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Detection and Partial Nucleotide Sequence of Egg Drop Syndrome Virus from a Poultry Flock in Ogun State, Nigeria

**** Ockiya, M. A.[1], Jarikre T.A[2], Jibril, A.T[2], Adeyefa, C.A.O[3]***

[1]Department of Animal Science, Niger Delta University
[2]Department of Veterinary Pathology, University of Ibadan
[3]Department of Veterinary Medicine, University of Ibadan

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ABSTRACT

This study investigated Egg Drop Syndrome (EDS) on a poultry farm at Ayepe Road, Shagamu, Ogun State, Nigeria using clinical, serological and molecular methods. Samples of the reproductive tract were collected from five randomly selected birds in the affected flock. The tissue samples and EDS virus (EDSV) stock obtained from the Virus Research Unit of the National Veterinary Research Institute, Vom, Nigeria were subjected to viral DNA extraction using QIA amp DNA mini kit and DNA blood mini kit 02/2003. A Polymerase Chain Reaction (PCR) protocol was optimized and used for the amplification of a 238bp fragment of proteinVIII (pVIII) gene of EDSV from both the positive control and test DNA. The region was partially sequenced using the Big Dye terminator method on ABI prism automatic sequencer. All serum samples tested were positive for egg drop syndrome virus infection with high titres ranging from 64 to 2040.The nucleotide sequence of test DNA and those of positive viral stock and Duck Adenovirus Virus both had 100% homology respectively. A Guanine-Cytosine content of 41.59% was also found. The region sequenced acts as a signal for late protein synthesis, thereby serving as confirmatory diagnosis in persistent EDSV infection. This study has established the preliminary detection and sequencing of the EDSV genome as well as the best optimization condition involved in a PCR protocol for rapid diagnosis of EDSV in Nigeria. More so, the regional isolation and determination of the genetic differences of EDSV in Nigeria is highly recommended.

*Corresponding author.
E-mail address: mikeockiya@gmail.com / michaelockiya@ndu.edu.ng

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1. Introduction

The egg drop syndrome virus (EDSV) causes major economic losses due to direct effect on egg production and quantity (Owoade and Durojaiye 1993**)**. It is characterized by severe drop in egg production at the onset or peak laying period with high occurrence of shell defects such as shell-less, soft-shelled, thin-shelled, rough-texture as well as small sized and depigmented eggs (Van Eck *et al*. 1976, Ezeibe et al 2008). EDS leads to failure of attaining peak production, and it affects both domestic and wild avian species globally (Ezema et al. 2010). Infection rates of above 66% were reported for chickens, guinea fowls, ducks and pigeons (Durojaiye et al 1991).

The EDSV belongs to the genus ***atadenoviridae*** in the family adenoviridae. They are non-enveloped with an icosahedral shape. The genome consists of a 30-35kb linear double-stranded DNA molecule with inverted terminal repeats and protein attached to a 5' end of each DNA strand (Cai, and Weber 1993; Fu *et al*., 2013). Recent reports indicate that the adenovirus has a size range of 75-80mm with a two-phase replicative cycle, early protein expression (E_1-E_4) and late protein expression (L1-L4) (Lutz and kedinger 1996; Wang *et al*, 2018). The adsorption of the virus to target cell receptors involves high affinity binding to cell receptors via the knob (hexon) portion of the fibres (Solanki *et al*; 2016; Qi *et al*., 2017). The late phase of DNA replication ensues with five cassettes of transcripts (termed L1 to L4) resulting from a complex series of splicing events. These lead to production of the virus structural components and the encapsidation and maturation of virus particles in the nucleus. A key player in the control of transcription is the major late promoter (MLP), which is attenuated during transcription of the early genes. However, there is a low basal level of late transcription occurring early in infection even before the MLP comes into play. After the onset of virus, DNA replication, the Iva2 and IX genes are expressed at high levels and transcription via the MLP is fully functional by specific activation. This is accomplished via the IX and Iva2 gene products (Lans *et al*., 2017, Greenshaw *et al*, 2019; Charman *et al* 2019) and also influenced by limiting transcription factors (Young, 2003). The encapsidation process is governed by the presence in the virus DNA of a packaging signal at the conventional left which consists of a series of AT-rich sequence (Yadvinder and Surresh; 2016). These events are accompanied by major changes in the nuclear infrastructure and the permeabilisation of the nuclear membrane (Weigert *et al*., 2017; Wu and Mei, 2019).

