SEROPREVALENCE AND MOLECULAR CHARACTERISATION ON INFECTIOUS BRONCHITIS VIRUS IN CHICKENS IN SOUTHWESTERN NIGERIA

BY

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CERTIFICATION

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DEDICATION

This thesis is dedicated to Late Professor Amubieya Ademola Owoade who slept in the Lordon the 24th of September, 2018 and buried on 12th, of October, 2018. May His gentle soul restin peace.

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ABSTRACT

Infectious Bronchitis (IB), a viral respiratory disease of chickens is a major threat to the poultry industry causing decreased egg production. Despite vaccination against the disease, outbreaks continue to occur in Nigeria with clinical features similar to other respiratory diseases. There is limited information on the circulating andavailable vaccine strains in southwestern Nigeria. This study was designed to investigate the level of awareness of farmers, experience of outbreaks by veterinarians, available vaccines and current seroprevalence of IBas well as characterise circulating virus in commercial and local chickens in Lagos, Ogun and Oyo states.

Structured questionnaires were interviewer administered purposively to obtain information on IB awareness from 83, 105 and 96 registered poultry farmers (based on accessibility) as well as experience of outbreak from 56, 64 and 70 veterinarians (based on poultry specialisation) in Lagos, Ogun and Oyo states, respectively, between September and November, 2015. A survey of commercially available IB vaccines was also conducted.Blood, cloacal and oropharyngeal swabs were obtained from 10 chickens per unvaccinated commercial flock from 15 randomly selected poultry farms per state. One hundred similar samples were obtained from unvaccinated local chickens in five locations per state. Cloacal and oropharyngeal swabs, lung and kidney tissues from21dead commercial chickens with history of respiratory signs were obtainedfrom poultry diseases diagnostic centers in the studyarea. Sera were screened for IB virus antibodies using ELISA, while other samples were subjected to reverse transcription polymerase chain reaction for virus detection. Purified *1b*, *S1* and *NP* genes were sequenced using Sanger's method. Nucleotide and amino acid sequences were aligned with sequences retrieved from GenBank using software. Phylogenetic analysis was performed using the Neighbour-Joining method. Data were analysed using descriptive statistics, ANOVA and independent t-test at $\alpha_{0.05}$.

Among the farmers, only 27.7%, 24.8%, and 28.1% were aware of IB, 22.9%, 19.0% and 24.0% vaccinated their chickens, while 10.8%, 19.0% and 10.4% had experienced outbreaks in Lagos, Ogun and Oyo states, respectively. Among the veterinarians, 28.0%, 37.0% and 30.0% had encountered IB outbreaks, while 72.0%, 55.5% and 66.0% advised farmers to vaccinate in Lagos, Ogun and Oyo states, respectively. Massachusetts strain H120 was the only IB vaccine strain available. Seroprevalence was 83.3%, 88.0% and 76.0% in commercial chickens and 70.0%, 85.0% and 82.0% in local chickens in Lagos, Ogun and Oyo states, respectively. Mean antibody titers were significantly higher in commercial chickens (49.74±2.50 and 43.25±4.64) than in local chickens (24.71±2.02 and 31.85±2.24), respectively, from Lagos and Oyo states. Phylogenetic analysis of the *1b* and *S1* gene sequences showed that detected IB virus strains clustered with Dutch Strain H120 Variant 2 (Israel) and Italian strain Qx, while analysis of the *NP* gene revealed 98-99% similarity with South Korean strain K210.

High prevalence of infectious bronchitis among chickens in Lagos, Ogun and Oyo states was established with circulating strains of the virus being genetically diverse from the available vaccine strain. Vaccines for use in southwestern Nigeria should be produced from homologous strains detected.

Keywords: Infectious bronchitis, Commercial and local chickens, Seroprevalence

Word count: 493

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LIST OF ABBREVIATIONS

BLAST Basic Local Alignment Search Tool

DDBJ DNA Data Bank of Japan.

DNA Deoxyribonucleic acid.

DPV Day post vaccination

E- protein Envelope protein

ELISA Enzyme linked immunosorbent assay.

EXPASY Expect Protein Analysis System.

GC Guanine – cytosine content

HI Haemaglutination Inhibition test.

HVR Hypervariable region.

IB Infectious Bronchitis

IBV Infectious Bronchitis virus.

IgA Immunoglobulin A.

IgG Immunoglobulin G.

IgM Immunoglobulin M

ILT Infectious laryngotracheitis

M –protein Membrane proteins

mRNA Messenger RNA.

N – Protein Nucleoprotein.

NCBI National Center for Biotechnology information**.**

NCD Newcastle disease.

NGAC Cloaca samples from Nigeria

NGAL Lung samples from Nigeria

Nsp non-structural protein

nt nucleotides

OIE: Office des Internationale Epizootics

ORF: Open reading frame.

PhCov: Pheasant coronavirus

RdRp: RNA dependent RNA polymerase.

RNA: Ribonucleic acid.

RT-PCR: Reverse transcriptase Polymerase Chain Reaction.

S gene: Spike gene

SIAS: Sequence identity and Similarity.

TCov: Turkey Coronavirus

UNESCO: United Nations Eductional, Scientific and cultural Organisation

USDA: United States Department of Agriculture.

UTR: Untranslated

VNT: Virus Neutralisation Test.

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CHAPTER ONE
INTRODUCTION

1.1 Background of the study

Globally, the livestock industry is fast growing with a significant global asset valued at least $1.4 trillion that has employed up to 1.3 billion and supported six hundred million diminutive farmers in underdeveloped nations (Thornton *et. al.,* 2006).Livestock products contribute seventeen percent calorie and thirty-three percent protein consumed in the world. Urbanisation, population as well as income growth have aroused people's interest in products from livestock thus attracting their attention and stimulating their participation (Delgado *et. al.,* 2005). Poultry is an important livestock sector and is defined as domesticated birds kept by human primarily for meat, eggs and also for entertainment.These are chickens, turkeys and guinea fowls; others are pigeons, ostriches, pheasant and quails. Among all the types of poultry, chickens are the commonest, highest in population and found in every continent of the world (Adeyemo and Onikoyi 2012). It is also the most commercialized agricultural subsector and has been expanding over the years probably because its acceptability is not limited to any religious belief (Ojo, 2003; Adene and Oguntade, 2006).

In Africa, the population of Nigeria is the highest and also number seven in the world. It shares boundary with Niger and Gulf of Guinea in the North and South respectively, Republic of Benin and Chad in West and East respectively. Geographically, Nigeria is 923,768 square kilometers and it harbours an approximately 202 million people (World bank, 2019) and also the frequency at which the inhabitants is growing is three per cent yearly (USDA,2013); therefore, production of eggs and poultry birds are essential to meeting daily protein requirement of her citizens (Ojo,2003).The number of birds in the country was estimated at 160 million with an economic value of US$250million (Akintunde *et. al*., 2015) which improves the GDP with 10% and protein intake of the populace by 36%. However, poultry production in Nigeria faces several challenges which reflect in production, marketing and consumption of poultry products. Some of the

challenges are low capital base, poor management, inefficient housing and marketing problems as well as diseases and parasites (Alabi *et al*., 2000). Disease is a major challenge in poultry because it threatens poultry production (Adewole, 2012) as it reduces productivity of sick chickens which is manifested in less meat, or fewer eggs (Akintunde and Adeoti, 2014), decline output, and fall in profit (Farooq *et al*., 2000) and also results in annual financial burden of 29.2 billion Nigeria currency (Mohammadao *et al.,* 2010). Globally, Infectious bronchitis (IB) exerts a powerful economic influence in poultry sector which manifests similar respiratory and reproductive symptoms with other diseases like infectious laryngotracheitis, avian influenza, viscera tropic velogenic Newcastle disease and Egg drop syndrome, however, most poultry farmers in Nigeria have poor knowledge of the disease despite its grievous economic consequences (Emikpe *et al.,* 2010).

Infectious bronchitis is a very transmissible infection of the respiratory system with consequential financial loss in poultry globally (Umar *et al.*, 2014) although the reproductive, renal and digestive systems could also be infected with clinical signs specific to each of the systems. The IBV infects the avian respiratory tract and causes serious damage to the epithelium that leads to difficult breathing. Chickens are the primary host although the disease has been decribed in other avian species.The important features of this disease in adult birds are respiratory signs like difficult breathing, coughing, sneezing, rattling and nasal discharges while in chicks, high mortality, serious respiratory difficulty and sometimes facial swelling (Cavanagh, 2007). In laying chickens, symptoms like nephritis, fall in quality and quantity of eggs and sometimes, respiratory discomfort (Awad *et al.,* 2014).The disease causes poor carcass weight in broilers, high morbidity but low mortality which may sometimes be as low as 5% although some strains affect the kidney and could lead to 50% and even 80% mortality in some Australian isolates (Asif *et al*., 2007; Jackwood, 2012). The high mortality in young chicks, results mostly from secondary complications such as viral and bacterial infection (Wickramasinghe *et al*., 2014). The viral replication in the oviduct and testes causes reduction in fertility and consequently poor and low egg production (Boltz *et al*., 2004).

The causative agent infects domestic chicken (*Gallus gallus*) (Eterradossi and Briton, 2013). Among the coronaviruses, the virus has the largest genome which is 27.7kb that replicates in the host's cytoplasm (Kuo *et al.,* 2013). IBV was first

identified in USA (Cavanagh, 2007) and later detected in most parts of the world (de Wit *et al.,* 2011).It is an RNA virus and it has the tendency to undergo antigenic shift or drift leading to the knowledge of new serotypes especially in countries where intensive poultry farming is practised (Zanella *et al*., 2003). Presently, several serotypes and genotypes have been reported globally (Mo *et al*., 2013) with little or no cross protection existing among them (Mahgoub *et al.,* 2010) and so several serotypes of different antigenicity and pathogenicity exist in poultry industry worldwide. The virus is sensitive to temperature and will only survive for few days at room temperature. It is also inactivated by disinfectants like virkon S, Virusnip and CID 2000 (Bentong *et al*., 2013).The virus causes avian bronchitis resulting in devastating effect in chickens of all ages.

Although the disease is not dependent on age or season, the prevalence is 35.7% during the early stage mostly between 7days and 35days and higher incidence of 66.7% during winter season (Javed *et al*., 1991). This is because of poor immunity development at the early stage, stressful condition and chilly environment perculiar to winter season (Usman and Diarra, 2008). Therefore, incidence of the disease is reduced with good management that ensures adequate protection of birds from extremely cold condition and healthy environment. Maternal immunity conferred on the chicks from the mother with previous exposure to the virus through infection or vaccination is also protective and reduces the incidence within ths first fourteen days of hatching (Soares, 2008).However, protection due to maternal antibodies against IB virus varies from flock to flock depending on the type of vaccines strains the birds are exposed to, vaccination schedule, quality of vaccine application, systems of production and breed of the birds (Soares, 2008).Virus transmission is through respiratory discharges and faecal droppings from infected poultry. Fomites, that is, contaminated poultry equipment, clothes, sandals or boots aid the spread from one flock to another flock and from one farm to another farm (Ignjatovic and Saparts 2000). There has not been any report of vertical transmission within embryo but the virus may be seen on hatching eggs (Saif *et al.,* 2008).

Emergence of multiple serotypes and variants complicates control of IB through vaccination therefore, it is imperative that the virus is isolated and identified for an effective control through vaccination regime and selection of vaccines based on serotype discovered in that specific geographical area (Yu *et al.,* 2001). Presently,

vaccination is still the best method of control and so for effective control, broilers and pullets are protected with live vaccines admnistered appropriately at young age and layers and breeder are protected with killed vaccines to boost their immuniy (Jackwood and de Witt, 2013).Vaccines are developed from strains that originated from countries like USA, Nevertherlands and Europe (Bande *et al*., 2015). Massachusetts type is the most acceptable of all live vaccine for prevention of infectious bronchitis (Callison *et al.,* 2006) although vaccine failures are reported sometimes after use (Bourogaa *et al*., 2014). Therefore, monitoring the existing serotypes in the region of intensive poultry production with techniques like virus isolation, virus neutralization, and haemaglutination inhibition.Other techniques such as ELISA and RT-PCR were also adopted (Zanella, 2003). ELISA kits are available commercially; the coating agents commonly used is inactivated and purified whole virus particles. Also PCR on transcribed RNA is proven to be potent, fast and sensitive for identification of IBV (Jahantigh *et al.,* 2013).

1.2 Problem Statement

The poultry industry is a commercial sector and has been expanding over the years because its acceptability is not limited to any religious belief (Ojo, 2003). Poultry business is very capital intensive and risky; the risk is spontaneous especially when it involves disease outbreak that could wipe out the whole flock (Abimbola *et al.,* 2013).

Diseases, especially infectious of viral origin like infectious bronchitis constitute a major threat to poultry growth due to unquantifiable financial loss. These losses are from mortality, morbidity, reduced production efficiency, low meat yield and quantity and extra vet costs that will reduce or eliminate returns (Bunnet, 2003).

Poultry business is dominated by private retirees and veterinarians with small flock size and so the consequence of IB outbreak in a farm is very devastating to farmers especially when the capital is from loans and this sometimes lead to stroke or death. This is because mortality could be up to 85-100% in chicks when there is bacterial complication or kidneys infection even after vaccination.

Infectious shares symptoms similar to other viral infectious disease especialy velogenic Newcastle disease which is well known among farmers and veterinarians and thus it could be mistaken for Newcastle disease particularly in a country where

there is limited laboratory diagnosis. Thus there is limited awareness of infectious bronchitis as it is rarely reported by veterinarians and farmers.

Most work on the disease in south western Nigeria which are on the seroprevalence of the virus in commercial and local chickens have revealed a high antibody titre. The most recent on this was carried out over a decade ago and there is need to know the current status (Emukpe *et al*., 2000) in the region being the kind of poultry production.the causative agent being an RNA virus is liable to variation leading to springing up of new serotypes and these serotypes are distinctive and definitive to each region (Mo *et al*., 2013). The most effective method of control is vaccination and vaccines are produced based on the knowledge available strain otherwise there will be vaccine failure. The commonly used vaccine strain for the control of IB is H120 even though some farmers complained of IB outbreak despite vaccination thus incurring losses due to mortality of chickens or reduction in quality and quantity of eggs or both. It is therefore important to know the strain/serotype circulating in the region for effective control. To the best of my knowledge, no work has been done on this in the region.

Ducatez *et al* (2001) reported novel genotype in the region and there is possibility of emergence of new genotype of the virus in the region as a result of mutation or recombination of imported and local strains.

This project is thus designed to know the prevalence, circulating genotypes and serotypes of infectious bronchitis for effective vaccines and vaccination.

1.3 Aim

This study was to conduct an inquiry into the prevalence of IBV in chickens in southwest, Nigeria and characterize detected virus (es) in the region.

1.4 Study Objectives

1. To establish the awareness of poultry farmers and experience of veterinarians on infectious bronchitis in southwestern Nigeria.
2. To determine the seroprevalence of IBVin Southwestern Nigeria.
3. To detect and characterize the virus in Southwestern Nigeria.
4. To compare the genetic relatedness of prevalent IBV genotypes circulating in Southwestern States of Nigeria.

