG/2009        ... ... ..C ..A ... ... ..G ... ... ... ..C ..G ... ... ..A ... ... ... ... ...
#BAUCHI37/NG/2012        ... ... ..C ..A ... ... ..G ... ... ... ..C ..G ... ... ..A ... ... ... ... ...

                         366                                                        425
#AF092944_HK46           AGG CAG ATA GAG GGA GCT GTC TTA AAA CCA ACC CTA TCT CTC CCC ATT GGA GAC CAG GAG
#AJ318897_UK661          ... ... ... ... ... C.. ..T ... ... ... ... ... ... ... ... ... ... ... ... ...
#FJ695139_YS07           ..A ... ... ... ... ... ..T ... ... ..G ... ... ... ... ... ... ... ... ... ...
#AF362774_23/82          C.A ... ... ... ..G ..G ..T C.. ... ..C ..T ... ... ... ... ... ... ... ... ...
#EF576658_IL             C.A ... ... ..A ..G ... ..T ... ... ..T ..T ... ... ... ... ... ... ... ... ...
#EF517529_HARBIN-1       C.A ... ... .TA ..G ..A ..T ... ..G ... ..T ... ... ... ... ... ..G ... ..A ...
#KC109815_HN04           C.A ... ... ..A ..G ... ..T ... ... ..C ..T ... ... ... ..T ... ... ..T ... ...
#AY705393_Gx             C.. ... ... ... ..G ... ... ..G ..G ... ..T ... ... ... ... ... ..G ... ..A ...
```

```
#D49707_OKYM           ... ... ... ... ..G ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ...
#AY099457_T09          ... ... ... ... ... ..G ..T ... ... ... ... ... ... ... ... ... ..G ... ... ...
#AF133905_VARIANT_E    C.A ... ... ..A C.G ... ... ..G ... ..T ..T ... ... ..T ... ... ... ... ..T ...
#JQ619640_T1           C.A ... ... ..A ..G ... ..T ... ... ..T ..T ... ... ..A ... ... ... ... ... ...
#PLATEAU19/NG/2009     ... ... ... ..A ..G ... ..A C.. ..G ..C ..T ... ... ... ... ... ... ... ... ...
#KADUNA61/NG/2012      ... ... ... ... ..G ... ..A C.. ..G ..C ..T ... ... ... ... ... ... ... ... ...
#BAUCHI58/NG/2012      ... ... ... ..A ..G ... ..A C.. ..G ..T ..T ... ... ... ... ... ..G ... ..A ...
#PLATEAU53/NG/2010     ... ... ... ... ..G ... ..A C.. ..G ..C ..T ... ... ... ... ... ... ... ... ...
#PLATEAU7/NG/2009      ... ... ... ..A ..G ... ..A C.. ..G ..C ..T ... ... ... ... ... ... ... ... ...
#BAUCHI37/NG/2012      ... ... ... ... ..G ... ..A C.. ..G ..C ..T ... ... ... ... ... ... ... ... ...

                      426                                                                            485
#AF092944_HK46         TAC TTC CCT AAA TAC TAC CCA ACA CAC CGC CCG AGC AAG GAA AAG CCC AAT GCG TAC CCG
#AJ318897_UK661        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#FJ695139_YS07         ... ... ... ... ... ... ... G.. ... ... ... ... ... ... ... ... ... ... ... ...
#AF362774_23/82        ... ... ..A ..G ... ... ... ... ..T ... ..T ... ... ..G ..A ... ... A.. ... ..A
#EF576658_IL           ... ... ..A ..G ... ... ... ... ..T ... ..T ... ... ..G ... ... ... ... ... ...
#EF517529_HARBIN-1     ... ... ..G ..G ... ... ... ..T ..T ... ... ... ... ..G ... ... .T. ... ... ...
#KC109815_HN04         ... ... ..A ..G ... ... ... ... ..T ... ..T ... ... ..G ... ... ... ... ... ...
#AY705393_Gx           ... ... ..A ..G ... ... ... ..T ..T ... ..T ... ... ..G ... ... ... ... ... ..A
#D49707_OKYM           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... T..
#AY099457_T09          ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#AF133905_VARIANT_E    ... ... ..A ..G ... ... ... ... ..T ... ..T ... ... ..G ... ... ... ... ... ...
#JQ619640_T1           ... ... ..A ..G ... ... ... ... ..T ... ..T ... ... ..G ... ... ... ... ... ...