This facilitates the egress of the virus into the cytoplasm and is followed by the disintegration of the plasma membrane and the release of virus from the cell.

There are few reports of EDS in Nigeria but these were only based on serological evidence with limited specificity and reliability (Nawathe and Abegude, 1980. Durojaiye and Adene, 1988; Durojaye *et al*., 1991; Owoade and Durojaiye, 1993; Adene *et al*; 1995; Ezeibe *et al*.,2008; Ezema *et al*., 2010). The flock investigated had a report of a sharp drop in egg production associated with the deformed eggs and failure to attain peak production. The farm housed 10,000 layers in battery cages. Investigation involved exclusion of possible differential diagnosis like nutritional deficiencies and New Castle disease. The diagnostic workup of EDSV was done using, serologic, antigenic and molecular methods. This study seeks to thoroughly investigate and amplify a region of EDSV as a rapid method in the diagnosis of EDS as well as carry out genomic sequencing of the amplified region for comparative genomic analysis.

2. Materials and Methods

The Flock: The flock comprised 10,000 caged layers 42 weeks of age in one pen. The birds had been purchased locally as day old chicks from a commercial hatchery and reared to point of lay in another pen house on the farm premises. They were vaccinated at day old by I/O instillation against Marek's and Newcastle disease with booster doses at 3, 11 and 17 weeks of age, and against infectious bursa disease (Gumboro) at 9 days by i/m injection and fowl pox at 10 weeks through the wing web. The birds were reared on deep litter during most of the rearing period and moved to wire cages at about 18 weeks of age. They were fed manually with chick mash comprising 21% crude protein, 2950kcal/kg energy, 0.25%

micronutrient for 8 weeks and then on growers mash comprising 16% crude protein, 2400kcal/kg energy, 0.25%, micronutrient till 18 weeks after which they were fed layers' ration comprising 17.5% crude protein, 2900 kcal/kg energy, 2.5%, micronutrient. The feeds were purchased locally.

Fresh clean water was supplied all through automatic nipple drinkers during rearing and later during laying period in the cages.

Clinical Examination of Flock: The birds were examined for clinical and post mortem abnormalities. The eggs were also examined for physical abnormalities.

Antigens: EDS's 76 virus (strain 127) stabilized in glycerol was used as antigen in HI test. The virus was obtained from the Virus Research Unit of the National Veterinary Research Institute, Vom, Nigeria. The virus was stored at – 20^0C in 2 ml aliquots. Similarly, commercial New Castle disease vaccine (NVRI, VOM, Nigeria) purchased locally, was used as antigen in New Castle Disease HI test.

SERA: EDS' 76 (strain 127) positive and negative reference sera were obtained from the Virus Research Unit of the National Veterinary Research Institute, Vom, Nigeria. The sera were stored at – 20^0C in 2ml aliquots. Forty blood samples were collected randomly from birds by jugular venipuncture into plain universal bottles and allowed to clot at 40C overnight. Serum samples were then harvested by centrifugation at 2,000 rpm for five minutes and stored at -20°C until used.

Preparation of O.8% washed RBC: A ratio of 0.lml sodium citrate to 1ml of blood was used. 0.5 ml of sodium citrate was aspirated into a 5m1 syringe. The 5ml syringe was made up to 5m1 volume content by blood collected through jugular venipuncture from experimental chickens kept at the poultry disease unit of the Veterinary Medicine Department of the Faculty of Veterinary Medicine University of Ibadan. Blood was discharged into a U-bottom 10ml tube and centrifuged at 2,000 rpm for five minutes. Supernatant was decanted and volume made up to original volume with normal saline and mixed. The process was repeated twice. Normal saline was used to make up to original volume and mixed. Two capillary tubes were filled and centrifuged in micro haematocrit centrifuge at 3000 rpm and packed cell volume (PCV) was read using microhaematocrit reader. 0.8% erythrocyte concentration was calculated based on the PCV value. PCV read was 26%. 0.8mls of washed RBC was then put into 25.2mls of normal saline to make an erythrocyte concentration of O.8%.