1.5 Justification

Nigeria was ranked 19th in the world and the top producer in egg production in Africa with the production reaching 636,000 metrc tonnes that is worth $527.49 million having a projection of 400,000 MT by 2021 (USDA,2013). This indicates the level of potential for growth in poultry production in the country due to its acceptability and makes it to be intensively practiced farming method in the southwest. However, IB is a threat to intensive poultry production and probably the most crucial cause of disorder in structure and function in chickens that greatly affects farmers' financial income. As such, it is necessary to investigate the prevalence of the disease in the southwestern part of Nigeria being the hub of poultry production in the country.Also, IB has not been well studied in Nigeria because it shows similar symptoms with other respiratory diseases especially velogenic Newcastle disease. However, seroprevalence of 90.1%, 91.97 and 63% has been reported respectively in breeders, layers and growers in commercial birds and also, 78.32% seroprevalence in indigenous chickens in southwestern states (Emikpe *et al*., 2010). Eighteen percent of prevalence based on nucleic acid and also description of a novel strain called 'Ibadan genotype" had also been reported after characterization of IBV in Nigeria (Ducatez *et al*., 2009). Since IBV is proned to high rate of mutation leading to incessant development of new serotypes which constitute a major challenge to effective prevention and control (Mahmood *et al*., 2011) and the report of the novel strain is over a decade. This study aims to determine the current prevalence and the likelihood of emergence of new serotypes due to mutation. This knowledge will thus help in the choice of vaccines and vaccination since there is poor cross - protection among serotypes.

1.6 Research Questions

1. Are farmers in Southwestern Nigeria aware of Infectious Bronchitis?
2. Have Veterinarians in Southwestern Nigeria ever diagnosed IB?
3. Is IBV prevalent in Southwestern Nigeria?
4. What are the genotypes and serotypes of IB in Suthwestern Nigeria?
5. Is Massachusetts vaccine protective against Infectious Bronchitis?

CHAPTER TWO

LITERATURE REVIEW

2.1 Classification and nomenclature of Infectious Bronchitis Virus

There is no agreed standard of classifying the mammalian coronavirus species and thus there are difficulties in naming coronavirus isolates without confusion of the host of origin. The mutability of RNA viruses makes it difficult to distinguish virus species within a genus however; sequence data provides useful information although no specific worth of genome sequence variation can authenticate the differences in virus species (Van Regenmoritel *et al.*, 1997, 2000). Seven diagnostic properties to differentiate between two species of the same genus have also been mentioned and they are; natural host range, genome sequence relatedness, and cell and tissue that support the growth of the virus. Others are the property that causes the disease and identification of the nature of the disease at the cellular level, method of transmiting disease, properties of antigen and physicochemical parameters (Van Regenmoritel *et al.*, 1997, 2000). There is also differentiation into genotypes, serotypes and protectotypes. This involves methods of analysing the genetic and antigenic features of the isolates and also the immunological response of chickens to challenge of IBV (Valastro *et al.,* 2016) however, genotypes, serotypes and protectotypes group IBVs in different ways therefore analysis of S1 sequence data is the most reliable means of grouping IBV strains. Summarily, classification of IBV was based on genomic organization, replication strategies similarities in genomic sequence, antigenic properties of viral proteins, and structural characteristics of virions, pathogenic, cytopathogenic and physicochemical properties (Tok and Tatar., 2017).

2.2 Taxonomy

Group	:	Group IV
Order	:	*Nidovirales*
Family	:	*Coronaviridae*
Subfamily	:	*Coronavinae*
Genus	:	*Gammacoronavirus*
Species	:	Avian infectious bronchitis virus (ICTV, 2011)

2.3 Coronavirus: structure and composition

Coronaviruses have the largest RNA genome ranging between twenty – seven and thirty-two kilobase (Cabeca *et al*., 2013; Birch, 2005) with a nucleocapsid of helical symmetry.Their diameter is between 80 – 160nm and the nucleocapsid is 2 - 20nm (Holmes and Casais, 2001). The virus appears like a crown under the eletron microscope because of club-shaped spike projections emanating from the surface of the virion (Fig.2.1) and so the name corona which is a latin word (Fehr and Perlman, 2015).

Coronaviruses are now recognized as emerging disease with natural tendency to cross new host species (Leppardi *et al*., 2018) causing serious and sometimes respiratory,cardiovascular,intestinal and neurological and antibodies to infectious bronchitis virus has been demonstrated in poultry workers although no clinical infection was established (Miler and Yates,1968; Ignjatovic and Saparts,2000).They were thought to be of mainly veterinary importance until in 2002 when there was a pandemic of a human disease in Asian countries that infected eight thousand people mostly in China leading to 774 deaths and consequently attracting global attention. The cause of the pandemic was later announced to be a fatal disease that infects upper respiratory system named Severe Acute Respiratory Syndrone (SARS) (Cabeca *et al*., 2013; WHO, 2015). Also, Middle East Respiratory Coronavirus (MERC) was reported after its isolation from a patient diagnosed of pneumonia in Saudi Arabia and another in Qarta that led to the death of almost one-tenth of the affected population thus arousing the interest of researchers in the study. Recently, Covid-19 pandemics has caused a very great challenge in the health industry as the virus spread to all continents except Antarctica leading to deaths of over one million people globally. Consequently, the

virus is now of public health importance because of the emergence of many new family members of coronavirus after outbreaks suggested to be due to the capabilities of coronavirus to cross the species barrier and enter human population (Hulda *et al*., 2016). In humans, coronavirus is mainly associated with transient respiratory diseases and gastrointestinal illness.like in animalswhere it causes gastro-intestinal disease in pigs, respiratory and diarhoic diseases in cattle and respiratory and kidney diseases in chickens causing grevious effect on the economy.

Coronaviridae consist of four genera that harbor causative agents of veterinary or human importance and these are Alpha-, Beta, Gamma and Delta coronaviruses. It was recently postulated that birds are the ancestral source of *Gamma*- and Delta coronaviruses while *Alpha*- and Beta coronaviruses originated from bats. Alpha coronaviruses infect animals and humans, Beta coronaviruses harbor the causative agents of SARS and MERS in humans and several diseases in rodents and ungulates. Delta coronaviruses cause infection in avian, porcine and feline species (Woo *et al.,* 2012) although human cells has been reported to be permissive to porcine delta coronavirus infection.The most economically important avian coronaviruses are IBV in chickens and TCov in turkeys, IBV was the first coronavirus reported as early as 1930 and it causes very infectious respiratory disease in domestic fowl that sometimes infects renal and genital organs with grevious economic implications worldwide (Cavanagh and Gelb, 2008). In the 1970s, Turkey coronavirus was also described relating it to intestinal disease (Guy, 2008).

2.4 Genome organisation and viral proteins

The genome of IBV is a non-segmented, positive sense single stranded, RNA (Liu *et al*., 2009). The first two-third of the genome is the replicase gene that is made up of open reading frame 1a and 1b (ORF1a and ORF 1b) as shown in fig.2.2. A set of proteinases that are encoded by the virus co- and post-translationally processed the polyprotein. One or two papain-like proteinases processed the two polyproteins at the N termini and the main proteases are responsible for the cleavage of coronavirus, the structural proteins and non-strucural accessory proteins are important in pathogenesis. (Zhou, 2014).

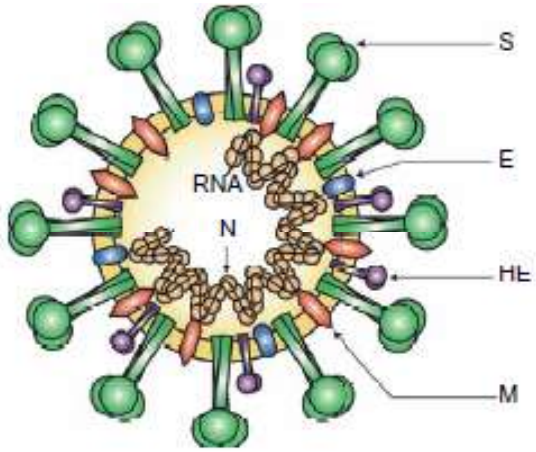


Fig 2.1: **A schematic diagram of Coronavirus**

(de Groot., 2006).

S-Spike glycoprotein, E-Envelope protein, M-Membrane protein, N-Nucleoprotein, HE: Haemaglutinin-Esterase.

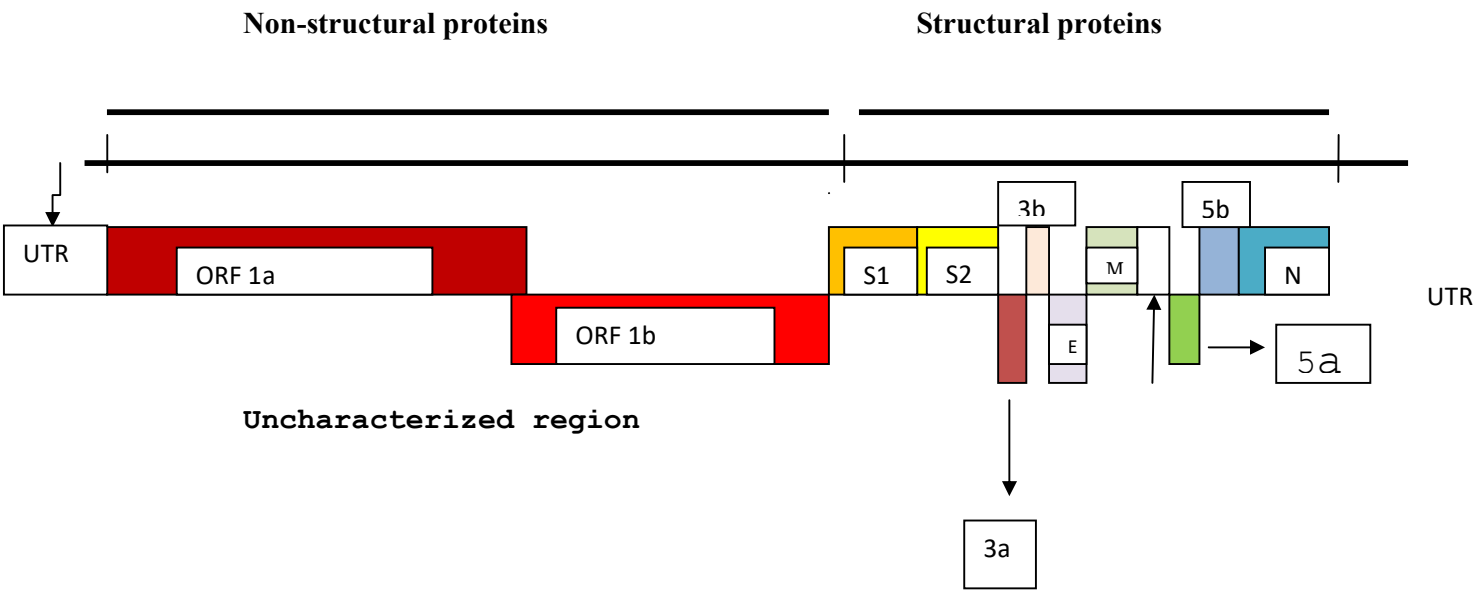


Figure 2.2: The genomic organization of IBV (Abro, 2013)

2.4.1 Non- structural genes

The genome of IBV possesses two small non- structural protein genes namely 3 and 5 non structural proteins which manifest five gene products; 3a, 3b, 3c and 5a, 5b, in the order given. The 3a, 3b, 5a and 5b proteins show specific characteristics when compared to members of groups 1 and 11 coronaviruses. These genes are thought to contribute to virus virulence.

2.4.2 The spike glycoprotein

This is a transmembrane protein that is highly glycosylated, consists of 3,400 nucleotides and is made up of 1,162 amino acids. The S1 glycoprotein is highly variable, important in receptor binding and also development of subunits vaccines against infectious bronchitis virus (Asadpour *et al.,* 2010). S1 gene is also crucial in immunogenicity and contains epitopes responsible for neutralizing antibody. It also controls the receptor binding specificity as well as membrane fusion. (Belouzard *et al*.,2012). S1 gene is proned to mutation and the occurrence could alter the immunogenicity and tissue tropism of IBV strains (Cavanagh and Naqi, 2003; Brandao, 2013) leading to strain differences which commonly occur in three hypervariable regions (HVRs) situated at position 114-120 nt which is equivalent to amino acid residues at 56-69 (HVR1), 297-423 nt corresponding to 117-131 amino acid residues (HVR2) and 822 - 1161nt equivalent to 278 – 387 amino acid residues at (HVR3)

2.4.3 The nucleocapsid gene

Nucleoprotein attaches viral RNA to form a helical Ribonucleoprotein (RNP) that is composed of the viral core structure involved in the assembly and viability of the virion (Saikatendu *et al*., 2007). It is generated in the course of an infection and is the most prolific viral protein of coronavirus (Zhao *et al*., 2012).

Infectious bronchitis virus nucleocapsid is a phosphoprotein accommodating four hundred and nine amino acids with 91 – 96.5% similarity (Spencer *et al*., 2008). N protein is very crucial to the replication and assembly of IBV thus influencing its immunogenicity. It also takes part in cellular immune response. However, dissimilarity in S1 gene as well as N gene is important to the emergence of new variants and so understanding it is crucial to the choice of effective vaccines.

2.4.4 The matrix protein

M protein is a polytopic glycoprotein which is 224 to 225 amino acids long and is relatively well conserved in infectious bronchitis virus (Masters *et al.,* 2013). It is the most abundant component of corona virons and determines the shape of the virion envelop (Wang *et al*., 2009). The M monomer ranges between 25 and 30KDa and is embedded in the envelope by three trans membrane domains (Wang *et al*, 2009). The domain is known as antigenic determinants. The M protein does not bear an amino terminal signal peptide and it is important in the organization and assembly of the virus.

2.4.5 Small envelope protein E

Coronavirus E envelope is important for efficient virus production as shown in a decrease production of small size although it is known to be associated with virion Envelope protein has not been well characterized because of its low level in virions and small size of between (75 and 109 amino acid) structural protein containing hydrophobic domain (HD).The hydrophobic domain of IBV is crucial to systematic let out of the virus (Ruch and Mahamer, 2011). E protein of different Covs has been shown to perform similar functions during virus infection.

2.4.6 Untranslated region

The genome of IBV is made up of 5′ and 3′ untranslated regions (UTRs) (Mahdi *et al.,* 2014). 5’ UTR is highly conserved and contains infected cells and the 3’ region is towards the parts of N gene and it takes part in the commencement of negative strand RNA synthesis. It has two hypervariable and conserved regions (Majdani *et al*., 2011).

2.4.7 Evolution of infectious bronchitis virus

Evolution occurs when there is an alteration in the genetic make-up of the population from one generation to another.It has been linked to lack of RNA polymerase proof reading, interference or uninterrupted use of live and in most cases, vaccines of diverse strains of IBV. (Toro *et al*., 2012a). Infectious bronchitis virus undergoes genetic diversity because of its liability to rapid replication, population growth, high mutation and recombination. Mutations including substitution, deletions and insertions are mechanisms of variation in IBV.