#PLATEAU19/NG/2009     ... ... ..A ... ... ... ..C ... ..T ... ..A ... ... ..C ..A ... ..C ... ... ..A
#KADUNA61/NG/2012      ... ... ..A ... ... ... ..C ... ..T ... ..A ... ... ..C ..A ... ..C ... ... ..A
#BAUCHI58/NG/2012      ... ... ..A ... ... ... ..T ... ..T ... ..A ... ... ... ..A ... ..C ... ... ..A
#PLATEAU53/NG/2010     ... ... ..A ... ... ... ..C ... ..T ... ..A ... ... ..C ..A ... ..C ... ... ..A
#PLATEAU7/NG/2009      ... ... ..A ... ... ... ..C ... ..T ... ..A ... ... ..C ..A ... ..C ... ... ..A
#BAUCHI37/NG/2012      ... ... ..A ... ... ... ..C ... ..T ... ..A ... ... ..C ..A ... ..C ... ... ..A

                      486                                                                            545
#AF092944_HK46         CCC GAT ATC GCG TTA CTC AAG CAG ATG ATC TAC TTG TTT CTC CAG GTT CCC GAG GCC ACA
#AJ318897_UK661        ... ... ... ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#FJ695139_YS07         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#AF362774_23/82        ..A ..C ..T ..A C.. ... ... ... ... ... ... C.. ... ..T ... ... ..A ... ... .AC
#EF576658_IL           ..A ..C ... ..A C.. ... ... ... ... ..T ... C.. ... ... ... A.. ..A ... ... .GC
#EF517529_HARBIN-1     ..A ..C ... ..A C.. ... ... ... ... ... ..T C.. ... ... ... ... ..G ..A ... ...
#KC109815_HN04         ..A ..C ... ..A C.. ... ... ... ... ..T ... C.. ... ... ... ... ..A ... ... .AC
#AY705393_Gx           ..A ..C ... ..A C.. ... ... ... ... ... ... ... ... ... ... ... ..G ..C ... ...
#D49707_OKYM           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#AY099457_T09          ... ... ... ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#AF133905_VARIANT_E    ..A ..C ... ..A C.. ... ... ... ... ..T ... C.. ... ..T ... ... ..A ... ... .AT
#JQ619640_T1           ..A ..C ... ..A C.. ... ... ... ... ... ... C.. ... ... ... ... ..A ... ... .AC
#PLATEAU19/NG/2009     ..A ..C ... ..A C.. ... ... ... ... ... ... C.. ..C ... ... A.. ... ... ..A CAC
#KADUNA61/NG/2012      ..A ..C ... ..A C.. ... ... ... ... ... ... C.. ..C ... ... A.. ... ... ..A CA.
#BAUCHI58/NG/2012      ..A ..C ... ..A C.. ... ... ... ... ... ... C.. ..C ... ... A.. ... ... ..A CA.
#PLATEAU53/NG/2010     ..A ..C ... ..A C.. ... ... ... ... ... ... C.. ..C ... ... A.. ... ... ..A CA.
#PLATEAU7/NG/2009      ..A ..C ... ..A C.. ... ... ... ... ... ... C.. ..C ... ... A.. ... ... ..A CAC
#BAUCHI37/NG/2012      ..A ..C ... ..A C.. ... ... ... ... ... ... C.. ..C ... ... A.. ... ... ..A CA.

                      546                                                                            605
#AF092944_HK46         GAT AAC CTC AAA GAT GAG GTC ACC TTA CTA ACC CAA AAC ATT AGA GAT AAG GCC TAC GGG
#AJ318897_UK661        ... ... ... ... ... ... ... ... C.. ... ... ... ... ... ... ... ... ..A ... ... ...
#FJ695139_YS07         ..A ... ... ... ... ... ... ... C.. ..G ... ... ... ... ... ... ... ..A ... ... ...
#AF362774_23/82        ..G GG. ..A ..G ... ... ..G ... C.C ... ..T ..G ... ..A ... ..C ... ... ..T ..A
#EF576658_IL           ..G G.. ..A ..G ..C ..A ..A ... C.C ..G ... ... ... ..A ..G ..C ... ... ..T ..A
#EF517529_HARBIN-1     ..G GGT T.A ..G ... ... ..G ... C.C ... ..T ..G G.. ..A ... ..C ... ... ... ...
#KC109815_HN04         ... ... ... ... ... ... ... ... C.. ... ... ... ... ... ... ... ..A ... ... ...
#AY705393_Gx           ..G GG. ..A ..G ..A ..A ..A ... C.C T.G ..T ... ... ..A ... ..C ... .TA ... ..A
#D49707_OKYM           ... ... ... ... ... ... ... ... C.. ... ... ... ... ... ... ... ..A ... ... ...
#AY099457_T09          ... ... ... ..G ... ... ... ... C.. ... ... ... ... ... ... ..C ..A ... ... ...
#AF133905_VARIANT_E    ..G GG. ..A ..G ... ..A ..A ... C.T ..G ... ... ..T ..A ..G ... ... ... ..T ..A
#JQ619640_T1           ..G GG. ..A ..G ..C ..A ..A ..T C.C ..G ... .T. ... ..A ..G ... ... .TT ..T ..A
#PLATEAU19/NG/2009     ..A GGG ... ..G ..A ... ..G ..T C.C ..T ... ... ... ..A ..G ..C ... .T. ... ...
#KADUNA61/NG/2012      ..A GGG ... ..G ..A ... ..G ..T C.C ..T ... ... ... ..A ..G ..C ... .T. ... ...