Haemagglutination — inhibition Test: HI tests were carried out by standard method in U-bottom 96 well microtitre plate for EDS and ND. The sera were tested for antibodies against EDS virus by the haemaglutination - inhibition test according to McFerran (1989) with slight modifications. The serum was not diluted with PBS) but was used undiluted. A reaction time of 30 minutes instead of 15 minutes as reported by McFerran (1989) was allowed. A 96 wells U-bottom microtitre plate (Dynatech laboratories) were used as reaction base for the HI test. Fifty (50) microlitre of PBS was put in all the wells. Fifty microlitres (50μl) of test sera was put in first wells A - F to make a 1:2 dilution. First wells of C and H had negative and positive control the last row and the excess of 50μl volume from each row was sera respectively. A two-fold dilution was done from first row to discarded. Fifty microlitre (50μl) of the EDS' 76 antigens diluted to 4HA unit was added in all the wells and allowed to react for at least five minutes. Fifty microlitres (50μl) of 0.8% washed red blood cells was added in all the wells as indicator. Plate was put on a microshaker for few seconds and was then left on the bench to react for 30 minutes. Titre was read as the last well that showed inhibition of haemagglutination i.e. the last well where no haemagglutination has taken place.

Polymerase Chain Reaction

Primer: The primers used for the amplification of a 238 base pair portion of the L4 gene of EDSV DNA were designed from the published nucleotide sequence of the complete genome of EDS 76 virus (Hess *et al*., 1997) as described by Zhang *et al*., (1996) and Raj *et al*., (2001). The sequence of the forward primer was 5'-TTG GCG TCT TCA AGG CAC TG-3' and the reverse primers was 5'-CAC ACA ACT GCA TCT GAC TG-3'. The primers were obtained from Invitrogen Life Technologies (U.S.A.) through the Department of Immunology Laboratoire Nationale De Sante, Luxembourg.

Extraction of viral DNA: DNA extraction from EDSV obtained from NVRI, VOM Nigeria was carried out using QIA amp DNA mini kit and QIA amp DNA Blood mini kit 02/2003. Twenty microliter (20μl) of proteinase-k was pippetted into the bottom of a L5m1 eppendorf tube. Two hundred microliter (200μl) of EDS' 76 virus was added to the tube. Two hundred microliter (200μl) of buffer AL was added to the sample and mixed by pulse-vortexing for 15s. This was incubated at 56°C for 10 minutes, briefly centrifuged to remove drops from the inside of the lid and 200μl ethanol (96%) was added to tube and mixed by pulse-vortexing for 15s. The sample was centrifuged (3000 rpm) briefly to remove drops from inside the lid, and carefully pipetted into a QIA amp spin column in a 2ml collecting tube and centrifuged at 8000 rpm for 1 minute. The filtrate was discarded, the collection tube was replaced and the spin column opened carefully to add 500μl of buffer AWI. The tube was centrifuged at 8,000 rpm for 1 minute and the filtrate was discarded with the collection tube. Spin column was placed in another collection tube and opened carefully for the addition of 500gI of buffer AW2. Sample was that centrifuged at 13,000 rpm for 3 minutes. The filtrate was discarded with the collection tube and spin column was now put in 1.5m1 microcentrifuge eppendorf tube. Sixty microlitres (60μl) of buffer AE was added to spin column and incubated at room temperature for 1 minute and then centrifuged at 8,000 rpm for 1 minute. The filtrate was collected as DNA and stored at -20°C until used as positive control DNA.

DNA Extraction from Tissues: The process of DNA extraction from tissue was similar to that of DNA extraction from the purified virus. The differences were in the earlier steps taken before the use of the extraction kit. Briefly five grams of the oviduct tissue was cut and put into a tissue homogeniser where it was homogenised with the addition of 1 ml of distilled water. The homogenate was put in a 1.5ml microcentrifuge tube and centrifuged at 3000 rpm for 1 minute to remove debris. The supernatant was then subjected to the same DNA extraction process as described for viral DNA extraction above. DNA extracts from purified virus and DNA extracts from uterine tissues were subjected to amplification by PCR.