Substitution occurs as a result of high error rate and poor proof capability of the viral RNA dependent RNA polymerase (RdRp) while insertion and deletions result from recombination events or by RdRp slippage.Mutation is an alteration in the genetic material, which can be transferred from one generation to another. Mutations occur randomly in the genome which could be deleterious, neutral or advantageous resulting from ionizing radiations, base analogs and base modifiers. It could also result from deficiency in nucleic acid, replication and repair mechanism.

Substitution occurs when there is replacement of single base with another. This could occur within purine or pyrimidine bases, that is A to G, or C to T/U (Cases-Gonzalez and Menendez-Ariar, 2004) and called 'transition'' or between purine base or pyrimidine base, that is A to C, G to T or T/U to A (Kricker and Drakes, 1990) and called transversion. Transition mutations are commoner than transversion because methylated thymine or cytosine experience high rate of mutation. Insertion is a type of alteration that occurs when not less than one extra base is added to the sequence while deletion is the taking away of not less than one base from the sequence and it has similar repercusion as insertion. (Montville *et al*., 2005).Synonymous mutation occurs when change in nucleotide does not translate into change in the amino acid and non-synonymous occurs when there is change in nucleotide translates to change in amino acid (Gojobori *et al*., 1994). The consequence of the nucleic acid sequence due to substitutions, insertions and deletions is the alteration in the feature or characteristics of that protein or non-existence of functional protein (Makadiya, 2007)

Recombination occurs inside the host cell at the time of replication and it could be refered to as homologous or non-homologous in nature. Homologous recombination occurs when the replicase proceeds to copy the new strand at the exact point it stopped with the old one and non homologous is the otherwise (Worobey and Holmes, 1999). High rate of events of rearrangement of genetic material have been reported in IBV genome and other coronaviruses and this helps them to change virulence (Jackwood *et al*., 2012). Recombination increases chances of survival in new environment by creating genomic diversity and reduce mutational load (Charpentier *et al*., 2006). IBV has regions with high incidences of

recombination breakpoints which are mainly at S glycoprotein gene, nucleoptotein gene and 1ab gene (Zhou *et al*., 2016).

2.5 Infectious Bronchitis

2.5.1 History of IB

The knowledge of infectious bronchitis was first mentioned in USA in 1930 and then identified in United Kingdom in 1948 (Schalk and Hawn 1931; Asplin, 1948). They described a new respiratory disease with distinct clinical signs in which chicks found it difficult to breathe and were also lethargic recording high mortality between 40% and 90%. In 1933, Bushnell and Brandly reported a similar respiratory disease caused by filterable virus thought to be infectious bronchitis but later found to be distinct from IB by Beach and Schalm through cross immunity studies in chickens. The disease was later suspected in layers with respiratory symptoms and a decline in egg production in 1940 (Van Rockel *et al.,* 1950; Broadfoot and Smith, 1954). Thereafter, Van Rockel initiated the development of immunization programme to prevent the disease in layers (Van Rockel, 1941). In 1944, he also observed neutralizing antibodies in the blood of infected chickens. In 1962, Cumming reported the first strain of IBV that infected and caused damage to the kidneys of chickens in Australia, the strain was was named 'T' strain and it had predilection for the kidney (Cumming, 1963).

Infectious bronchitis was first demonstrated as coronavirus disease by Beach and Schalm in 1936 and in 1937. Beaudette and Hudson performed the first isolation of the virus in chick embryonated egg and sturting and curling in chick embryo was established to be pathognomonic lesion of the virusin 1949 (Fabricant, 1949). Consequently, the first strain was named after Beaudette, however, it was later discovered that M41 and Beaudette strain were related after serological test (Bracewell, 1975). Jungherr *et al* (1956) discovered that aetiology of IB had more serotypes when he observed that Connecticut and Massachusetts strains could not cross protect. The first isolation of Massachusetts (Mass) type in Europe was in 1940 and it was made into vaccines to protect against IB.However, in1980s, outbreaks of IB were reported in poultry flocks in Netherlands despite vaccination against IB with vaccines produced from Massachussets strains. In 1985, 4/91 type

was isolated in France: and it was later identified in United Kingdom and later in 1990s, a new type of IBV, 793B was described in flocks of chicken.

2.5.2 Infectious bronchitis in Europe

There are so many strains and serotypes circulating in Europe since the report of the disease in Dakota, USA. Most of the strains were similar to four strains from Netherland which were D207, D212, D3128 and D3896 while thirty percent of the strain and serotypes were identical to known America serotypes in the1970s after serological tests (Bande *et al*, .2017). In United Kingdom, 4/91 was first described in 1990 in chickens with respiratory disease and had since become the dominant strain compared to D 274 which was prevalent in 1980. Also, presently, 4/91 also refered to as 793/B and CR88 is the commonest serotype in Europe (Dolz *et al.*, 2006). In 2002, Italy 02 became the third of all the strains that were often discovered and probably dominant wild type in countries like Spain, France, UK, Germany and Italy (Jones *et al*., 2005). The existence of the genotype all over Europe may suggest an ineffectiveness of the vaccination strategies which failed to protect the chicken against the infection.

In Belgium, IBV infections are highly prevalent and epidemiological surveys in the poultry sector showed an apparent shift from types commonly witnessed between 1986 and 1995 (Meulemans *et al*., 2001) and those experienced between 2002 and 2006 (Worthington *et al.*, 2008).In South America, Massachusetts seems to be the predominant strain. In Brazil, the first strain reported was Massachusetts in 1950s (Hipolito, 1957) and after about two decades it was reported later in Chile (Hidalgo *et al.*, 1976). In Poland, first serological report on infection caused byIBV was in 1967 (Karczewski and Cąkała, 1967) but in the mid-1980s, there were outbreaks of IB in poultry farms manifesting in respiratory and reproductive symptoms in non-vaccinated laying hens and breeders (Bratu *et al*., 2004). In the 1990s, outbreaks occurred in broiler flocks and were found to cause nephritis (Minta *et al*., 1995). The common strains in Poland are 793B and QX (de Witt*et al.,* 2011)

In France, the most prevalent strains were 793B which was about 54%, Massachusetts 23% and QX, 12%. These were linked to vaccines used in the country except QX which was detected in China. In Turkey 793B was the predominant strainwhile 4/91 and D274 were common in Switzerland. In Russia,

most isolates belonged to Mass type although strains like 793B, D1648, 6241, It-02 and QX were also present. Thirty percent of the isolates in the country were also novel genotype. QX was first described in China in 1992 but it has spread throughout Europe. It was first detected in Netherland but later in France, Italy, Germany, UK, Slovenia and Sweden. Arkansas was the commonest strain in USA obtained from IB outbreaks, it is important to state that this strain has not been detected outside USA.

2.5.3 Infectious bronchitis in Africa

Infectious bronchitis virus has been well studied in Northern African countries where some classical IBV variants were detected. In Egypt, IBV was first reported in the 1950s and isolateswere similar to strains from Netherlandsuch as D3128 which are of Massachusetts strain, others genotypes have also been described in various poultry flocks in the country (Sediek, 2005). Novel genotypes had also been isolated in various poultry farms (Abde-Moneim *et al*., 2006). In Morocco, IBV was first detected and characterized in 1986 (El -Houdfi, 1986). The result showed six isolates, five of which were related to Massachusetts and one unique and distinct serotype called Morocco 'G'. In 2004, Alrabi in his work on relationship between nephropathogenic disease and IBV discovered three groups, Grp 1 related to Massachusetts and Groups 11 and 111 were unknown. In Morocco, poultry industry is a major sector for provision of jobs and also 85% of broiler meat production (Naim Hassan *et al.,* 2017). Consequently, measures were taken to protect the industry with vaccine strains like Arkansas, 793B and Massachusetts available in the country. In Tunisia, three isolates have been reported; TN200/00, TN200/01 and TN/335/01 and all the isolates are identicalCR88121 and D274 strainsfrom Europe (Bourogaa *et al*., 2009). The commonly used vaccines were 793/B, D274 and Massachusetts (Bourogaa *et al.,* 2009). New IBV genotypes, Algeria 28/b1, Algeria 28/b2 and Algeria 28/b3 were reported in Algeria although the pathogenicity is not known (Sid *et al.,* 2015). In Libya, the isolates reported are closely related to Egypt and Israeli strains (Bande *et al.,* 2017) although low information on the prevalence of the disease.

In Ghana, Infectious bronchitis was recently reported incommercial poultry farms with respiratory symptoms (Anyim-Akonor *et al*., 2013). In Togo, seroprevalence

of 72% and prevalence of 14.6% has been reported while in Burkina Faso, the prevalence was as low as 3% (Kouakou *et al*., 2015). In Nigeria, in the nineties, seroprevalence of IB was reported to be 42.5%, 15.3% and 3.3% in Ibadan, Jos and Nsukka, repectively (Oyejide *et al*., 1988, Komolafe *etal*., 1990) in commercial chickens and prevalence of 91.3% in indigenous chickens in Kano, after which there was a dearth of information until Owoade reported a seroprevalence of 84% in Nigeria (Owoade *et al*., 2006). A comprehensive study on IBV that led to the discovery of a novel genotyperelated to QX variant was reported and named 'Ibadan strain" in 2006, Adebiyi and Fagbohun, 2017 reported 34.32% seroprevalence of IBV in indigenous and free ranging birds. The emergence of IBV pathotype was reported in a breeder farm in Ibadan (Sopeju *et al.*, 2019). Co circulation of Massachosetts, Connecticut and Arkansas and also 100% seropositivity of IBV were reported in layer farm in Plateau state, central Nigeria (Shittu *et al*., 2019).

2.5.4 Distribution

Infectious bronchitis virus has worldwide distribution (de Wit *et al*., 2011). In 2009, it was reported in Bangladesh, Guangdong and Hebei.Also reported inHenan, and Pakistan.In Malaysia, Lebanon, Jordan, Japan and Iran.Israel, Republic of Korea, Vietnam and Thailand (OIE, 2009) Taiwan (OIE Handistatus, 2005).In Africa, it has been reported in Burkina Faso, Cameroon, and Central African Republic. Reports have also come from Cote d'Ivoire, Mauritius and Zimbabwe (OIE, 2009). While there was no information from other African countries like Nigeria, Togo, Tanzania, Senegal, Rwanda, Morocco, Mali and Malawi. Kenya, Ghana, Gambia, and Congo Democratic Republic did not supply information on the disease (OIE, 2009). In North America, it has been reported in all parts of the continent except Bermuda and Greenland. Infectious bronchitis is rare in Central America and Caribbean but has been reported in Costa Rica. In South America, it has been reported in Brazil, Argentina, Chile, Paraguay and Uruguay. In Europe, it has been reported Den mark, Germany, Netherland and Norway.

2.5.5 Host range

Naturally, the main hosts of IBV are chickens although other avian species like pheasants have also been incriminated. The pheasants exhibit clinical respiratory and reproductive symptoms however, not all species of pheasants are susceptible to IBV or not all strains of IBV could cause diseases in pheasants (Ignjatovic and Saparts, 2000; Cavanagh *et al.,* 2002). Other members of avian species have been incriminated especially in the advancement of IB (Fellipe *et al*., 2010).

2.5.6 Genetic relatedness and epidemiology of infectious bronchitis virus

Initially, IBV coronavirus was the only speciesin goup II until IBV-like viruses including turkey coronaviruses (TCov) and turkey enteritis were described.Pheasant coronavirus (Phcov) is also a member of the group and has similar gene sequence and antigenic relationship. Guinea fowls, partridges and peafowls have been shown infected by coronaviruses that have similarity with IBV. However, of all avian species, TCov is the most identical species to IBV with regard togene and protein sequences as well as antigenic relationship. It has also been thought that group 3 coronavirus emerged from interspecies evolution of the coronavirus which originally infected bats.

Excessive mutation of IBV resulted in creation of several populations of virus particles that are of various kinds and different from each other allowing IBV to swiftly adjust to selection pressure. Emergence of new variants resultfrom genetic shift or drift and if the amount of genetic change reaches a critical level, the available vaccine might not be able to confer protection against the virus leading to vaccine failure thus explaining why there is no effective control (Dolz *et al*., 2006).

Indiscriminate introduction of trade birds, migratory wild birds and use of live attenuated vaccines are essential agents of spread IB (Liu *et al*., 2006). Migration of wild birds enables connection or contact of infected birds with many populations of birds thus transmitting the pathogens and so the strains spread easily over long distances (de Wit *et al*., 2011) and live attenuated vaccines encourage the spread of vaccine like viruses with greater intensity of virulence.

In Spain, twenty-six IB viruses were divided into four distinct genetic groups, genotype 1 consisted of isolates related to 4/91 reference isolates, genotype 11 related to Italy 02, group 111 related to Massachusetts while group IV was in unique genetic group. Those isolates related to Massachusetts were said to be

vaccine strain used as immunization against the disease which supports the likelihood of introduction of the strain to another country through vaccination (Dolz *et al*., 2006). Isolates closely related to Italy 02 of Spain was reported in Morocco as novel genotype and the similarity was due to geographical proximity, trans boundary and commercial transactions between the two countries which includes exportation of breeder chicks to Morocco (Felahi *et al*.,2015). There are over 50 serotypes across the globe and IBV strains within a locality are perculiar to that locality even though many countries have similar antigenic types with strains usually seen in other countries like United States, Ausralia and Europe. It is thus possible that IBV detected in those countries might be due to the genetic change between the IBV population in such countries and IBV introduced as a result of vaccination with live vaccines (Liu *et al*., 2006). Epidemiology of QX suggests the possibility of the virus circulating in a country before detection and increasing in virulence with years of existence. The IBV originated from China in the 70s spread within the country until 1990s and then spread to Europe (Germany, 2002) and Thailand (2005). It then spread to countries such as Poland, Italy (2003), Netherland and France (2004).The spread to other countries like Africa was aided by European countries, for example France introduced it to South Africa and it spread to Egypt through Spain. However, IBV was introduced to Iran, Iraq and South Korea through China.

2.5.7 Genotypes and Serotypes of infectious bronchitis virus

Classification into serotypes is often carried out in the laboratory by neutralization tests.Serotypes are groups of organisns within species that have the same antigens on their surfaces. Apart from cross neutralization test, monoclonal antibodies in antigen captured Elisa and haemaglutination inhibition test are also used. Among IBV strains antigenic differences and relationship are important for the choice of vaccines and vaccinations since most serotypes do not cross protect (Jackwood and de witt, 2013). Sequences of S1 gene give adequate information that shows antigenic similarities and relationships among serotypes and also vaccine strain (OIE, 2018).