#BAUCHI58/NG/2012      ..A GGG .CA ..G ..A ... ..G ..T C.C ..C ... ... ... ..A ..G ..C ... ... ... ...
#PLATEAU53/NG/2010     ..A GGG ... ..G ..A ... ..G ..T C.C ..T ... ... ... ..A ..G ..C ... .T. ... ...
#PLATEAU7/NG/2009      ..C GGG ... ..G ..A ... ..G ..T C.C ..T ... ... ... ..A ..G ..C ... .T. ... ...
```

```
#BAUCHI37/NG/2012      ..A GGG ... ..G ..A ... ..G ... C.C ..T ... ... ... ..A ..G ..C ... .T. ... ...

                      606                                                                                 665
#AF092944_HK46         AGT GGG ACC TAC ATG GGA CAG GCT ACC AGA CTT GTT GCT ATG AAA GAG GTT GCC ACT GGG
#AJ318897_UK661        ... ... ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ...
#FJ695139_YS07         ... ..A ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#AF362774_23/82        ..C ... ... ... ... ... ..A ..A ..T C.G ... ..G ..C ... ... ... ... ... ... ...
#EF576658_IL           ... ... ... ... ... ... ..A ..A ..T C.. ... ..G ..C ... ..G ... ..C ..T ... ...
#EF517529_HARBIN-1     ... ... ... ... ... ... ... ..G ... ..G ... ... ..C ... ... ... ..C ..T ..A ...
#KC109815_HN04         ... ... ... ... ... ... ..A ... ... ... ... ... ... ... ... ... ... ... ... ...
#AY705393_Gx           ... ..A ... ... ... ..G ..A ... ... C.G ... ..C ... ... ... ... ... ... ... ...
#D49707_OKYM           ... ... ... ... ... ... ..A ... ... ... ... ... ... ... ... ... ... ... ... ...
#AY099457_T09          ... ... ... ... ... ... ..A ..C ..T ..G ... ..C ... ... ..G ... ... ... ... ...
#AF133905_VARIANT_E    ... ... ... ... ..T ... ..A ..A ..T C.. ... ..G ..C ... ..G ... ..C ... ... ...
#JQ619640_T1           ... ... ... ... ... ... ..A ..A ..T C.. ... ..G ..C ... ..G ... ..C ... ... ...
#PLATEAU19/NG/2009     ..C ..A ... ... ... ..G ..A ..G ..A ... ... ..G ..C ... ..G ..A ..A ... ..A ...
#KADUNA61/NG/2012      ..C ..A ... ... ... ..G ..A ..G T.A ... ... ..G ..C ... ..G ..A ..A ... ..A ...
#BAUCHI58/NG/2012      ..C ..A ... ... ... ..G ..A ..G ..A ..G ..A ..G ..C ... ..G ..A ..A ... ..A ...
#PLATEAU53/NG/2010     ..C ..A ... ... ... ..G ..A ..G ..A ... ... ..G ..C ... ..G ..A ..A ... ..A ...
#PLATEAU7/NG/2009      ..C ..A ... ... ... ..G ..A ..G ..A ... ... ..G ..C ... ..G ..A ..A ... ..A ...
#BAUCHI37/NG/2012      ..C ..A ... ... ... ..G ..A ..A ..A ... ... ..G ..C ... ..G ..A ..A ... ..A ...

                      666                                                                                 725
#AF092944_HK46         AGA AAC CCA AAC AAA GAT CCT CTA AAG CTT GGG TAC ACC TTT GAG AGC ATA GCC CAG CTA
#AJ318897_UK661        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#FJ695139_YS07         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ...
#AF362774_23/82        ... ... ..G ... ..G ... ... ... ..A ... ... ... ..T ... ... ... ..C ..A ... ..G
#EF576658_IL           ... ... ... ... ..G ... ... ... ..A ... ... ... ..T ... ... ..T ..C ..G ... ..G
#EF517529_HARBIN-1     ... ..T ... ... ... ... ... ... ..A ... ... ... ... ... ... ... ..C ..A ... ..G
#KC109815_HN04         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#AY705393_Gx           ... ... ... ... ... ... ... ..G ..A ... ... ... ... ..C ... ... ..C ..T ... T.G
#D49707_OKYM           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#AY099457_T09          ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#AF133905_VARIANT_E    ... ..T ... ... ..G ... ... ..T ... ... ... ... ..T ... ... ... ..C ... ... ...