Optimization of PCR: Optimisation of PCR was carried out as described by Xie *et al*. (1998). Various components of the reaction mixture were tested in different concentrations of 0.5 – l.5μl $MgCI_2$ (5OmMol), 1.5 --2.5 10 x buffer (200mM Tris-HCI (pH8.4) and 500mMKCI), 0.5 - 1μl dNTPs (2'deoxynucleoside, 5'triphosphate), 0.5 - 1μl of each primer (100μM) and 0.1 — O.3μl Taq polymerase (5 units/μ). The amplification was carried out in a total of 25μl reaction volume using a programmable thermal cycler (Eppendorf Mastercycler © Personal, CA, USA). The specificity of PCR was tested using a O.Spi of DNA prepared from known positive EDS 76 virus. Final optimisation of PCR in 25μl reaction volume was achieved at 2.Sjsl of lOx buffer, 0.9μl $MgCI_2$, O.5μl dNTP, 1μl of each primer and 0.lμl Taq polymerase. l4μl distilled water and a template of 5μ1. The PCR protocol used was an initial denaturation step of 94°C for 5 is followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C 30 sec and extension at 72°C for 1 mm and a final extension step at 72°C for 7min.

PCR Amplification of Test DNA: After optimisation, PCR amplification of the test DNA samples from uterine tissues was carried out.

Detection of PCR products by agarose gel electrophoresis: The EDS' 76 virus specific product of 238 base pair amplified by PCR was tested by its size in 2% agarose gel.

Agarose (0.6gms) was dissolved in 30 ml of 0.5 x TBE and heated to dissolve using a domestic microwave oven. Gel was allowed to cool and 0.5μl of ethidium bromide was added and mixed. Gel was poured into a gel tank with the combs appropriately placed and allowed to solidify. Combs were taken out and solid gel was put in electrophoresis tank containing 0.5x TBE (Tris-Boric EDTA) to completely cover gel. One and half microliter (l.5μl) of DNA marker (ladder) was added at the extreme well. Five microliters (5μl) of test PCR product and control PCR product was then mixed with 1.5μl of loading dye and put in the well, each sample per well. The microcomputer electrophoresis power (consort E455) was connected to electric source at 120v for 20 minutes. The gel was viewed using UV-transilluminator (UVP Ts-20) and Kodak DC 120 camera connected to a computer.

Nucleotide Sequence Analysis: Partial nucleotide sequencing of the PCR product was carried out at the Department of Immunology Laboratoire Nationale de Sante, Luxemburg using the automatic AB1 Prism 377, sequencer with the Big Dye terminator V3.1 sequencing kit (Applied Biosystems, CA, USA).
Sequence Homology and Alignment: Sequence homology and alignment of the test DNA with other sequences of avian adenovirus and Duck adenoviruses obtained from the EMBL was carried out using the NCBI platform and Mega 2.2 software respectively.
Protein Sequence and Modelling: Test DNA sequences were translated to protein sequences using EMBOS software. Protein modelling was done using Phyre 2 and Pymol softwares.
Phylogenetic Relatedness: Phylogenetic relatedness of test DNA with other avian and duck adenoviruses sequences obtained from EMBL was done using the NCBI platform and Mega 2.2 software on default settings.

3. Results

Clinical observation: The birds had depression in egg production, non-attainment of peak egg production and various egg abnormalities ranging from shell-less, thin-shelled, rough- textured or sandpaper-like eggs as well as small-sized and depigmented eggs in the flock, no obvious gross abnormalities at post- mortem. Some eggs had white chalky deposits and were sandpaper-like in texture (Figure 1). There was no egg shape abnormality and no mortality was observed.in the flock. The variation in the weekly egg production from weeks 22-42 is shown in figure 2.

A=Normal eggs, B=Thin shelled eggs, C=Small-sized eggs, D=Shell-less eggs

Figure 1: Different egg abnormalities observed in the investigated layer flock in Ogun state

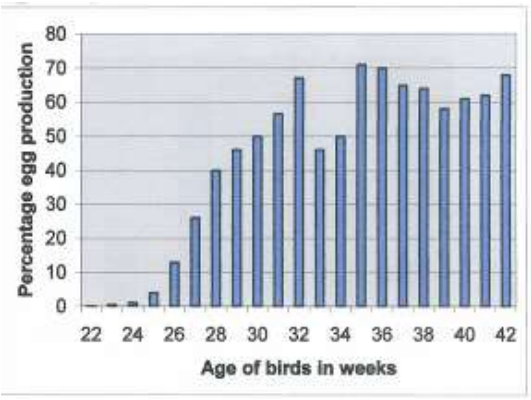


Figure 2: Weekly egg production from 22 – 42 weeks of age in the investigated layer flock in Ogun state