Globally, over fifty serotypes or variants of IBV such as Italy-02, H120, D274 have been described. The appearance of several IBV variants or serotypes in

various continents makes serological methods of serotyping difficult (de Wit *et al.,* 2011) and so molecular methods, gene sequencing technology and bioinformatics are now used for the typing of the virus (Lin and Chen, 2017)

2.5.8 Humoral immunity and Infectious Bronchitis Virus infection

Maternal antibody is the transfer of antibodies by a female through the placenta, colostrum, milk or egg and it is necessary to secure new chicks from infectious agents till the full development of their immune system (Hasselquist and Nilson, 2009). Chicks with high MDA titres of anti-IBV are well protected reaching 95% when challenged by IBV at day old. However, the MDA titre of anti IB diminishes very quickly at seven days dropped to less than 30% of the antibody titre (Mandal and Naqi, 2001) and could not be detected at day fourteen (Hamal *et al.,*2006). However, the MDA anti-IBV of unvaccinated chicks dropped sharply in comparism with vaccinated chicks vaccinated at day one (Talebi *et al*., 2005)

Serological assays such as ELISA, HI or VN tests have shown that chickens initiate a quality humoral antibody response when they are challenged by IBV (Ruano *et al.,* 2000). Combination of serological tests such as IBV-specific ELISAs and immunohistochemistry techniques enabled a more detailed analysis of IBV-specific antibodies and their distribution in different chicken tissues (de Wit, 2000). IgM appears first in the blood after IBV infection and disappear within a short period unlike other immunoglobulins. Thus, IBV-specific IgM antibodies in serum are confirmation of a recent challenge of IBV. Following vaccination with IBV-M41, IgM antibodies can be detected on the third day to one-week post-vaccination in the serum (Mocket and Cook, 1986). The concentration of IgM antibodies reaches the highest at fourteeth day and then slowly decrease till they cannot be detected by 21 Day Post Vaccination (DPV)(Mocket and Cook, 1986). A second inoculation induces a similar IgM response with no significant changes in the antibody concentrations as observed in the primary response throughout the observation period. However, unlike IgM, IgG was detected on 6 DPV and got to the peak between 9th to 14th DPV in chickens vaccinated with IBV-M41. There was a gradual decline in IgG antibody concentration after day 14, but significant amounts of IgG were still detected in serum until 42 DPV. Thus, the primary IgG response remains in serum for a longer time than the IgM response (which was

undetectable by 21 DPV). After boosting, IgG levels in sera increased more substantially and followed the same pattern noticed after priming (Mockett and Cook, 1986.). Unlike other antibodies, IgA antibodies are vital for mucosal immunity to IBV (Toro and Fernandez, 1994). IgA antibodies can be found in Harderian gland and tears after IBV infections but antibodies against IBV as expressed by the presence of IgA are first noticed in tears before appearing in serum. IBV-specific IgA is also present in saliva and tracheal washes after an IBV infection. More importantly, lachrymal IgA correlates with resistance to reinfection with IBV.

2.6 Epidemiology

2.6.1 Aetiology

This disease, IB is caused by IBV. The virus is ubiquitous and has worldwide distribution especially where poultry birds are intensively and commercially reared. It damages the mucosae of the respiratory tract and the disease becomes grievous when it is complicated by other infections (Landman and Ferberwee, 2004; Anyim-Akonor *et al.,* 2018).

2.6.2 Infection and Transmission

Transmission of IBV is by unmediated connection with infected chickens or unintended connection with wild birds, contaminated water and materials. The virus also spreads through tracheo-bronchial exudate and faecal droppings of infected chickens (Ignjatovic and Saparts, 2000). The virus spreads horizontally by ingestion or aerosol and morbidity is controlled by the severity of the virus and the capacity of chicken to defend itself against disease. Incubation period varies and depends on the route and dose of infection,while it is 18 hours with trachea route it occurs 36 hours if infection is through the ocular (Cavanagh and Gelb,2008). Samples taken from trachea, lungs, kidneys as well as bursa of infectous have proved relevance in isolation of IBV and the isolation is best done as from fourteen to twenty weeks after the virus is introduced into any living organism or in contact with chickens.

2.6.3 Physicochemical properties of the virus

Most IBV strains are inactivated by exposure to 56°c for 15mins or 15min or45°c for 90mins indicating the fragile nature of the virus. The virus is regardedto be sensitive to common disinfectants and is inactivated by ether, chloroformand other solvent. The infectivity of virus gets totally destroyed by 50%chloroform and 0.1% sodium deoxycholate (Cavanagh and Naqi, 2003).

Potasium permanganate (1:10,000), mercuric chloride (1:1000) and 5% sodiumcan also destroy the infectivity.

The virus will survive for few days at 20˚c but can be preserved in refrigerator forseveral months and for longer preservation, it is safe at -70˚C. However, wherethere is no refrigeration; infected tissues can be preserved in 50% glycerol andinfectious bronchitis virus in allantoic fluid that is freeze dried, closed up andcarefully kept in the refrigerator can survive thirty years. Also the virus can survivefor 56 days in faeces but it easily inactivated by common disinfectants like ethanol, 1% formalin and iodine (Cavanagh and Gelb, 2008). Perpertuity of virus is affected by water quality but 10% glucose will stabilize it in the lyopholised state (Saparts and Ignatovic, 2000). It has also been reported that the virus has been stored successfully by cotton- based cellulose membrane filter card for 15 days containing lyophilized chemical (Moscosso *et al*., 2015).

2.6.4 Pathogenesis

The virus replicates in all respiratory tissues causing manifestation of respiratory symptoms and so the virus could easily be detected within 72 hours of infection in the respiratory tract especially nose and trachea because the titre will be at the highest level at that time till the fifth day of infection. The virus then deciliate the epithelial cells of these organs and then advance to other inner parts such as lungs. It also spreads to kidney, gonads, digestive and intestinal tracts (Ignatovic and Saparts, 2000). It has also been reported that the virus could also be detected in bursa of Fabricius and caecal tonsils in addition to respiratory tissues (Cavanagh and Naqi, 2003). Mortality is caused by complication of secondary bacterial infection like mycoplasma and other viral infections that could cause immunosuppression.

The consequences of the virus replicating in the gonads are infertility and reduction in the number of eggs. Some IBV strains are intrinsically nephrogenic and so cause higher mortality because the kidney is damaged. The extent of damage of IBV on

the number and grade of eggs in laying birds is determined by virulence of the strain, it has been reported that some variant strains had a marked effect on egg color. The M41 strain had less virulence on the oviduct while H52 strain markedly affected the oviduct. Several renal lesions were produced by different IBV strains with varying severity (Meulemans*et al.,* 2001). An enteropathogenic strain G was isolated and has been shown to have affinity for alimentary tract of chickens (El Houadfi, 1986). Secondary pathogens also contribute to virulence of the virus, *Haemophilus paragallinarum* had been found to cause higher mortality and severe lesion presentation and shortened incubation period of the disease. A combination of intranasal inoculation of IBV *and Escherichiacoli* inoculated intranasal has also been shown to produce mortality and ascites in young chickens (Sylvester *et al*., 2005). Pathogenecity of IBV also varies with age, as chicks below 21days are more vulnerable than older ones (Cavanagh and Naqi, 2003). Genetic difference in susceptibility to nephritis has also been described, light breeds was reported to be more susceptible than heavy breeds. Moreover, nephropathogenic IBV has caused higher mortalities in broilers than in layers and male chicks are found to be more susceptible to nephritis than female (Zanella *et al.,* 2003). A high protein diet will increase mortality from IBV induced nephrosis and also low temperature or cold stress increases the severity of IB infection in birds (Sylvester *et al*., 2005).

2.6.5 Clinical signs

The disease affects the respiratory, urogenital and sometimes enteric system. In the respiratory tract, it infects the tracheal epithelium causing deciliation and desquamation leading to contagious respiratory disease. Respiratory signs are coughing, difficulty in breathing and nasal discharge, gasping with the eyes and sinuses becoming swollen (Mohammed *et al.,* 2012). The disease is more prounounced in chicks less than six weeks old compared to older birds. However, in any group, mortality is higher in complicated cases. In chicks, non-specific symptoms are depression, clustering round the source of heat and also dyspnea (Awad *et al*., 2014). Urogenital symptoms are nephritis, increase water intake leading to wet droppings and high mortality if the kidneys are affected. Reproductive symptoms include reduction in number and size of eggs, poor standard of eggs; soft egg shell, uneven and misshaped eggs (Muneer *et al*., 2000). The reproductive tract is permanently damaged at the early infection of the virus

resulting in poor egg production, inability of the chickens to reach the peak during laying period and consequent poor profitability. IB also affects the proventriculus in the digestive system inducing symptoms such as roughness of the feathers, wetness of droppings with white and yellow milky faeces are prominent (Mohammed *et al.,* 2012).

2.6.6 Morbidity and Mortality.

Morbidity in infected chicks could be up to100% but mortality is low varying from 25% to 30% in young chicks. However, in complicated cases, it may be 80% or more depending on age, immunity of chickens, pathogenicity, severity of the strain and environmental factors. Marek'sdisease, infectious bursal disease and secondary bacterial infection like E.coli or mycoplasma may increase the mortality if co-infected with IBV. Nephropathogenic strains cause more mortality when compared with strains infecting respiratory or reproductive system.

2.7 Pathology

In upper respiratory organs, there is mucoid secretion in the trachea, congestion and haemorhage with serous exudate, there is also oedema of tracheal mucosa and extrapulmonary bronchi. The wall of the air sac becomes thickened with yellow exudate. In nephrogenic strains, inflammation of kidneys as manifested by swelling and congestion of the kidney is observed; also there is paleness of ureters and urate deposit. When it is complicated by bacterial pathogen, pale, swollen and mottled kidneys are seen.

2.7.1 Histopathology

Histologically, IB causes deciliation of the trachea, oedema and makes some epithelial cells to change from columnar to squamous cells and hypertrophy of glandular cells.and infiltration of lymphocytes (Bande *et al.,* 2016). For nephrogenic strain, interstitial nephritis, tubular degeneration and infiltration of heterophils are observed. Also, necrotic foci, heterophil and lymphocytes are noticed in the interstitial spaces. Also, Bowman's capsule becomes eodematous, collecting ducts and sphenoids are sometimes infilterated by granulocytes (Cavanagh and Gelb, 2008).In the reproductive system, the oviduct is non–patent and hypoglandular especially in severely affected chickens.

2.7.2 Diagnosis

Infectious Bronchitis has short duration of between three and ten days and so a rapid diagnosis of the virus in none or vaccinated flock is necessary to reduce the devastating economic effect of the disease (Chen and Wang, 2010). IBVcan be diagnosed by serotyping which is by specific antibody against the virus, that is serology or by genotyping which is the detection of the virus or part of it using the nucleic acid base methods (Villereal, 2010). Successful detection of the virus depends on factors like the time of sample collection, the type and quality of samples collected, bird genetics and virus isolation. The level of detection is high when samples are collected from the respiratory tract during an acute infection or kidneys, caeca and cloaca during chronic infection and should be kept in the refrigerator or placed in glycerin to maintain the viability of the virus (Villereal, 2010). Serological detection involves demonstration of presence of IBV identified IgM or IgG in the blood. VNT is also a serological test but is rarely used because it is strenuous and takes much time.Various molecular procedures are used for the identification of the virus.

2.7.3 Serological tests

Detection and serotyping of IBV strains were carried out with serological assays such as VN and HI tests before the advent of molecular studies. The tests were important to know the protection status of the flock after vaccination. Infectious Bronchitis Virus does not naturally cause haemaglutination and so requires treatment with type C phospholipase enzyme. HI test is not very reliable even though it can detect serotypes based on antibodies produced against S1 spike protein (OIE, 2008). ELISA is another serological test that is more sensitive, reliable and very usable in the field for monitoring antibody due to exposure to field or vaccine strain. It is an enzymatic method, most ELISA assaysare generic for IBV and gives positive result when any strain is present (Villareal, 2010). Four kinds of Elisa are available; direct, indirect, sandwich and competitive Elisa. The categorisation is based on the principle of operation. ELISA kits are commercially available with several modifications, for example a type-specific blocking ELISA (Chen *et al.,* 2011)

2.7.4 Virus isolation and identification

This is the usual method of IBV diagnosis.The virus is isolated in 9-10 specific pathogen free embryonated eggs, followed by identification of isolates by immunological method. Virus isolation is burdensome, tedious and expensive involving several passages in embryonated egg until embryonated mortality occurs or other signs are detected in the embryo (Villarreal, 2010).Appropriate sampling technique should be done earnestly for successful isolation of IBV. Collected swab samples should be conveyedimmediately to the laboratory with phosphate buffer saline in sterile tubes. Tissues are taken aseptically from chickens and immediately put in a sterile container for onward transportation to the laboratory on ice. IBV isolation could be through embyonated eggs, chicken organ cultures or cell lines

2.7.5 Molecular Diagnosis

Molecular diagnostic assays are now becoming new gold standard because of the superiority in sensitivity and reliability compared to conventional assays (Hodinka, 2013). RT-PCR involves the amplification of RNA of the virus either directly, or following cDNA synthesis. It was designed to target several conserved region of the genome, mostly the untranslated region and N gene for universal detection and S1 region for genotypic classification. A pan-corona primer aiming at a conserved region of unrelated coronavirus isolates could be used in One-Step PCR amplification of IBV strain. Also, a serotype specific primer that could differentiate Massachusetts, Connecticut, Arkansas, and Delaware field isolates has been designed.

Restriction Fragment Length Polymorphism **(**RFLP) is an IBV genotyping methoddesigned to differentiate between known strains of infectious bronchtis virus and also recognise current variants after RT –PCR amplification and enzyme analysis. It involves full length sequence of IBV strains with the presence of distinct electrophoresis banding pattern defined by restriction enzyme digestion.

Real time PCR assay was introduced for increased test sensitivity and specificity. It could also differentiate Massachusetts from others targeting S1 glycoprotein (Acevedo *et al.*, 2013). For genotyping, S1 gene is usually amplified using RT-

PCR, sequenced and subjected to bioinformatics analysis using databases such as NCBI, EMBI and DDBJ (Zulperi *et al.,* 2009; Abro *et al*., 2013).

2.8 Differential diagnosis

Infectious bronchitis presents clinical signs similar to some diseases of respiratory tract such as NCD, ILT and IC. It also includes avian influenza and avian metapneumovirus (aMPV) (Dhama *et al*., 2014). However, neurological signs and diarhoea in NCD, high mortality in AI and pronounced facial or head swelling in coryza and avian pneumovirus respectively are not common in IB (Bande *et al*.,2016). Although continuous decrease in number, value and quality of eggs and shell are observed in both IB and Egg drop syndrome, poor internal egg quality is perculiar to IB (Dharma *et al.*,2014).

2.9 Control

Severity of infectious bronchitis will depend on age of chicken at the time of infection, strain of the virus, and the environment or level of management of the poultry farm.Therefore, efforts should be made to ensure good hygiene and strict biosecurity (Cavanagh and Naqi, 2003;Cavanagh, 2006). In any area of intense poultry farm, it is a huge task to keep chickens free of the disease since it spreads majorly through aerosol. Therefore, the control is hung on the appropriate administration of both vaccines with adequate biosecurity and good management (Cavanagh and Gelb, 2008).The continuous emergence of variants poses threat to controlling the disease because while several of the variants disappear, some continue to circulate and give rise to disease (de Wit *et al*.,2011).Thus, the best approach tocontrolling IB is to administer vaccines of similar strain to those found in the region. Where this is not possible or where there were no available prevalent strains, administration of multiple strain vaccines will be the appropriate plan of action.