#JQ619640_T1           ... ... ... ... ..G ... ... ... ..A ... ... ... ..T ... ... ... ..C ..G ... T.G
#PLATEAU19/NG/2009     ..G ... ..C ... ... ... ... ... ..A ... ... ... ... ... ... ..T ..C ... ... ..G
#KADUNA61/NG/2012      ..G ... ..C ... ... ... ... ... ..A ... ... ... ... ... ... ..T ..C ... ... ..G
#BAUCHI58/NG/2012      ..G ... ..C ... ... ... ... ... ..A ... ... ... ..T ... ... ... ..C ... ... ..G
#PLATEAU53/NG/2010     ..G ... ..C ... ... ... ... ... ..A ... ... ... ... ... ... ..T ..C ... ... ..G
#PLATEAU7/NG/2009      ..G ... ..C ... ... ... ... ... ..A ... ... ... ... ... ... ..T ..C ... ... ..G
#BAUCHI37/NG/2012      ..G ... ..C ... ... ... ... ... ..A ... ... ... ... ... ... ..T ..C ... ... ..G

                      726                                                                                 785
#AF092944_HK46         CTT GAC ATC ACT TTA CCG GTA GGC CCA CCC GGT GAG GAT GAC AAG CCC TGG GTA CCA CTC
#AJ318897_UK661        ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#FJ695139_YS07         ... ... ... ... C.. ... ... ... ... ... ..C ... ... ... ... ... ... ..G ... ...
#AF362774_23/82        ... ... ... ... C.. ... ... ... ... ..T ... ... ... ... ... ... ... ..G ... ...
#EF576658_IL           ... ... ... ..A C.. ... ... ... ... ... ... ... ... ... ... ... ... ..G ..G ...
#EF517529_HARBIN-1     ... ... ... ..C C.. ..A ..C ... ... ..T ... ... ... ... ... ... ... ..G ..G ...
#KC109815_HN04         ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#AY705393_Gx           ... ... ... ..C C.. ..A ..T ... ... ..T ... ... ... ... ... ... ... ..G ... ...
#D49707_OKYM           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#AY099457_T09          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ... ... ...
#AF133905_VARIANT_E    ... ... ... ..A C.. ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ...
#JQ619640_T1           ... ... ... ..G C.. ... ... ... ... ... ... ... ... ... ... ... ... ..G ... ...
#PLATEAU19/NG/2009     ... ... ... ... C.. ..A ... ... ... ..T ... ..A ... ..G ... ... ... ..G ... ...
#KADUNA61/NG/2012      ... ... ... ... C.. ..A ... ... ... ..T ... ... ... ..G ... ... ... ..G ... ...
#BAUCHI58/NG/2012      ... ... ... ... C.. ... ... ... ... ... ... ..A ... ..G ... ... ... ..G ... ...
#PLATEAU53/NG/2010     ... ... ... ... C.. ..A ... ... ... ..T ... ..A ... ..G ... ... ... ..G ... ...
#PLATEAU7/NG/2009      ... ... ... ... C.. ..A ... ... ... ..T ... ..A ... ..G ... ... ... ..G ... ...
#BAUCHI37/NG/2012      ... ... ... ... C.. ..A ... ... ... ..T ... ..A ... ..G ... ... ... ..G ... ...
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                         786                                                                             845
#AF092944_HK46            ACA AGG GTG CCG TCA AGG ATG TTG GTT CTG ACG GGC GAC GTA GAT GGG GAA TTT GAG GTT
#AJ318897_UK661           ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#FJ695139_YS07            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#AF362774_23/82           ... ..A ... ... ... ... ... ..A ..G ... ... ..A ... ... ... ... ..C ... ... ...
#EF576658_IL              ... ..A ... ... ... C.. ... ... ..G ... ... ..G ... ... ... ..C ..C ... ... ...
#EF517529_HARBIN-1        ... ..A ... ... ... ... ... C.A ..G ... T.. ..A ... ... ... ... ..C ... ... ...
#KC109815_HN04            ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#AY705393_Gx              ... ... ... ... ... ... ... C.A ..G ... T.. ..A ... ... ... ... ..C ... ... ...
#D49707_OKYM              ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#AY099457_T09             ... ... ... ... ... ... ... ..A ... ... ... ..T ... ... ... ... ... ... ... ...
#AF133905_VARIANT_E       ... ..A ... ... ... C.. ... ... ..G ... ... ..A ... ... ... ..C ..C ... ... ...
#JQ619640_T1              ... ..A ... ..T ... C.. ... ... ..G ... ... ..A ... ... ... ..C ..C ... ... ...
#PLATEAU19/NG/2009        ... ... ... ... ..T ... ... ... ..G ... ... ..A ... ..G ... ... ..C ... ... ...
#KADUNA61/NG/2012         ... ... ... ... ..T ... ... ... ..G ... ... ..A ... ..G ... ... ..C ... ... ...
#BAUCHI58/NG/2012         ... ... ... ... ..T ... ... ... ..G ... ... ..A ... ..G ... ... ..C ... ... ...
#PLATEAU53/NG/2010        ... ... ... ... ..T ... ... ... ..G ... ... ..A ... ..G ... ... ..C ... ... ...
#PLATEAU7/NG/2009         ... ... ... ... ..T ... ... ... ..G ... ... ..A ... ..G ... ... ..C ... ... ...
#BAUCHI37/NG/2012         ... ... ... ... ..T ... ... ... ..G ... ... ..A ... ..G ... ... ..C ... ... ...

                         846                           869
#AF092944_HK46            GAG GAC TAC CTT CCC AAA ATC AAC
#AJ318897_UK661           ... ... ... ... ... ... ... ...
#FJ695139_YS07            ... ... ... ... ... ... ... ...