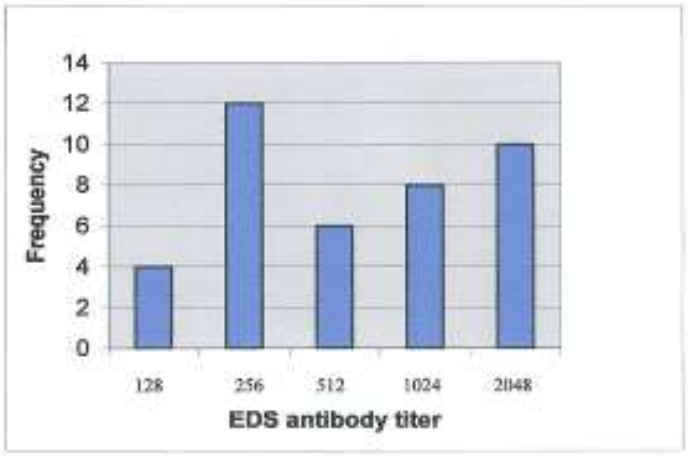


Figure 3: EDSV antibody titre in the investigated layer flock in Ogun state.

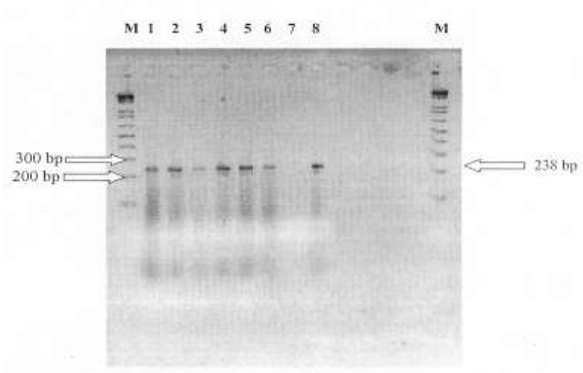
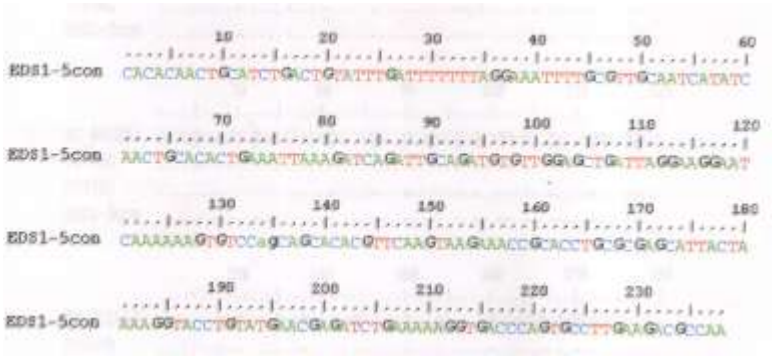


Figure 4: Agarose gel electrophoresis of PCR products in the investigated layer flock in Ogun state.
M: 1 kb plus DNA ladder, lanes 1-5: reproductive tissue samples tested for EDS 76, lane 6: reproductive tissue sample 3 (repeat), lane 7: negative control and lane 8: positive control.

SEROLOGY: All serum samples tested were positive for egg drop syndrome virus infection with high titres ranging from 64 to 2040.The flocks tested HI positive for EDS-76 virus. The birds had very high antibody titre to EDSV. The most frequently occurring antibody titre observed was 256 followed by 512 and 64 (Figure 3).

PCR : A 238 base pair segment of the untranslatable region of protein VIII of EDSV was amplified. All four samples were positive for EDSV. The PCR products from the suspected samples are shown in figure 4. The 238bp nucleotide sequence of pVIII of EDSV is shown in figure 5 with the consensus sequence, while comparison of the EDS sequence with those of AAV, EDS genome, DAV and AAV EDS gene encoding protein VIII is shown in figure 6.