2.9.1 Vaccines and vaccination

Vaccination still remains the cheapest, most effective and cost effective method of controlling infectious bronchitis (Meeusen *et al*.,2007) even though there is a challenge of emergence of new serotypes or variants that bring about poor or no cross protection (de Witt *et al*.,2000). Vaccines are developed from strains that

originated from countries like USA, examples are M41, Ma5, Ark and Conn; Netherland, examples are H52 and H120 and European strains such as793/b.CR88 and D274 (Bande *et al*.,2015). Vaccines with selected or specific genotype provide effective protection against homologous viral strain and little cross protection against strains with other genotypes, thus vaccination with two genetically distinct vaccine strains provide broader cross protection against heterologous IBV strains. Apart from hinderance caused by continuous emergence of variants or serotypes, heterologous challenge, immunosuppression and inappropriate application of the vaccine are contributing factors.Commercial vaccines such as live and killed vaccines (oil adjuvanted) are obtainable. These vaccines have some merits and demerits, while inactivated vaccine is safer, more costly but less effective than live attenuated vaccine, the later can revert to virulence (Asadpour,2010) and cause infection on the field (Meulemans *et al.,* 2001). Live attenuated vaccines can be applied despite maternal antibody on the first day of age or within the first week by coarse spray, beak dipping, nasal or eye drop. Older birds could be vaccinated via drinking water, coarse spray or eye drop (Mayahi *et al.,* 2013). For broilers, IB vaccination is given at the hatchery and repeated at interval of 2-3 weeks of age Live attenuated vaccines are also administered to prepare layers and breeders for intramuscular administration of inactivated vaccines for effectiveness at 13-18 weeks of age (Bande *et al* 2015). These vaccines could be applied singly or combined with other virus vaccines like infectious bursa disease, Marek's disease or Newcastle disease. Although it is doubtful if the combination could affect the immune response to combined antigen (Vagnozzi *et al*,2010), excess IB particles in vaccine could interfere with ND immune response (Zamani Moghaddam,2005). However, combined vaccine is still preferable to application of mixed single vaccines thus ND +IB vaccines induce higher systemic and local antibody compared to single vaccine application. It is also noteworthy that exposure of chickens to IB vaccine at day 1 may lead to intermittent shedding of the virus and so could lead to presence of vaccine strain in unvaccinated chickens (Matthjis, 2008; Rua,2016).

Vaccines and vaccination are important component of successful poultry enterprise which is not limited to production but includes marketing (Marangon and Busani, 2006). Live vaccines have been successfully applied for control of infections in

chicks and to prepare future breeders and layers before administration of inactivated vaccines (Cavanagh and Naqi, 2003). Several technicalities are employed in mass application of vaccines and these include routes of administration, quantity and quality of vaccines, temperature of water for vaccine dilution and combination with other vaccines to achieve effective vaccination (Jackwood *et al*., 2009). However, strict compliance to these technicalities does not guarantee complete protection because of limitation of live vaccines which include poor thermo stability, reversion to virulence and exchange of nucleic acids between vaccine and field virus leading to the appearance of serotypes and variants with poor cross protection.

Vaccine failure occurs when the host is unable to exert enough protective antibody response after primary or booster vaccinatiion and it could be dependent on the vaccine, age, health status of the host or genetic factors (Widedermann *et al.,* 2016). In chickens, it occurs as a result of break in cold chain, stress, mismanagement or suppression of immune system as a result of association with other concurent immune compromising diseases (Bouzoubaa *et al*., 2006). However, in IB, partial failure has been attributed to challenges by more than one serotype, weak immune system, duration between vaccination and challenges of the field virus and incorrect application of vaccine (Jackwood *et al*., 2009).

Although live attenuated vaccines (H120) have reduced economic loss, outbreaks have been reported in several countries which were mostly attributable to infections with strains serologically different from those used for vaccination (Mahmood *et al*., 2011). In Nigeria, it has been insinuated that the most widely used Massachusettes strain H120 vaccines may not protect chickens against local variants (Ducatez *et al.,* 2009), also there is no restriction to the importation of poultry inputs including vaccines and the entry points are Lagos and Ogun states (Obi *et al*., 2008). Ogun state shares boundary with Oyo state whch is also important in poultry production in Nigeria. It is thus possible that IBV detected in the country might be due to the genetic change between the IBV population in the country and IBV introduced as a result of vaccination with live vaccines (Liu *et al*., 2006).

2.9.2 Vaccinal interference

Presence of both maternally derived and short lived IgG does not have adverse effect on efficacy of live vaccine but provides protection against IBV. Maternal antibody has also been found to reduce the severity of vaccinal reaction in chicks and so vaccination of maternally immune chicks is routinely performed without interference in the development of immunity (Rollier *et al*., 2000). It has been established that IBV and NDV do not interfere with each other (Cardosso *et al*., 2005)

2.9.3 Economic importance

Flock management and the strain of virus influence the severity or otherwise of IB. The disease is devitalizing in chicks resulting in poor feed conversion and hence poor weight gains (Ignjatovic and Saparts, 2000).Losses from production inefficiencies are more than mortality in layers or breeders, IB causes loss of egg quality and quantity and egg production may drop down to 10-50%.Nephrogenic strains cause mortality of up to 30% in susceptible flocks (Meulemans *et al.,* 2001). High cost of vaccine production, ambiguous attenuation mechanism and also the inability of vaccines to protect against all serotypes complicate control of the disease and cause huge financial loss. The economic loss is further increased by cost of disease control and implementing biosecurity measures(Custura *et al*.,2012). In South Africa, the estimated loss per flock to infectious bronchitis was 10% or 20% of market value (Perdue and seal.,2000).In Brazil, a total loss of US$3,567.4 and US$4,210.8 per 1000 birds at 25-26 and 42 weeks respectively has been reported in breeders while an estimate loss of US $266.3 per 1,000 birds was reported in broilers at 48day old (Colvero *et al*., 2015). In United Kingdom, the cost of losses to infectious bronchitis virus was estimated as £23 million per year and every 10% reduction in infectious bronchitis will worth £654 million globally.In Western Canada, a drop of 46.6% in egg production was reported in 10 days in a poultry farm of a stock of 8,000 birds. The financial loss was $6,823 at the rate of $2.15/dozen for the period (Amarasinge *et al*., 2018) and IB was said to cost US government millions of dollars annually (Jackwood, 2009).

2.9.4 Field experience/awareness of farmers and veterinarians on infectious bronchitis.

Poultry business is capital intensive and risky, the risk could be spontaneous and devastating especially when it involves disease outbreak that could wipe out the whole flock (Abimbola *et al*., 2013). Thus, the advancement of infectious diseases among livestock unfavorably affect health and welfare of animals as well as farmers' economy.This understanding by farmers makes them to sacrifice their potential income to avoid the risk resulting in reluctance to increase their stock and so, most poultry farmers still operate at low level of production (Aboki *et al.*, 2013). Farmers face many challenges which are; the scarcity of day-old chicks, lack of quality feeds, and sometimes non availability of the feed ingredients especially grains. They also encounter ineffective and costly veterinary services, unavailability of drugs, vaccines and also finance for expansion programmes (Ayinde *et al.,* 2012). However, the main challenge is non- availability of credit facilities for the purchase of poultry inputs which leads to compromise even in strict adherence to biosecurity measures because farmers like other entrepreneur want to maximise profit and enjoy great output in relation to input. Most poultry diseases are as a result of compromise in compliance with biosecurity measures and include issues that are related to the environment such as substandard hygenic conditions, crowdedness of chicks or adulterated water and unhealthy throwing away of waste (Moses *et al*., 2017). Diseases, especially infectious diseases of viral origin like infectious bronchitis, constitute a major threat to poultry growth in Nigeria due to unquantifiable financial loss (Mshella *et al*., 2016). These losses are from mortality, morbidity, reduced production efficiency, low meat yield and quality and extra veterinary costs that will reduce or eliminate returns (Bunnet, 2003; Bunnet and Ijpelaar, 2005). One of the sources of avian diseases is the interplay of between poultry and other animals, especially wild birds and it is commonly promoted by free range method of production (Paul *et al*., 2011).It has been established that migratory birds harbor IBVand spread it to domestic chickens. Also diseases could be spread through transportation of poultry especially in live bird markets or working utensils or movement of humans within or between different flocks of birds.It is therefore imperative that diseases of

chickens be perceived and regarded as being important because of its consequence on the healthiness, grade of chickens and obstacle to growing and flourishing poultry industry (Fasina *et al*., 2012). To forestall these negative impacts, management of poultry diseases which includes good hygiene, cleanliness and containtment must be imbibed to prevent huge financial loss.

The responsibility to prevent and control diseases in a farm lies solely on the farmer and it is dependent on his belief on the possibility of prevention and control of diseases. This is divided into three; behavioural belief in which farmer feels that certain actions will lead to improved productivity, normative belief in which a farmer believes others to implement certain actions and lastly control belief, where farmers believe in someone's perceptions of their own capability to perform. Consequently, farmers' decision on implementation of new tactics will depend on attitude and perception towards the specific measure and its efficacy with an adequate awareness and assurance (Racicot *et al*., 2012). Thus, for successful management of diseases, knowledge which is mostly a product of education, experience and sensitisation is important to avoid confusion (Racicot *et al* 2012). Knowledge can also be described as the initial stage of perception which generates attitudes that result in action and it has been reported that most farmers in southwest, Nigeria had post-primary educational level which affect their attitudes towards embracing new methods (Bamiro *et al*., 2013). However, knowledge of any disease depends on the awareness created by government agencies, the media, veterinary agents, poultry associations and friends.It will be recalled that awareness of avian influenza was low until 2006 when an outbreak occurred in a farm in Kaduna and later spread to other states, this necessitated wide publicity on radio, newspapers and television and so on. It has also been reported that married and older poultry farmers with high working experience tend to have a high level of awareness and good attitude towards prevention and control of diseases (Yasha'u *et al*., 2015). It is also important to say that education is influential to the knowledge and prevention of diseases as reported in cases of avian influenza (Musa *et al*., 2013) in which 62.3% of respondents from Bauchi and Gombe were aware of the disease but only 15.5% were aware of its zoonotic implication as most of the respondents were not educated. Also 86.4% were aware in Kaduna but only 38.4% had knowledge of the cause and nature of the disease thus the difference in

the awareness is the public sensitization which was said to be more in urban centers than rural areas (Ameji, 2010)

Nigerian poultry sector is controlled by private farmers with small flock size and the sector gives attention to egg production although some farmers concurently engage in meat production. Most practitioners are veterinarians, retirees and public servants that operate on part time basis (Obi *et al.,* 2008). Thus, most farmers in the southwest are educated and enlightened (Adebayo and Adeola, 2005; Aromolaran *et al*., 2013; Bukunmi and Yusuf, 2015) and are likely to be knowledgeable of diseases encountered in their farms. Although veterinary facilities and surveillance of animal health are weak in the country, It has enough and experienced veterinarians and other animal health workers most of which are into private practice that could render extension services to farmers (Adebayo and Adeola, 2005; Bukunmi and Yusuf, 2015) Thus, since many poultry farmers are enlightened and are open to technical advice from veterinarians, it is likely that reliable information could be obtained from them based on their experience and knowledge of poultry production.

Generally, it is believed that veterinarians and farmers are crucial to animal welfare; disease management and control. Therefore, there must be a good relationship between veterinarians and farmers for successfulmanagement of diseases (Gunn *et al*., 2008; Cresswell *et al*., 2014). Most farmers in the southwest are educated, experienced and so are willing to adopt new innovations from veterinarians who act as both scientific adviser and extension agents. Thus the knowledge of the infectious bronchitis will bring about increasing standards of cleanliness, good hygiene and containment which are important for the control of the disease (Fasina *et al.*,2012) because awareness of IB has not been documented in Nigeria unlike Newcastle disease which was reported to have the highest awareness among poultry farmers (Adene and Oguntade, 2006; Geidam, 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Preamble

The research was designed to proffer solutions to five important questions through five objectives. The first objective was to assess the knowledge of farmers and experience of veterinarians on infectious bronchitis in their farms and on the fields respectively. The second objective was to determine the endemicity of the disease through antibody titer in unvaccinated commercial and local chickens in the three states. The third objective was to determine the prevalence of infectious bronchitis virus in the three states through the detection of the virus in both local and unvaccinated commercial chickens. The fourth objective is to characterise the virus to determine whether the circulated serotypes or strains are unique to Nigeria or are similar to strains from other countries. The fifth objective was to study why oubreaks occurred in vaccinated flocks.

The study was carried out in Lagos, Ogun and Oyo states southwest, Nigeria (figure 3.1) which is the part of the country with highest concentration of commercial chickens. The states were selected being the hub of the poultry business in Nigeria. Lagos being refered to as center of excellence was founded in 1967 and has the most buoyant economy in the country. It lies at latitude of 6.45407˚N and longitude 3.39467˚N. Ogun state was created in 1976 and is refered to as 'Gateway state' because it links other parts of Nigeria to Lagos and indeed West African countries. It lies on latitude 6.9098˚N and longitude 3.2584˚E of the Greenwich meridian. Oyo state was also created in 1976 and is refered to as 'Pacesseter state'.It lies at latitude 8˚00.00N and longitude 4˚.00E. All the three states share boundaries with one another and the Republic of Benin.

3.2.1 Study Design

Questionnaires were designed and distributed to both farmers and poultry health professionals in the study area irrespective of their age, farm size or educational status.

3.2.2 Sample collection

Purposive sampling technique was adopted. Three hundred and sixty questionnaires (Appendix I) were distributed to farmers in Lagos, Ogun and Oyo states irrespective of their years of experience and flock size although two hundred and eighty- four were retrieved and analysed. The questionnaires requested for information like age of farm, flock size and awareness of IB. It also includes experience of infectious outbreak, when the outbreak occurred and confirmation of IB. For the professionals, two hundred questionnaires were distributed to veterinarians (government and private) in the study area while one hundred and seventy-four were retrieved and analysed.

3.2.3 Statistical analysis

Data generated from retrieved questionnaires were analysed with Statistical Package for Social Sciences statistical data editor using descriptive statistics to obtain frequency and percentage.

3.2 Objective 1:Field experience/awarenessof farmers and veterinarians on infectious bronchitisStudy area

Samples were collected from fourteen local government areas (LGAs) and one Local Government Development Area (LCDA). In Lagos State, samples were taken from Odo- Ngunyan, Ikorodu (6.671˚N, 3.5155˚E), Poka (6.6212˚N, 3.9827˚E) and Araga (6.584˚N, 3.983˚E) in Epe local government. Also at Eleko,(6.453056˚N, 3.395833˚E), Ibeju/Lekki local government and Igbogbo in Igbogbo/Bayeku development area (6.6206˚N, 3.5191˚E). In Ogun State, samples were collected from Ade-Odo/Ota (6.6117˚N, 3.0576˚E), Obada, Ewekoro (7.0706˚N, 3.2885˚E), and Oke-Ata, Abeokuta North (7.137˚N, 3.2934˚E), Mowe, Obafemi/Owode (6.8082˚N, 3.4357˚E) and Ijebu North East (6.8827˚N, 4.0083˚E) local government areas. In Oyo state, samples were collected from Lagelu (7.484˚N, 4.049˚E), Ona- Ara (7.2689˚N, 4.049˚E) and Egbeda (6.5916˚N, 3.2911˚E), Ibadan North (7.4102˚N, 3.9165˚) and Akinyele (7.5503˚N, 3.947˚E) local government areas.