#AF362774_23/82           ..A ..T ... ... ..A ... ... ...
#EF576658_IL              ..A ..T ... ... ... ... ... ...
#EF517529_HARBIN-1        ..A ... ..T ..C ..A ..G ... ..T
#KC109815_HN04            ... ... ... ... ... ... ... ...
#AY705393_Gx              ..A ... ... ..C ..A ..G ... ...
#D49707_OKYM              ... ... ... ... ... ... ... ...
#AY099457_T09             ... ... ... ... ... .G. ... ...
#AF133905_VARIANT_E       ..A ..T ... ... ... ... ... ...
#JQ619640_T1              ..A ..T ... ... ..G ..G ... ...
#PLATEAU19/NG/2009        ..A ..T ... ..A ..A ... ... ...
#KADUNA61/NG/2012         ..A ..T ... ..A ..A ... ... ...
#BAUCHI58/NG/2012         ..A ... ... ..A ..A ... ... ...
#PLATEAU53/NG/2010        ..A ..T ... ..A ..A ... ... ...
#PLATEAU7/NG/2009         ..A ..T ... ..A ..A ... ... ...
#BAUCHI37/NG/2012         ..A ..T ... ..A ..A ... ... ...
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Appendix 16: Nucleotide sequences data of segment B of six Nigerian IBD viruses with others from GenBank

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                        26         36         46         56         66         76
#PLATEAU19/NG/2009      QDVEELLIPK VWVPPEDPLA SPSRLAKFLR ENGYKILQPR SLPENEEYET DQILPDLAWM
#KADUNA61/NG/2012       .......... .......... .......... .......... .......... ..........
#BAUCHI58/NG/2012       .......... .......... .......... .......... .......... ..........
#PLATEAU53/NG/2010      ...V...... .......... .......... .......... .......... ..........
#PLATEAU7/NG/2009       .......... .......... .......... .......... .......... ..........
#BAUCHI37/NG/2012       .......... .......... .......... .......... .......... ..........
#AB368955.1_TANZANIA    .......... .......... .......... .......... .......... ..........
#AB368957.1_TANZANIA    .......... .......... .......... .......... .......... ..........
#AB368961.1_ZAMBIA      .......... .......... .......... .....V.... .......... ..........
#AB368958.1_ZAMBIA      .......... .......... .......... .......... .......... ..........
#JQ241031.1_87ETH       .......... .......... .......... .......... .......... ..........
#JQ241031.1_86ETH       .......... .......... .......... .......... .......... ..........
#AY705393.2_Gx          .......... .......... .......... .......... .......... ..........
#EU544149.1_BRAZIL      .......... .......... .......... .....V.... .......... ..........
#AM167551.1_94432       .......... .......... .......... .....V.... .......... ..........
#FJ95139.1_YS07         .......... .......... .......... .......... .......... ..........
#AJ318897.1_UK661       .......... .......... .......... .......... .......... ..........
#JF907705.1_CAK785      .......... .......... .......... .....V.... .......... ..........
                        86         96         106        116        126        136
#PLATEAU19/NG/2009      RQIEGAVLKP TLSLPIGDQE YFPKYYPTHR PSKDKPNAYP PDIALLKQMI YLFLQIPEAH
#KADUNA61/NG/2012       .......... .......... .......... .......... .......... .........Q
#BAUCHI58/NG/2012       .......... .......... .......... ...E...... .......... .........Q
#PLATEAU53/NG/2010      .......... .......... .......... .......... .......... .........Q
#PLATEAU7/NG/2009       .......... .......... .......... .......... .......... ..........
#BAUCHI37/NG/2012       .......... .......... .......... .......... .......... .........Q
#AB368955.1_TANZANIA    .......... .......... .......... ...E...... .......... .....V...S
#AB368957.1_TANZANIA    .......... .......... .......... ...E...... .......... .....V...S
#AB368961.1_ZAMBIA      .......... .......... .......... ...E...... .......... .....V...N
#AB368958.1_ZAMBIA      .......... .......... .......... ...E...... .......... .....V...T
#JQ241031.1_87ETH       .......... .......... .......... ...E...... .......... .....A.D.T
#JQ241031.1_86ETH       .......... .......... .......... ...E...... .......... .....A.D.T
#AY705393.2_Gx          .......... .......... .......... ...E...... .......... .....V.D.T
#EU544149.1_BRAZIL      .......... .......... .......... ...E...... .......... .....V...N
#AM167551.1_94432       .......... .......... ......S... ...E...... .......... .....V...T
#FJ95139.1_YS07         .......... .......... .......A.. ...E...... .......... .....V...T
#AJ318897.1_UK661       .....P.... .......... .......... ...E...... .......... .....V...T
#JF907705.1_CAK785      .......... .......... .......... ...E...... .......... .....V...N
                        146        156        166        176        186        196
#PLATEAU19/NG/2009      EGLKEEVTLL TQNIRDKVYG SGTYMGQATR LVAMKEVATG RNPNKDPLKL GYTFESIAQL
#KADUNA61/NG/2012       .......... .......... ........S. .......... .......... ..........