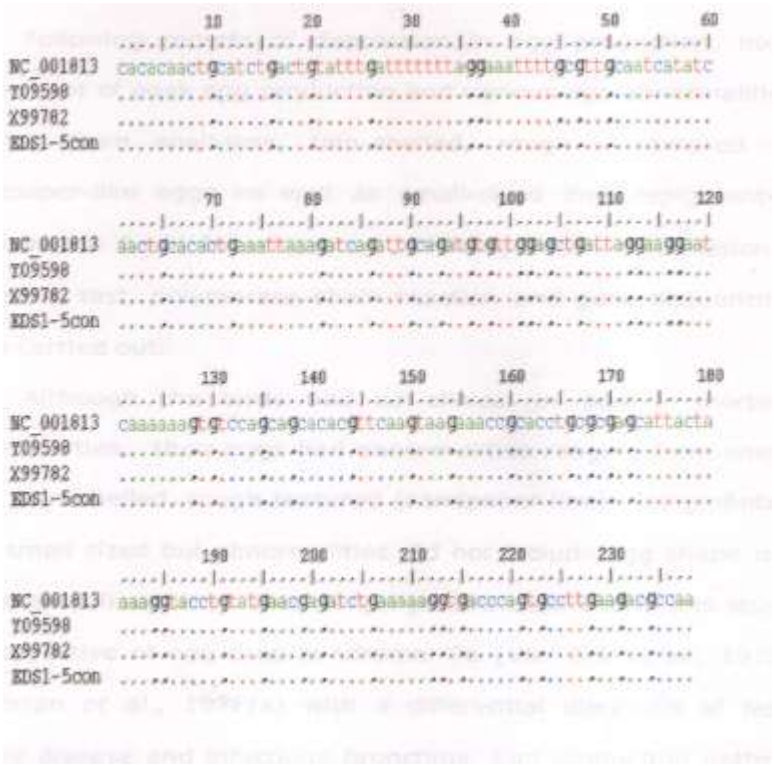
Sequencing: The results of the sequenced PCR products of reproductive tissue samples 1-5 is as indicated below. The partial nucleotide sequence determined using the Big dye terminator method on ABI Prism automatic sequencer



EDSI-5 Consensus sequence:

CACACAACTGCATCTGACTGTATTTGATTTTTTTAGGAAATTTTGCGTT
GCAATCATATCAACTGCACACTGAAATTAAAGATCAGATTGCAGATGT
GTTGGAGCTGATTAGGAAGGAATCAAAAAAGTGTCCagCAGCACACGT
TCAAGTAAGAAACCGCACCTGCGCGAGCATTACTAAAAGGTACCTGTA
TGAACGAGATCTGAAAAAGGTGACCCAGTGCCTTGAAGACGCCAA

Figure 5: sequenced PCR products of reproductive tissue samples 1-5 and consensus sequence in the investigated layer flock in Ogun state



Key:

NC 001813 (Duck adenovirus genome)

Y0958 (Avian adenovirus, EDS genome)

X99782 (Avian Adenovirus, EDS gene encoding pVIII protein)

EDS 1-5 con (Test uterine samples)

Figure 6: Alignment of test sequence data with data of other adenoviruses

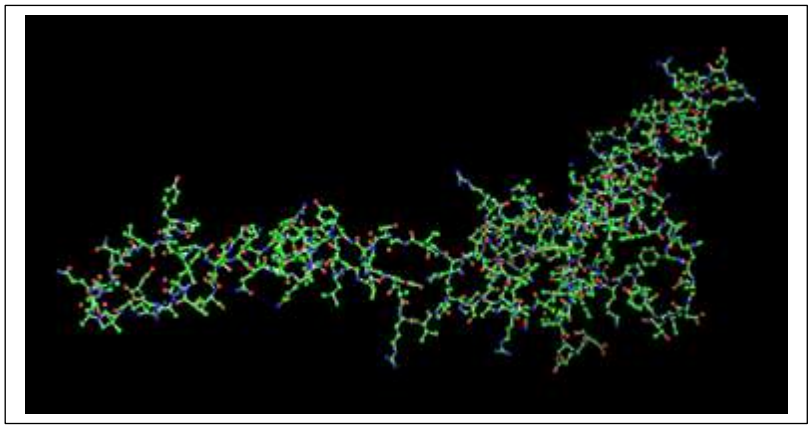


Figure 7: Ball and Stick Model of EDSV PVIII

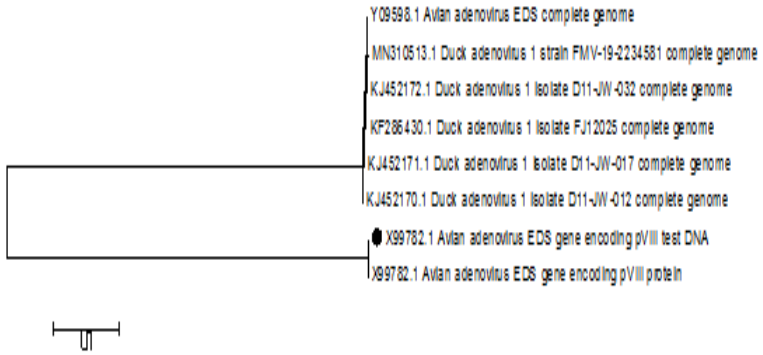


Figure 8: Phylogenetic relatedness of positive EDSV sample with other Avian and Duck adenoviruses