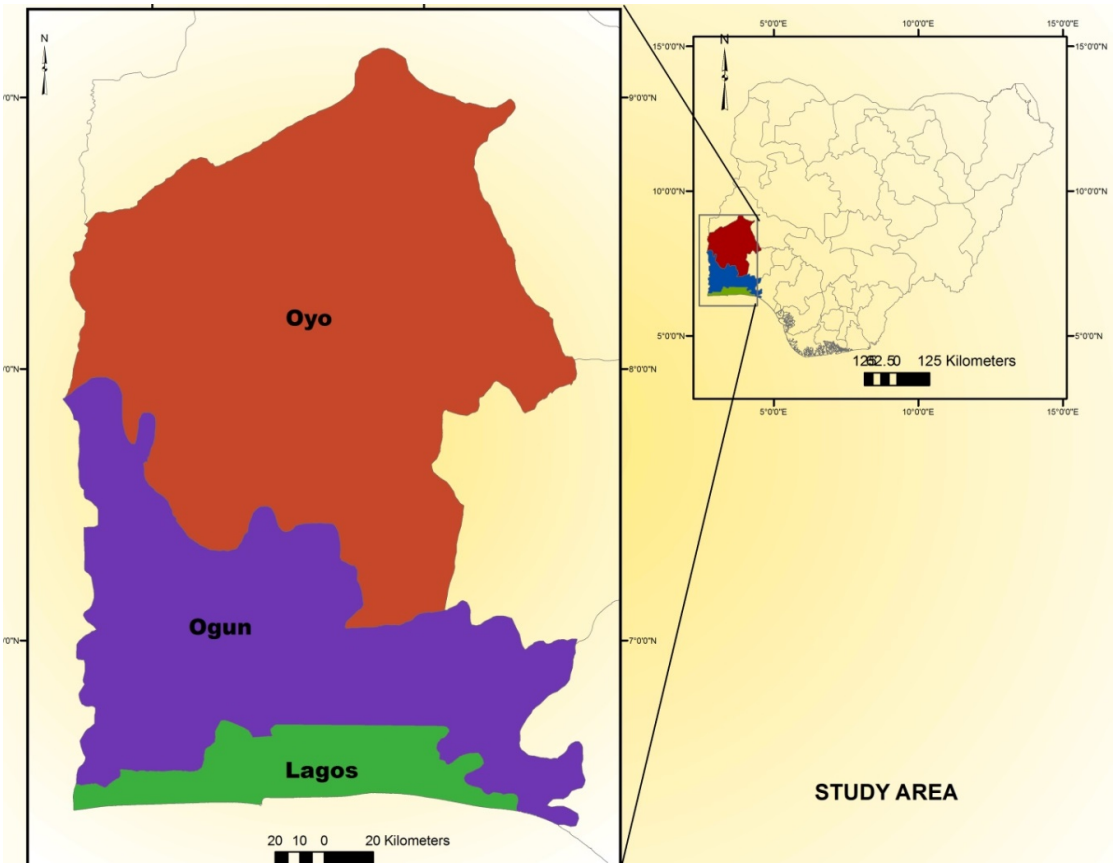


Figure 3.1: **Map of the study area**

3.3 Objective 2: Seroprevalence of infectious bronchitis virus in Lagos, Ogun and Oyo States

3.3.2 Materials and Reagents

Infectious Bronchitis Virus Antibody Kit was purchased from Affinitech, LTD, in USA. The kit measured IgG in the serum and it detected total antibody response to IBV.

The following reagents were provided:

Antigen well of 12 × 8 strips that were well coated with IBV, sample diluent (4x) which is a red buffer solution with protein stabilisers and wash solution marked 20x which is an opaque solution. Also supplied are positive and negative for use, conjugate which is a green solution of α – chicken IgG alkaline phosphatase, substrate that is clear solution of P- Nitrophenylphosphate and stop solution that is a clear solution containing 3.0M Naoh

Materials used were precision pipets for dispensing 2, 100 and 800µl, multichannel pipet for dispensing up to 100µl and timer. Graduated cylinders, distilled water, plate washing apparatus and dilution tubes. Elisa plate reader which is also referred to the microplate reader

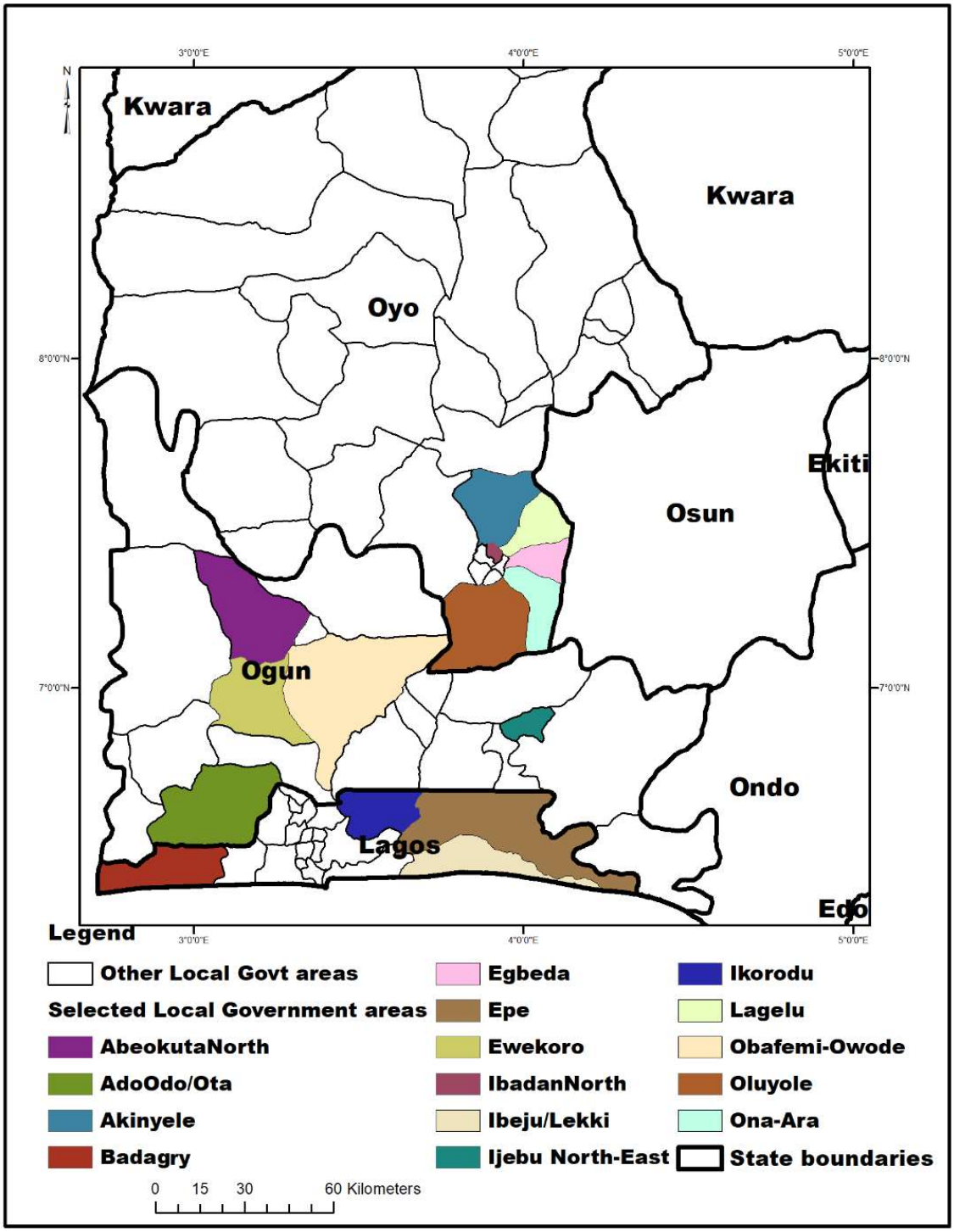


Figure 3.3.1: Map of the local governments of the study area

3.3.3 Sampling technique

Purposive sampling technique was used whereby fifteen commercial farms with unvaccinated flocks against infectious bronchitis were randomly chosen in each of the three states. Twenty local chickens were randomly chosen in five locations in each of the states. The chickens were aseptically bled through the jugular vein using 21G needles and 5 ml syringes. The blood was allowed to clot and serum was then carefully separated into eppendorf tube, optimal sample size was calculated using Cannon and Roe formula of 1982:

$n = (1 – (1 – c)\ 1/Dsens)\ N - 0.5(D – 1)$

where n is the required number of samples, C the desired certainty of detecting infectionin infected flocks (1 is 100% certainty), sens is the estimation of test sensitivity (1 is 100%) in that situation, D the prevalence within sampled animals × N and N is the flock size.

Thus, to calculate the sample size, C is taken to be 0.95%. Prevalence is taken as 25%. Flock is taken as 1000, D = 25/100 X 1000 = 250. Sens = 1.

Substituting the values, sample size was taken as 10 in a flock of 1000.

3.3.4 Determination of Infectious Bronchitis Virus Antibody Titres

ELISA method was used for the assay of IBV antibodies that were harvested. All reagents were mixed thoroughly. Sample diluent (4x) was also mixed 1 part to 3 parts of de-ionized water. Wash solution (20x) was also mixed 1 part with 19 parts of deionized water. The control negative and positive samples, as well as, test samples were shaken to suspend them. Serum samples were diluted in a ratio 1:40 and were mixed very well by pipetting 4 times with 100 µl displacement. 100µl of negative and positive controls were dispensed into duplicate wells of the microtiter plates that were already coated with IBV antigen. This was followed by 100 µl diluted test sera per well and allowed to stand for 30 minutes at room temperature. The plate was then emptied and washed by dispensing 300 µl of wash solution per well. The plate was emptied again and excesses were tapped out on paper towel. The washing process was repeated three times. 100 µl of conjugate was immediately dispensed per well and the plate was allowed to stand for 30 minutes at room temperature. The plate was again washed three times with 30 µl wash solution. 100 µl of substrate solution was immediately dispensed per well and the plate was left for 30 minutes at room temperature, after which 100µl of stop

solution was dispensed per well. The plate was then read with ELISA reader (Els 800 Biote, USA) at 410nm.

3.3.5 Determination/ calculation of result

Average absorbance of the negative control wells was subtracted from the average absorbance value of the positive control and samples.Sample to positive ratio was calculated as follows:

$$\frac{\text{Ave. Abs test sample} - \text{Ave. Abs. Negative}}{\text{Av. Abs Positive} - \text{Av. Abs. Negative.}} = \text{Sample to Positive ratio (S/P)}$$

S/P X (100) = ELISA unit, Positive control value is set as 100 Elisa Unit (EU)
According to the manufacturer, less than 10 EU is negative while greater than 10 EU is positive for antibodies to IBV**.**

3.3.6 Data analysis

Data was analysed with IBM SPSS statistics 21 using descriptive statistics. Seroprevalence was calculated as a percentage of the total number of chickens screened in each LGA and state. Mean ± SEM of IB virus antibody titer was calculated and comparison for significance difference was carried out using Analysis of Variance, Students t test and Least significant difference method of multiple comparison.

3.4 Objective 3: Detection and prevalence of infectious bronchitis virus

3.4.1 Sample collection

Ten each of cloacal and oropharyngeal swabs were obtained from unvaccinated layer chickens (20 to 55 weeks old) from 15 randomly selected poultry farms in Lagos, Ogun and Oyo states. Also, a total of 20 each of cloacal and oropharyngeal swabs were obtained from adult female local chickens in 5 LGAs in each of Lagos, Ogun and Oyo states. The swabs were obtained aseptically and inserted into 50% glycerin solution for the preservation of the virus. For samples from the commercial chickens, 6 best cloacal and 4 best oropharyngeal swabs were selected per farm and pooled as 3 cloacal swabs per pool and 2 oropharyngeal swabs per pool, for molecular studies. Samples from local chickens were similarly pooled per

LGA. A total of 450 of each of cloacal and oropharyngeal swabs were obtained from commercial chickens in forty-five poultry farms and 300 each of cloacal and pharyngeal swabs from local chickens from 15 LGAs in Lagos, Ogun and Oyo states. The pools were a total of 60 and 40 for the commercial and local chickens, respectively.

3.4.2 Laboratory analysis

The laboratory work was done at Institute of immunology, National public health laboratory, 20A rue Augusta Lumiere. L-1950 Luxembourg, Luxembourg.

RNA extraction was done with Qiagen extraction kit (Qiamp viral RNA minikit). The Pre-extraction preparation involved the addition of 0.5 ml of a viral lysis (AVL) buffer to a carrier RNA red tube and mixed properly to re suspend the powder. The solution of AVL and carrier RNA was aliquoted into 1.5ml ependorf at 560µl per tube and were appropriately labeled. The aliquots were then stored at 4˚C.

To prepare wash buffer 1 and 2, 130ml of ethanol was added to 98ml concentration of a wash buffer 1 to obtain 228ml buffer and 160ml of ethanol was added to 66ml concentration of AW2 to obtain 226ml AW2 buffer.

The sample tubes were placed on tissue paper and sprayed with virkon to decontaminate the containers and then labeled. The samples were equilibrated at room temperature, vortexed for 15 sec and then spinned down. The AVL (lysis) buffer aliquots were heated for 5mins at 80˚C in a water bath to remove crystals and then allowed to cool at room temperature before use. Clinical samples (140µl) were added to 560µl of AVL and mixed by pulse vortexing for 15secs. These were later incubated at room temperature for 10mins to lyse the virus and then spinned. Ethanol (560µl) was then added to all the sample solution, vortexed for 15secs and short spinned for few seconds to remove drops from the lids. The mixture (630µl) was then transferred to labeled column and spinned at 8000 rpm. The collection tubes were then discarded and replaced with new ones. The mixture was again added to the labeled column, centrifuged at 8000 rpm and the collection tube was again discarded. Wash buffer (AW1) of 500µl was added and centrifuged at 8000 rpm. The collection tubes were changed and 500 µl of second wash buffer (AW2) was also added and spinned at 3 min at 13000 rpm. The collection tubes were

replaced and spinned for 1 min at 13000 rpm. The columns were then placed on 1.5 ml Eppendorf tubes and 60µl of elution buffer was added to all columns. They were then incubated for 1min at room temperature, spinned at 8000 rpm and the columns were discarded. The extracted RNA samples were stored at (-80˚C). It is important to state that for each extraction, there were one positive and negative control.

The extracted RNA was first reverse-transcribed with random primers and superscript III (Invitrogen) following the manufacturer's instruction adhering to the use of mixes on Table 3.4.1 and 3.4.2. The cDNA was screened for the IBV genome using a highly sensitive nested PCR specific for a constant region of the nucleocapsid protein gene (Akin *et al*., 2001). In a first approach, a region of the S1 gene (approximately 400nt) was amplified from IBV positives in a nested format (Adzhar *et al.,* 1997). The PCR conditions are summarized in Table 5.5 and Table 5.6. All the Polymerase Chain Reactions were performed in 25 ml final volume with 1 U Platinum Taq DNA polymerase per reaction.The equivalent of 0.5 ml of the reaction of the reverse transcription reaction or the first PCR was transferred to a new tube for the first round or the nested reactions, respectively. All programmed cycling was performed in a thermocycler (Mastercycler Gradient; Eppendorf). PCR amplicons 'were analysed in a 1.5% agarose gel (Ultrapure; Invitrogen).