#BAUCHI58/NG/2012       ..P....... .......A.. .......... .......... .......... ..........
#PLATEAU53/NG/2010      .......... .......... .......... .......... .......... ..........
#PLATEAU7/NG/2009       D......... .......... .......... .......... .......... ..........
#BAUCHI37/NG/2012       .......... .......... .......... .......... .......... ..........
#AB368955.1_TANZANIA    ....D..... .......A.. .......... .......... .......... ..........
#AB368957.1_TANZANIA    ....D..... .......A.. .......... .......... .......... ..........
#AB368961.1_ZAMBIA      ....D..... .......A.. .......... .......... .......... ..........
#AB368958.1_ZAMBIA      DN..D..... .......A.. .......... .......... .......... ..........
#JQ241031.1_87ETH       DS..D..... .....E.A.. .......... .......... .......... ..........
#JQ241031.1_86ETH       DN..D..... .......A.. .......... .......... .......... ..........
#AY705393.2_Gx          .......... .......... .......... .......... .......... ..........
#EU544149.1_BRAZIL      .D..D..... .......A.. .......... .......... .......... ..........
#AM167551.1_94432       DN..D..... .......A.. .......... .......... .......... ..........
#FJ95139.1_YS07         .N..D..... .......A.. .......... .......... .......... ..........
#AJ318897.1_UK661       DN..D..... .......A.. .......... .......... .......... ..........
#JF907705.1_CAK785      ....D..... .......A.. .......... .......... .......... ..........
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                       206        216        226        236        246
#PLATEAU19/NG/2009      LDITLPVGPP GEDEKPWVPL TRVPSRMLVL TGDVDGDFEV EDYLPKIN
#KADUNA61/NG/2012       .......... .......... .......... .......... ........
#BAUCHI58/NG/2012       .......... .......... .......... .......... ........
#PLATEAU53/NG/2010      .......... .......... .......... .......... ........
#PLATEAU7/NG/2009       .......... .......... .......... .......... ........
#BAUCHI37/NG/2012       .......... .......... .......... .......... ........
#AB368955.1_TANZANIA    .......... .......... .......... .......... ........
#AB368957.1_TANZANIA    .......... .......... .......... .......... ........
#AB368961.1_ZAMBIA      .......... ...D...... .......... .......... ........
#AB368958.1_ZAMBIA      .......... ...D...... .......... ......E... ........
#JQ241031.1_87ETH       .......... ...D...... .......... ......E... .....R..
#JQ241031.1_86ETH       .......... ...D...... .......... ......E... .....R..
#AY705393.2_Gx          .......... ...D...... .......... S......... ........
#EU544149.1_BRAZIL      .......... ...D...... .......... .......... ........
#AM167551.1_94432       .......... ...D...... .......... ......E... ........
#FJ95139.1_YS07         .......... ...D...... .......... ......E... ........
#AJ318897.1_UK661       .......... ...D...... .......... ......E... ........
#JF907705.1_CAK785      .......... ...D...... .......... .......... ........
```

Appendix 17: Amino acid sequences data of segment B of six Nigerian IBD viruses with others from GenBank

Appendix 18: Preparation of phosphate buffer saline

NaCl	-	32g
KCl	-	0.8g
$NaHPO_4$. $12H_20$	-	4.6g
KH_2PO_4	-	0.8g
Distilled water	-	4liter

Procedure

The salts were dissolved in distilled water and final volume made up to 4 liters. The pH was adjusted to 7.2 with 5N HCl. It was dispensed into 200ml bottles and autoclaved at 120^0C for 30 minutes. They were stored at $+4^0$C ready for use.

Appendix 19: Antibiotics solution

Penicillin Crystalline	-	5 million unit
Streptomycin	-	5g
Gentamicin	-	1ml
Amphotericin B	-	5mls
Sterile distilled water	-	500ml

Powders were dissolved with sterile distilled water and liquid antibiotics were added into 500ml sterile bottle and final volume made up to 500ml using distilled water.

Appendix 20: Thioglycollate broth

Thioglycollate powder	-	14.9g
Distilled water	-	500ml

Suspend 14.9g of powder in 500ml of distilled water. Boil at 100^0C to dissolve the powder and distribute into 5ml bijou bottles. Autoclave at 120^0C for 15 minutes. Store at room temperature.

Appendix 21: Tryptose soya broth

Tryptose soya powder	-	15g
Distilled water	-	500ml

Dissolve 15g of powder in 500ml of distilled water. Distribute into 5ml bijou bottles and autoclave at 115^0C for 15 minutes. Store at room temperature.

Appendix 22: Agarose gel for Agar gel immunodiffusion test

Agarose	-	1g
NaCl	-	8g
Distiller water	-	100ml

Add agarose and salt in 100ml of water. Heat to dissolve agar. Autoclave at 121^0C for 10 minutes. Pour into 9cm Petri dish and store at $+4^0$C.