4. Discussion

Following the clinical reports of depression in egg production, non-attainment of peak egg production and various egg abnormalities ranging from shell-less, thin-shelled, rough- textured or sandpaper-like eggs as well as small-sized and depigmented eggs in the flock, investigations involving haemagglutination inhibition test, polymerase chain reaction and gene sequencing were carried out. Although the birds had no post-mortem abnormalities, their eggs had abnormalities ranging from shell-less, thin-shelled, rough textured (sandpaper-like), depigmented and small sized but abnormalities did not include egg shape nor albumen-yolk quality. This clinical picture observed in this study is suggestive of egg drop syndrome '76 (Van Eck *et al*., 1976: McFerran *et al*., 1977, Durojaye and Adene 1981) with a differential diagnosis of New Castle disease and infectious Bronchitis. Egg production pattern revealed a non-attainment of peak production as well as two waves of over 20% egg production depressions at weeks 33 and 34 as well as between weeks 36 and 42. At about 10 weeks of lay, birds are expected to attain peak production level of about 95%. This peak production is maintained for about 8 weeks before waning. At 36 weeks (i.e. 14 weeks after onset of lay), the birds only attained a maximum of 70% production, suggesting that they did not attain peak production. The non- attainment of peak production is equally suggestive of a long standing infection probably before onset of egg laying. This observation is in accord with that of McFerran *et al*., (1977) and Adene et al (1995). The wave of production depression was most likely due to a lateral spread of the infection as described by Cook and Darbyshire, (1980).

It was observed that flock had not been vaccinated against EDS '76 virus. Consequently, a haemagglutination inhibition test was carried out by standard method on the 40 serum samples obtained from the birds, using antigen and positive and negative control sera obtained from the Virus Research Unit of the National Institute of Veterinary Research, Vom, Nigeria. The high titres are indicative of a long standing infection as more of the antibodies are developed overtime as reported by Hess (2017). The high level of antibody response is not likely to be maternal as maternal antibody to EDS '76 is hardly detectable in chicks and cannot exceed three weeks of life (McFerran and Stuart; 1990; Younus *et al*., 2017). The birds in the present study were 42 weeks of age as at the time of sampling. The variation in EDSV antibody titre is suggestive of a field infection since the birds in this flock might have been infected at different times with varying doses of virus inoculum which probably replicated at different rates and thus stimulating different levels of antibody production in the birds. The pattern of antibody level was equally not due to vaccination since the birds were not vaccinated against EDS. Vertical transmission was suspected in this case probably originating from the hatchery where the birds were obtained at day old (Hafez, 2011). However, it was not possible to obtain information in this regard at the time of sampling. Nonetheless, the variation in antibody levels and the waves of egg production depression suggest an associated lateral spread of the infection. This is consistent with the limitations of vertical transmission as reported by Baxendale et al. (1980) and Kumar *et al*., (1992).

Other causes of egg shell defects as well as egg production depressions include infectious bronchitis (IB) and New Castle disease (ND). Infectious Bronchitis (TB) was ruled out based on the observed clinical signs which were not consistent with those of lB. Moreover, post - mortem examination and histopathology did not show any lesions in respiratory organs and kidneys. New Castle Disease (ND), on the other hand was investigated by carrying out haemagglutination inhibition test and post - mortem examination. Post-mortem examination did not reveal any abnormalities. However, HI test demonstrated high protective antibody titres ranging from 64 - 1024 with 72.5% of the birds showing a titre of 512. This near uniform pattern of antibody titre is suggestive of vaccinal response especially as there is a history of NDV vaccination in an attempt to address the egg quality defects in the flock. The antibodies here were not cross reactive with those of EDS.