Table 3.4.1: Reverse transcription mixes: Composition of mix 1

Component	**Volume/Sample (µl)**
Primer(RP 0.03/µl(1:100)	5
1.0mM DNTP	1
Sterile distilled H_20	2
Total	8

RNA 5µl was added; denaturing was done at 72˚C, 10 mins and was quickly placed on ice.

Table 3.4.2: Composition of Mix 2

Component	Volume/Sample (µl)
5X first – strand buffer	4
0.1 DTT	1
RNase Out (400U/ml)	1
Superscript 111 (200µl/ml)	1
Total	7

Incubation was done 50˚C for 80 minutes and inactivation of the reaction was done at 70˚C for 15 minutes.

Table 3.4.3: Mixes for IBV first round PCR

Component	Volume/sample (µl)
H_20	17.65
Buffer (10x)	2.5
$Mgcl_2$	0.75
dNTP (10mM)	0.5
SyBR Green (10x)	0
Primer N784 (25µM)	0.5
Primer 1145(25 µM)	0.5
Platinum taq	0.1
Template 1: 5	2.5
Total	25

Table 3.4.4:Mixes for IBV Nested PCR

Component	Volume/sample (µl)
H_20	17.55
Buffer (10x)	2.5
$Mgcl_2$	0.75
dNTP (10mM)	0.5
SyBR Green (10x)	0
Primer N791 (25µM)	0.5
Primer 1129(25µM)	0.5
Platinum taq	0.2
Template 1: 5	2.5
Total	25

Table 3.4 .5: Thermocycler setting for first round Polymerase Chain Reaction

Component	**Temperature (˚C)/Duration**
Heated lid	112
Number of cycles	40 cycles
Initial denaturation	95 30 secs
Annealing temperature	55˚ 30 secs
Elongation	72 1 min
End cycle (elongation)	72 10 mins
Holding temperature	4

Table 3.4.6: Thermocycler setting for Nested Polymerase Chain Reaction

Component	Temperature(˚C)/Duration
Heated lid	112
Number of cycles	40 cycles
Initial denaturation	95 30 secs
Annealing temperature	54 30 secs
Elongation	72 1 min
End cycle (elongation)	72 10 mins
Holding temperature	4

3.4.3 Gel Preparation

Electrophoresis buffer was prepared by adding 2% of TAE (Tris base, Acetic acid and EDTA) buffer solution into a conical flask. Agarose (2g) was then added and the mixture was boiled for few seconds to allow dissolution of agar powder.The diluted solution was then placed on laboratory desk and allowed to cool for some minutes, after which 0.5µg/ml of ethidium bromide was added and mixed thoroughly by gentle swirling. The combs were fixed into gel casting tray while the solution cooled. The warm agarose solution was then poured into the mold to ensure 3-5 mm thickness. The gel was allowed to set for about 40 min, after which small amount of electrophoresis buffer was poured on the top of the gel. Each of the DNA samples was mixed with 0.2µg/ml of the loading dye. The samples were then loaded one after the other with the aid of micropipettes changing tips after each loading. The lid of the gel tank was closed and the electrical leads were attached to power supply so that DNA could migrate towards the anode. The gel was then removed from the gel tray placed under the imager (BIORAD^{R})

3.5 Objective 4: Characterization of infectious bronchitis virus in chickens in Lagos, Ogun and Oyo states.

3.5.1 Two genes of interest were targeted, amplified, sequenced and analysed. These were 1b gene which identifies the family and S1 gene which identifies the serotypes.

3.5.2 Amplification of the 1b gene of infectious bronchitis virus.

The cDNA was amplified in a first round of PCR (forward primer 5'-GGK TGG GAY TAY CCK RTG-3' and reverse primer 5'-TGY TGT SWR CAR AAY TCR TG-3', in 40 cycles at $X^{0}$for 20 secs, 48˚C for 30 secs and 72^{o}C for 50˚CPCRproducts were amplified in a second round PCR under amplification identical to those of the first round PCR, except that a new set of primers was used in the assay (forward primer 5'- GGT TGG GAC TAT CCT AAG TGT GA-3', reverse primer 5'- CCA TCA TCA ATA GAA TCA TCAT-3'.The final products (380bp) were sequenced unidirectionally and analysed.

3.5.3 RT-PCR and nucleotide sequencing for S1 gene.

RNA was extracted from the pooled cloacal and faecal samples with the nucleospin RNA virus package (Macherey-Nagal) according to manufacturer's commands. The reverse transciption-polymerase chain reaction (RT- PCR) used to amplify the

complete S1 with oligonucleotides S1 unit 2^{+} and IBPI^{-} was conducted as previously narrated (Adzhar *et al.*, 1996). In addition to the flanking primers used in the RT-PCR, a combination of eight internal primers to different regions of the S1 gene were designed to completely sequence both strands of the S1 gene of the field strains.The sequencing primers and their location are indicated in the table 5.4 below. The 1800-base pair RT-PCR products were purified by the QIAquick PCR purification kit and Minelute PCR purification kit (Qiagen Inc.) by the QIAGEN Inc,) following the manufacturer's instruction.Purified RT -PCR products were sequenced by the dideoxy-mediated chain termination method using ABI PRISM Big dye Terminator v3.1 cycle sequencing Kit (PE Biosystems) as described by the manufacturer. Sequences were analysed with an automated nucleic acid analyser (ABI PRISM 3100; Avarit PE Biosystem)

Table 3.5.1: Oligonucleotide localization.

OLIGONUCLEOTIDE	SEQUENCES (5' TO 3')	LOCATION
S1PRI+	GTG TTT GTT ACA CAT TG	20692 - 20708
S1PRI-	CAA TGT GTA ACA AAC AC	20692 – 20708
S1PR2+	TGG CTT ATT TTG TTA ATG GTA C	20987 – 21005
S1PR2-	GTA CCA TTA ACA AAA TAA GCC A	20987 - 21005
SIPR4+	GGT TGT AAG CAA TCT GT	21436 – 21452
S1PR4-	ACA GAT TGC TTA CAA CC	21436 - 21452
S1PR5-	TGT CTA TGG CAC CAG ATG TAT CTA	21764 – 21787
S1PR6+	CCA TAG ACA TCT TCG TTG TAC	21779 – 21799

(Bournell *et., al* 1987).

3.5.5 Nucleotide and Amino Acid Deduced Sequence Analysis

Assembly and analysis of sequence data were conducted using BioEdit 5.0 package. Nucleotide and amino acid deduced sequences were aligned using cluster W software. Translation of DNA nucleotide sequences to protein before alignment using EXPASY' translate tool (*http://web.expasy.org*) was done. IBV sequences used for comparison in this study were from GenBank and were available from the National Centre for Biotechnology Information (www.ncbi.nlm.nih.gov)

3.5.6 Guanine - Cytosine content

It is calculated as a percentage value of nitrogenous bases on a DNA or RNA molecule that are either guanine or cytosine and sometimes called G + C ratio or GC ratio. It is calculated as G+C/ (A+T+G+C). It was done using on-line calculator *www.endmemo.com* where the sequence was placed and then read.

3.5.7 Sequence Identity and Similarity (SIAS)

This was done on line using Immunomedicine group tool: *imed.med.ucm.cs*.It was used to calculate pairwise sequence identity and similarity from multiple sequence alignment.

3.5.8 Phylogenetic Analysis of 1b and S1

This was done to determine the relatedness of the isolates and it was done by performing multiple nucleotide alignment on the gene representative viruses using clusterW on MEGA 6. The phylogenetic tree was constructed with Mega 6 software using neighbor joining method and each tree was produced using a consensus of 1000 bootstrap replicates (Tamura *et al*., 2011).

3.6 Objective 5: Detection and molecular characterisation of infectious bronchitis virus in mortalities from vaccinated flocks showing respiratory signs

3.6.1 Study Location and Collection of Samples.

Samples were collected from two veterinary diagnostic institutions located in Ogun state, southwest Nigeria. These veterinary diagnostic centers render services to farmers in the three states under study. Samples from congested lung tissues, kidney tissues, and tracheal tissues as well as cloaca swabs were collected from chicken carcasses submitted for post mortem examination from vaccinated flocks which had history of respiratory distress, fall in egg production and mortality between January and March 2017. The samples were collected aseptically into transporting media containing 50% glycerin for tissue preservation.

Survey of available IBV vaccines was also done by visiting ten veterinary shops per state and collecting information on them.

3.6.2 Laboratory analysis

The following laboratory activities were carried out at Department of Veterinary Medicine, University of Ibadan, Oyo State.

3.6.3 RNA Extraction

RNA extraction was carried out using Quick RNA Mini Prep Kit (Zymo Research, Irvine, USA) according to manufacturer's instruction. The positive and negative controls were IB vaccine and RNase free water, respectively.

Content of **RNA** Quick-RNA^{TM}MiniPrep were: RNA lysis buffer, RNA Prep buffer, RNA Wash Buffer (concentrate), RNase/DNase Free water ,DNase I^{2} (lyophilized) DNA digestion buffer, Spin away filters, Zymo-SpinIIICG column and collection tubes

Prior to RNA extraction, 250 µl of beta-mercaptoethanol was added to 50ml of Viral RNA buffer to obtain a final dilution of 0.5% and 96ml of 100% ethanol was added to 24 ml of Viral wash buffer concentrate.

Samples were homogenized mechanically using pestle and mortar. The lysis buffer was added to the homogenized samples in a ratio of 1:1.

One volume of ethanol (100%) was added to the sample in RNA lysis buffer (1:1) and was mixed well. The mixture was transferred to a Zymo–Spin IIICG Column in a collection tube and centrifuged at 16,000g for 30s. The flow through was discarded and the column was prewashed with 400µl RNA wash buffer, centrifuged for 30 min and flow through was also discarded. DNase 1 reaction (80 µl) Mix was added to the column matrix, incubated at room temperature for 14mins and centrifuged for15mins.Wash buffer 400µl, 700 µl and 700 µl wash buffer was added to the sample consecutively, centrifuged for 30 second for the first two steps and for 2mins for the last step. The column was then transferred carefully into free RNase tube water directly to the column and centrifuged for 30 secs. The eluted RNA was immediately stored at -70˚C

3.6.4 Reverse Transcription Polymerase Chain Reaction

Virus detection was carried out using using One Taq–Step RT-PCR. Primers used IBV 5'- AAT TTT GGT GAT GAC AAG ATG A -3'(forward) and IBV 5' CAT TGT TCC TCT CCT CAT CTG -3'(reverse) as designed by Akin *et al*.,2001.The amplification kit was obtained from New England Biolabs inc. and used following manufacturer's instructions.

Contents of One Taq – Step RT –PCR were**:** Nuclease free water**,** One Taq One-Step Enzyme mix**,** One Taq One step Reaction mix**,** Quick load ^{R} One Taq One step reaction

Total RNA, Gene–specific,One taq one step reaction and nuclease free water were mixed together to make up 46µl. It was then denatured for 5mins at 65^{o}C in a water bath and was promptly put on ice. 2µl each of One Taq One – step enzyme mix (25x) and Gene – specific forward primer (10µM) were then added to the tube making a total volume of 50 µl. The tubes were the placed in a thermocycler which was set to run Reverse Transcription at 48˚C for 15 mins for I cycle, initial denaturation at 94˚Cfor 1min. Denaturation. Annealing and extension were set and run at 94˚C,50˚C and 68˚C for15 sec, 30 sec and 1min respectively to run for 35cycles. The final extension was at 68˚C for 5 mins and was held at 4˚C. At the end of the programme, amplicons were obtained ready to be loaded on the gel.

Gel preparation

Electrophoresis buffer was prepared by adding 2ml of TAE stock solution into a conical flask and 98ml of distilled water. Agarose solution was then prepared by

adding 2g of agarose to 100ml of 1xTAE buffer. The solution was then boiled for few seconds to allow dissolution of agar powder. After agar powder had dissolved, it was placed on laboratory desk and allowed to cool for some minutes. Ethidium bromide (0.5 µg/ml) was added and mixed thoroughly by gentle swirling. The combs were fixed into the gel casting tray while the solution was cooling. The warm agarose solution was then poured into the mold to ensure 3 -5mm thickness. The gel was allowed to set for about 40mins and small amount of electrophoresis buffer was then poured on the gel. Each of the DNA samples was mixed with 0.2µl of the loading dye. The samples were then loaded one after the other with the aid of micropipettes changing tips after each loading. The lid of the gel tank was closed and the electrical leads were attached to power supply so that DNA could migrate towards the anode. The gel was then removed and placed under imager of Bio-Rad Gel Doc (^{TM})XR + with image Lab (™) Software.

Five positive samples were successfully sequenced at Cornell University. The nucleotide sequences detected in the three states were compared with deposited sequences available at the Gen Bank database using Blast search via the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/ BLAST/) and also compared with sequences of H120 vaccine strains as well as with somesequences from other countries stored in the GenBank.

Multiple alignment of five Nigerian nucleotide and amino acid sequences were carried out including those IBV sequences retrieved from GenBank;AY790350.1.AIBV(South Korea),KF826880.1.AIBV(ventrivaccine,India),AY028296.1.AIBVH120,(China) AY856349.1.AIBV/IBN(China)KM658222.1IBV(Argentina),EF213578.1.IBV/CK/CH/LSD1051(China),FJ588732(Israel), AF352310(H52), FN1882280(Nigeria), FJ589733(Israel) and IBD gene as the outgroup were using CLC Workbench 8.

Phylogenetic analysis was carried out to know the relatedness of the isolates using MEGA 7 soft ware after multiple alignments with CLC Workbench 8. Maximum likelihood was used for phylogenetic analysis.

CHAPTER FOUR

RESULTS

4.1 Field Experience/Awareness of Farmers and Veterinarians on Infectious Bronchitis

There were 83, 105 and 96 respondents (poultry farmers) from Lagos, Ogun and Oyo states, respectively. Their demographic information is presented in Table 4.1.1 In Lagos, Ogun and Oyo states, 73.5%, 78.1% and 77.1% of respondents were males, 68.1%, 76.1% and 72.9% were married and 65.2%, 65.7% and 69.8% had tertiary education, respectively. Table 4.1.2 shows farming experience and awareness of respondents. In Ogun and Oyo states, 51.4% and 32.3%, respectively, of respondents had been in poultry farming for over 10 years, while 51.8% of those in Lagos State had only 1-5 years' experience. While 51.8% had two chicken flocks on their farms in Lagos State, 52.4% and 81.3% had only one flock in Ogun and Oyo states, respectively. Flock sizes ranging from 1,000-5,000 are 62.7%, 63.8% and 62.5% in Lagos, Ogun and Oyo states, respectively. While 24.8 – 28.1% of respondents were aware of infectious bronchitis in the three states, only 19.0 – 24.0% of respondents vaccinated their flocks against IB and 10.4 – 19.0% had actually experienced outbreaks. Out of those that have experienced outbreaks 55.6%, 70.0% and 70.0% confirmed the outbreaks using laboratory means in Lagos, Ogun and Oyo states, respectively. Most outbreaks occurred at 1-3 week-old (30.0%) in Oyo State, at 7-8 week-old in Lagos (66.7%) and at 19 week-old and above in Ogun State (50.0%). The duration of outbreak is mostly 3-4weeks old (77.8%) in Lagos, 1-2 weeks (60%) in both Ogun and Oyo states.