Appendix 23: Agarose gel (for electrophoresis)

Seakem® LE Agar	-	0.4g
TBE	-	50ml

Weigh 0.4g of agar and add to 50ml of TBE. Heat to dissolve agar. Allow to cool. Add 3.5µl of ethidium bromide. Cast gel.

Appendix 24: Cleaning of PCR product for sequencing using WIZARD®SV gel and PCR clean-up system (PROMEGA)

Add an equal volume of membrane binding solution to the PCR reaction. Insert SV Minicolumn collection tube and transfer prepared PCR product to the minicolumn assembly. Incubate at room temperature for 1min. Centrifuge at 16,000xg for 1min. Discard flowthrough and reinsert minicolumn into collection tube. Add 700 µl membrane washing solution (ethanol added). Centrifuge at 16,000xg for 1min. Discard flowthrough and reinsert minicolumn into collection tube. Add 500 µl membrane washing solution. Centrifuge at 16,000xg for 5mins. Empty the collection tube and re-centrifuge the column assembly for 1min with the microcentrifuge lid open (or off) to allow evaporation of any residual ethanol. Carefully transfer minicolumn to a clean 1.5ml microcentrifuge tube. Add 50 µl of Nuclease-Free water to the minicolumn. Incubate at room temperature for 1min. centrifuge at 16,000xg for 1min. Discard minicolumn and store DNA at 4^0C or -20^0C.

Appendix 25: **Tris-Borate-EDTA (TBE)**

TBE	-	34g
Distilled water	-	1liter

Weigh 34g of TBE and add make up volume to 1 liter. Dissolve salt with the aid of a magnetic stirrer.

Appendix 26: Composition of master mix for PCR (for 1 sample)

2x Reaction mix	-	12.5µl
Primer forward	-	1 µl
Primer reverse	-	1 µl
25x RT-PCR Enzyme Mix	-	1 µl
Nuclease free H_2O	-	7.5 µl

Add 23 µl of master mix to 2 µl of cDNA template. Put in a thermo cycler for PCR.

Appendix 27: Preparation of 75% ethanol

100% ethanol	-	75ml
Distilled water	-	25ml

Measure 75ml of ethanol using a clean measuring cylinder. Make up to 100ml mark with 25ml of distilled water.

Appendix 28: Preparation of forward and reverse primer stock solution

Lyophilized primer	-	forward (128.15 nmoles)
Lyophilized primer	-	reverse (114.85 nmoles)

Molecular grade H_2O

Spin each tube containing the lyophilized primers before opening. Multiply the nmoles of each primer by 10 which will be the amount of H_2O to add to make a 100µM stock.

Appendix 29: Distribution of sampled IBD outbreaks from 2009 – 2014 with respect to age of birds

Ho: IBDV outbreaks is independent of the ages of birds

H1: IBDV outbreaks is not independent of the ages of birds

Chi-Square Test

	Observed N	Expected N	Residual
1	1	41.4	-40.4
3	6	41.4	-35.4
21	21	41.4	-20.4
66	66	41.4	24.6
113	113	41.4	71.6
Total	207		

Test Statistics

	x
Chi-Square	2.182E2[a]
df	4
Asymp. Sig.	.000

a. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 41.4.

Decision: Reject Ho since the value of chi-square is 41.4 and the P-value is less than 0.01

Appendix 30: Distribution of IBDV outbreaks with respect to States

Ho: IBDV outbreaks is independent of the state

H1: IBDV outbreaks is not independent of the state.

Chi-Square Test

	Observed N	Expected N	Residual
3	3	20.7	-17.7
4	4	20.7	-16.7
5	5	20.7	-15.7
6	6	20.7	-14.7
12	12	20.7	-8.7
14	14	20.7	-6.7
18	18	20.7	-2.7
26	26	20.7	5.3
30	30	20.7	9.3
89	89	20.7	68.3
Total	207		

Test Statistics

	y
Chi-Square	2.880E2[a]
df	9
Asymp. Sig.	.000

a. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 20.7.

Decision: Chi-square value is 20.7, therefore we reject the null hypothesis and conclusion that IBDV is not independent on the State.

Appendix 31: Distribution of sampled IBDV outbreaks with respect to species of birds

	Observed N	Expected N	Residual
1	3	35.8	-32.8
4	4	35.8	-31.8
11	11	35.8	-24.8
52	52	35.8	16.2
109	109	35.8	73.2
28	28	35.8	-7.8
Total	207		

Test Statistics

	w
Chi-Square	2.325E2[a]
df	4
Asymp. Sig.	.000

a. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 35.8

Appendix 32: Distribution of sampled IBDV outbreaks with respect to breed of chickens

x

	Observed N	Expected N	Residual
1	1	51.2	-50.2
8	8	51.2	-43.2
48	48	51.2	-3.2
148	148	51.2	96.8
Total	205		

Test Statistics

	x
Chi-Square	2.686E2[a]
df	3
Asymp. Sig.	.000

a. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 51.3.