In this study, a 238 base pair segment of the untranslatable region of protein VIII of EDSV was been amplified and partial nucleotide sequence determined using the Big dye terminator method on ABI Prism automatic sequencer. Hess *et al*., (1997) determined the complete nucleotide sequence of the EDSV and reported the genome to be 33,213 nucleotides in length with a molecular weight of 21-3x106, G.C content of 425% and high homology with other mammalian adenovirus proteins except the E1A, IX, V and E3 proteins. Although partial nucleotide sequence of pVIII was generated, the region amplified and sequenced, falls in the non-coding region for the protein between nueclotides 92-330 determined for EDSV pVIII by Rhon *et al.,* (1997) and nucleotide 21,385 and 21,622 in the overall genome sequence. There is 100% homology between the nucleotide sequence of the test DNA in the present study and those of the EDSV and duck adenovuirus obtained from the European Molecular Biology Laboratories (EMBL) nucleotide sequence data base under accession number Y09598 and NC-001813 respectively. This region also has a GC content of 41.59% which is in accord with that reported by Hess *et al.,* (1997). The five families (L1-L5) of mRNA (of which pVIII is part) encoded by adenovirus major late transcription unit, all contain the same 200-nucleotide-long 5' non-coding region termed the tripartite leader sequence. This 5' non-coding region is very important for translation of mRNAs late in infection and lacks secondary structure (Dolph *et al*, 1990; Walter and Petra, 2012). The tripartite leader sequence probably acts as a signal for late protein synthesis. Part of this region had been amplified and partially sequenced in this study. The ability to amplify and sequence this non coding region in pVIII is of diagnostic importance since it is conserved in all the five late mRNA families of adenoviruses including EDSV.

The EDS pVIII is a structural protein which belongs to the hexon-associated proteins and connects the core with the inner surface of the adenoviruse capsid in association with pVI (Rhon *et al*., 1997; Wang *et al*., 2018), Limited proteolysis of six structural proteins PVI, Pvii, PVIII, lila, preterminal protein and the ilk protein is required for the production of mature adenovirus particle. The virus encoded L3 23K protein is the protease responsible. These precursor proteins are required for adenovirus assembly (Webster *et al*., 1993; Foster *et al*., 2019), and the cleavage is also necessary for virus maturation (Weber, 1985). The pVIII in association with polypeptides VI (217aa) and IX (139aa) also stabilise the hexon capsomere lattice. The protein is thus a very important protein for adenoviruses. It also plays a role in disassembly. There is a sequential disassembly of the virus particle when it is being transported across the cytoplasm to the nuclear pore during internalisation following absorption. PVIII disassociates from the virus particle as the penton capsomers are lost and shortly after entry of the virus particle into the cytosol, protein VI is degraded. The loss of polypeptides VI and VIII prepares the virus particle for the release of genomic DNA (Greber *et al*., 1993; Sartorius *et al*., 2019; Spriggs *et al*., 2019). This further reinforces the importance of PVIII in adenoviruses including EDSV. The analysis of this region could therefore be useful as confirmatory diagnostic technique particularly in persistent EDSV infection.

Adenoviruses often undergo long periods of persistent/latent infections in man, animals and birds where the viruses are reactivated. For persistent infection to occur, adenovirus must be capable of evading the hosts' immune response. Evidence from animal models suggests early (E) region 3 might be important in this process. It has been shown that E3 products particularly E3 14.5k and 10.4k proteins down-regulate E1a and other viral proteins (Zhang *et. al*, 1996). The E1a gene encodes proteins that contain the immunodominant peptide for cytotoxic T (TC) cell recognition in the context of class 1 MHC molecules (Kast *et al*., 1989). These E3 14.5k and 10.4k proteins are both cytoplasmic membrane bound proteins that

exist in a complex in-vitro, functioning in concert (Tollefson *et al.,* 1991). They have two functions, protection against cytolysis by tumor necrosis factor α (TNFα) and down-regulating cell surface expression of the receptor for epidermal growth factor. Evasion of T-cell-medicated host immune response by adenoviruses appears to focus on limiting the epitope recognized by $CD8^+$ cytotoxic T cells (Zhang *et al.,* 1996). The persistent infection in the case reported here is consistent with the mechanism of evasion of the birds immune response by the EDSV and the disease appearing at this time is probably due to reactivation of latent virus as earlier reported by McFerran, *et al.,* (1978).

In conclusion, both epidemiologic and molecular evidence strongly suggest that unique patterns of adenovirus genomic type shift is economically important in poultry, the focus of further work would be to isolate EDSV from various geographical locations in serotypes in poultry in order to understand the molecular basis of regional shifts, if any, that could be observed in genomic types of the virus.

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