b.

Appendix 33: Yearly distribution of IBDV outbreaks from 2009 – 2014

x1

	Observed N	Expected N	Residual
9	9	34.5	-25.5
16	16	34.5	-18.5
22	22	34.5	-12.5
33	33	34.5	-1.5
41	41	34.5	6.5
86	86	34.5	51.5
Total	207		

Test Statistics

	x1
Chi-Square	1.115E2[a]
df	5
Asymp. Sig.	.000

a. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 34.5.

Appendix 34: Monthly distribution of IBDV outbreaks from year 2009-2014

x4

	Observed N	Expected N	Residual
1	1	17.2	-16.2
3	3	17.2	-14.2
5	10	17.2	-7.2
7	7	17.2	-10.2
8	8	17.2	-9.2
9	9	17.2	-8.2
11	11	17.2	-6.2
19	19	17.2	1.8
26	26	17.2	8.8
29	29	17.2	11.8
31	31	17.2	13.8
53	53	17.2	35.8
Total	207		

Test Statistics

	x4
Chi-Square	1.451E2[a]
df	11
Asymp. Sig.	.000

a. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 17.3.

Appendix 35: Distribution of sampled IBDV outbreaks with respect to seasons from year 2009-2014

x5

	Observed N	Expected N	Residual
4	4	51.8	-47.8
28	28	51.8	-23.8
31	31	51.8	-20.8
144	144	51.8	92.2
Total	207		

Test Statistics

	x5
Chi-Square	2.277E2[a]
df	3
Asymp. Sig.	.000

a. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 51.8.

Appendix 36: Distribution of sampled IBDV outbreaks with respect to type of vaccine used

x2

	Observed N	Expected N	Residual
1	3	25.9	-22.9
2	2	25.9	-23.9
3	6	25.9	-19.9
9	18	25.9	-7.9
10	10	25.9	-15.9
43	43	25.9	17.1
48	48	25.9	22.1
77	77	25.9	51.1
Total	207		

Test Statistics

	x2
Chi-Square	2.009E2[a]
df	7
Asymp. Sig.	.000

a. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 25.9.

Appendix 37: Distribution of sampled IBDV outbreaks with respect to type of husbandry

x3

	Observed N	Expected N	Residual
30	30	51.8	-21.8
45	45	51.8	-6.8
47	47	51.8	-4.8
85	85	51.8	33.2
Total	207		

Test Statistics

	x3
Chi-Square	31.821[a]
df	3
Asymp. Sig.	.000

a. 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 51.8.

LIST OF ABBREVIATIONS

IBDV- Infectious bursal disease virus

IBD- Infectious bursal disease

vv- very virulent

vvIBDV- Very virulent Infectious bursal disease virus

SPF- Specific pathogen free

BF- Bursa of fabricius

CEF- Chicken embryo fibroblast

CPE- Cythopathic effect

PBS- Phosphate buffer saline

RPM- Revolution per minute

CAM- Chorioallantoic membrane

PSGA- Penicillin/Streptomycin/Gentamicin/Amphothericin B

FTA cards- Flinder Technology Associates cards

µl- Micro liter

ml- milliliter

min- Minutes

PCR- Polymerase chain reaction

RT-PCR- Reverse transcript-polymerase chain reaction

RT-PCR/RFLP- Reverse transcript-Polymerase chain reaction/Restriction fragment length polymorphism

RFLP- Restriction fragment length polymorphism

RT-LAMP- Reverse transcription coupled with loop-mediated isothermal amplification

AGID- Agar gel immunodiffusion

TE buffer (Tris and ethylene diamine tetra acetic acid (EDTA) buffer

RNA- Ribonucleic acid

ds-RNA- Double stranded-ribonucleic acid

^{0}C- Degree Celsius

RT- Room temperature

DMSO- Dimethyl sulfoxide

TBE- Tris borate EDTA buffer

IL- Interlukin

MDA- Maternally derived antibody

ORF- Open reading frame

VP- Viral protein

OIE- Office International Epizooties

Ig- Immunoglobulin

CD- Cluster of differentiation

nM- Nanometer

hpi- hour postinfection

bp- Base pair

kDa- Kilodalton

kb- Kilobase

kpb- Kilobase pair

g- gram

hr- Hour

RdRp- RNA-dependent RNA-polymerase

aa- Amino acid

E. coli - *Escherichia coli*

A- Alanine

R- Arginine

N- Asparagine

D- Aspartic acid

C- Cystine

E- Glutamic acid

Q- Glutamine

G- Glycine

I- Isoleucine

L- Leucine

K- Lysine

M- Methionine

F- Phenylalanine

P- Proline

S- Serine

T- Threonine

W- Tryptophan

Y- Tyrosine

V- Valine