Table 4.1.1: Demographic Information of Farmers in Lagos, Ogun and Oyo states

	LAGOS		**OGUN**		**OYO**	
SEX		%		%		%
Male	60	72.2	82	78.1	74	77.1
Female	23	27.8	23	21.9	22	22.1
MARITAL STATUS						
Single	21	25.3	18	17.1	15	15.6
Married	56	67.5	80	76.2	70	72.9
Widowed	6	7.20	7	6.7	11	11.5
EDUCATIONAL STAGE						
None	5	6.0	8	7.6	13	13.5
1˚	9	10.8	13	12.4	18	18.8
2˚	20	24.1	19	18.1	11	11.4
3˚	49	59.0	65	61.9	54	56.3

1˚ - primary, 2˚ - secondary and 3˚ - tertiary

Poultry business is dominated by males as shown in Table 4.1.1., 72.1%,78.1% and 77.1% of poulry farmers intervied were male probably because the work is demanding and tasking. Most of them are married and had post primaryeducation. Most farmers are backyard farmers with a flock of between 1,000 and 5,000 chickens. It is largely dominated by retirees, civil servants and veterinarians. As per the experience and awareness, most farmers had been on the business for more than five years and so they would be able to give reliable information of the disease. However, there is low awareness of the disease.

As per the veterinarians, there were 56, 64 and 70 veterinarian respondents in Lagos, Ogun and Oyo states, respectively. Out of these numbers, 39.3% and 50.0% from Lagos and Ogun states, respectively, have ≥16 years' experience, while 34.3% of those from Oyo State have <5 years' experience in practice (Fig 4.1.1). Most of them i.e. 55.4%, 57.8% and 57.1% in Lagos, Ogun and Oyo states, respectively, do not consult for poultry farms. Out of those that undertake farm consultations, 76.0 – 83.3% consulted for 1-5 farms while each of the remaining handle more farms (Fig 4.1.5). Also, 55.6 – 72.0% of them advised farmers to vaccinate against IB (Fig 4.1.4) and 28 -37% have encountered suspected cases with most veterinarian recording 1 – 5 cases (Fig 4.1.2). While 70% of the cases encountered in Ogun State were confirmed, only 43% and 33.3% were confirmed in Lagos and Oyo states, respectively.

Table 4.1.2: Farming experience and awareness of Infectious bronchitis in chickens in Lagos, Ogun and Oyo states

LOCATION	**LAGOS**		**OGUN**		**OYO**	
EXPERIENCE	NO.	%	NO.	%	NO.	%
< 1yr	5	6.02	5	4.8	11	11.5
1-5yrs	43	51.8	17	16.2	26	27.1
6-10yrs	12	14.5	29	27.6	28	29.2
>10yrs	23	27.8	54	51.4	31	32.3
FLOCK NO						
One	35	42.2	55	52.4	78	81.3
Two	43	51.8	38	36.2	15	15.6
Three	5	6.0	8	7.6	3	3.1
Four	0	0	4	3.8	0	0
FLOCK SIZE						
<1000	21	25.3	22	21.0	23	23.9
1000 & 5,000	52	62.7	67	63.8	60	62.5
6,000 & 10,000	9	10.8	3	2.8	10	10.4
>10,000	1	1.2	13	12.4	3	3.1
AWARENESS	23	27.7	26	24.8	27	28.1
IB OUTBREAK	9	10.8	20	19.0	10	10.4
IB VACCINATION	19	22.9	20	19.0	23	24
DURATION OF OUTBREAK						
1-2weeks	1	11.1	12	60	6	60
3-4weeks	7	77.8	7	35	3	30
5-6weeks	1	11.1	1	5	1	10
CONFIRMATION OF THE DISEASE						
Yes	5	55.6	14	70	7	70
No	4	44.4	6	30	3	30
AGE OFOUTBREAK						
1-3 weeks	0	0	0	0	3	30
4-6 weeks	2	22.2	8	40	2	20
7-8 weeks	6	66.7	2	10	3	30
19 & above	1	11.1	10	50	2	20

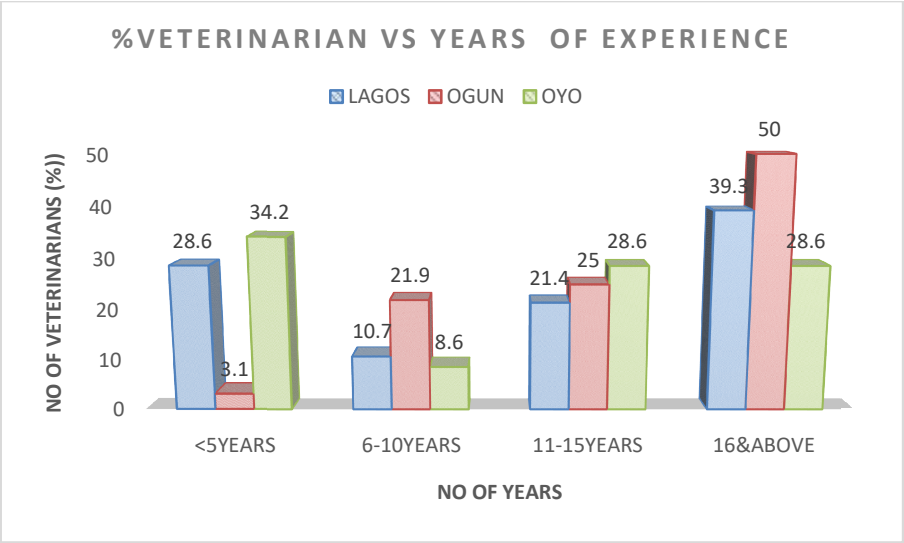


Fig 4.1.1: The number of veterinarian and their years of experience on the field in Lagos, Ogun and Oyo states**.**

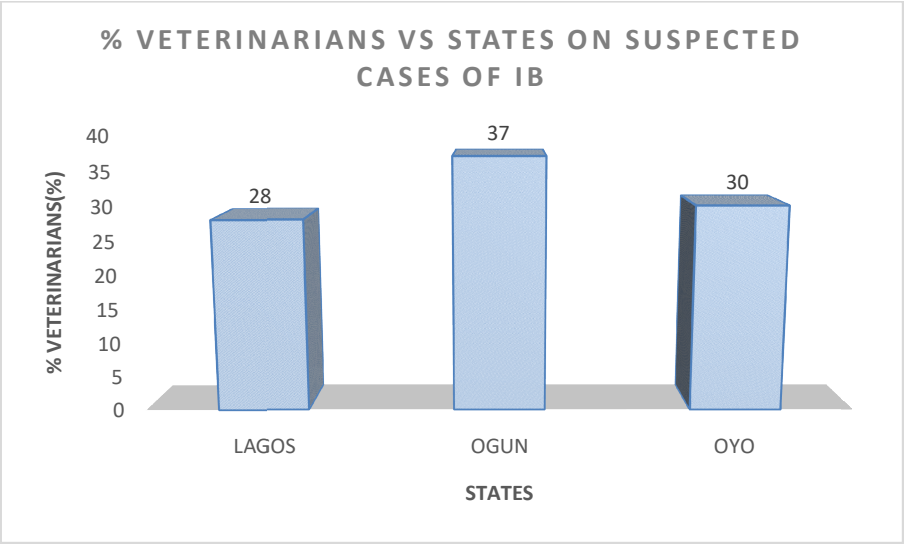


Figure 4.1.2: Percentage veterinarians versus States on IB suspicion

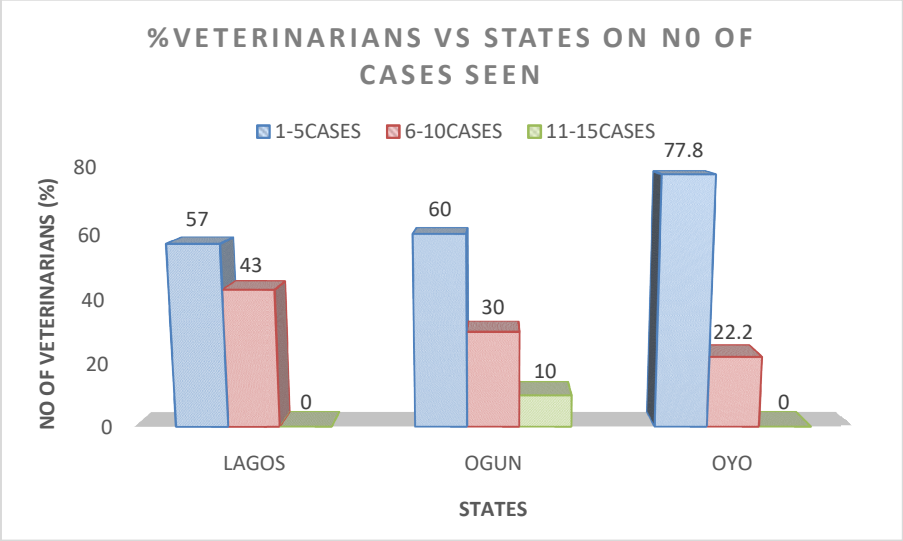


Figure 4.1.3: percentage veterinariansVersus States on number of cases.

Most of the veterinarians interviewed were very experienced with 39.3%,50% and 28.6% having been practising for more than sixteen years. However, their encounter on the field showed that very few cases of IBV were experienced on the field although most of them have seen more than five cases.

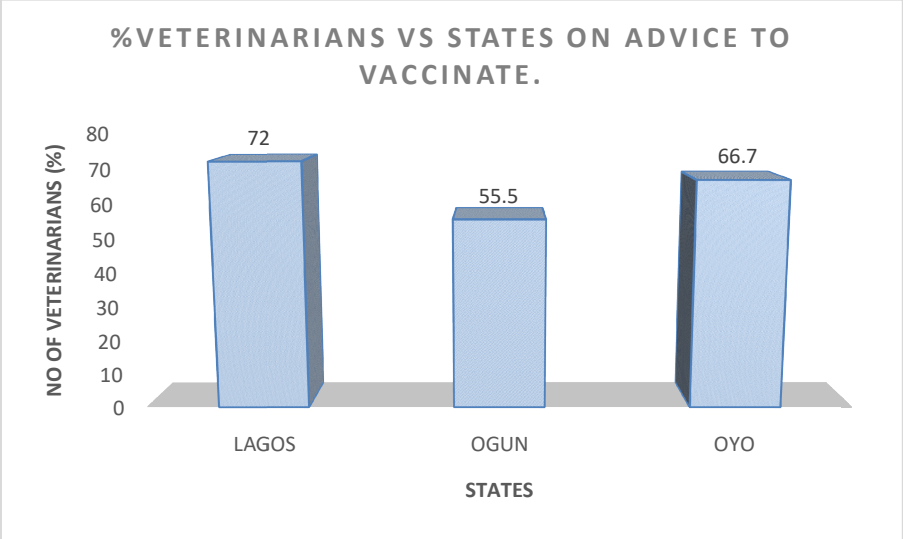


Figure 4.1.4: Percentage veterinarians versus states on advice to vaccinate against Infectious bronchitis

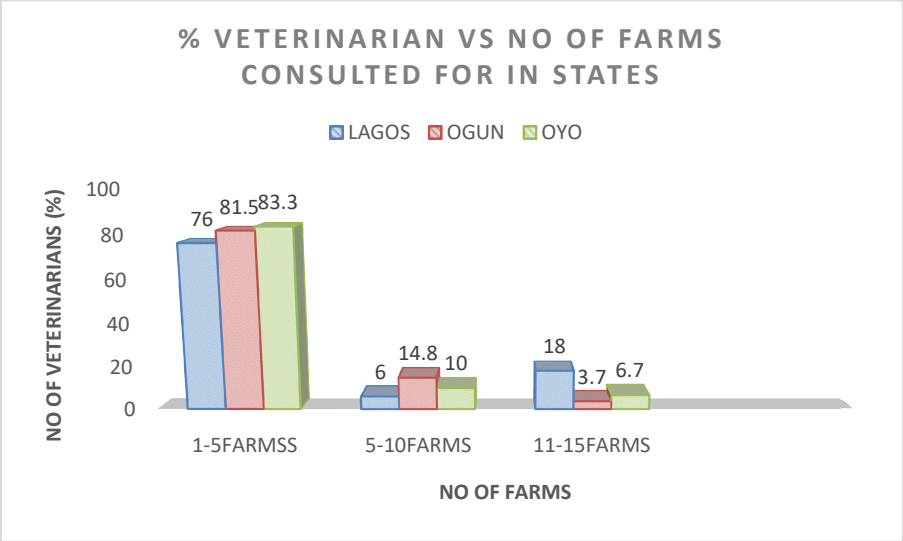


Figure 4.1.5: Percentage veterinarians versus states on number of farms consulted for

Fig. 4.1.3 to 4.1.5 showed that most veterinarians interviewed consulted for at least 5 farms and they advised their clients to vaccinate their chicken against IB. This implies the possibility of the spread of the disease from one farm to another especially through fomites and also the emergence of variant of the virus.

4.2 Seroprevalence of infectious bronchitis virus in Lagos, Ogun and Oyo states

In Lagos state, most farms sampled had 100% seroprevalence rate except in Badagry local Government area where it varied between 20 and 80% prevalence (Appendix 111). High prevalence was also recorded in local chickens varying between 35% and 80% (Table 4.2.1) .In Ogun state; the prevalence is also high between 60% and 100% in commercial(Appendix IV) and 75% and 90% in local chickens respectively (Table 4.1.2). As regards Oyo state, the prevalence is also high with some farms having 100% prevalence but there were farms with 0% and 10% prevalence (Appendix V). The prevalence within local government in each of the states followed the same trend discussed above. The prevalence in each state was 83.3%, 88% and 76% for commercial chickens and 70%, 85% and 82% (Tables 4.2.1 to 4.2.3) in local chickens for Lagos, Ogun and Oyo states respectively. Overall prevalence for the commercial and local chickens was 82.4% and 79% respectively while for both local and commercial was 81% (fig. 4.2.1)

Table 4.2.1:Seroprevalence of Infectious Bronchitis Virus Antibodies in Commercial and Local Chickens in Lagos State.

Local Government Area	Commercial		Local	
	No. Positive/Total Sample	**% Positive**	**No. Positive/Total Sample**	**% Positive**
Ikorodu	30/30	100	16/20	80
Igbogbo/Bayeku	29/30	96.6	16/20	80
Epe	30/30	100	16/20	80
Ibeju/Lekki	24/30	80	15/20	75
Badagry	12/30	40	7/20	35
TOTAL	125/150	**83.3**	70/100	**70**

Table 4.2.2: Seroprevalence of Infectious Bronchitis Virus Antibodies in Commercial and Local Chickens in Ogun state.

Local Government Area	Commercial Chickens		Local Chickens	
	No. Positive/Total Sample	**% Positive**	**No. Positive/Total Sample**	**% Positive**
Ade Odo/Ota	30/30	100	18/20	90
Ewekoro	25/30	83.3	18/20	90
Abeokuta North	24/30	80	18/20	90
Obafemi/Owode	23/30	76.7	14/20	75
Ijebu North East	30/30	100	17/20	85
Total	132/150	**88**	85/100	**85**

Table 4.2.3: Seroprevalence of Infectious Bronchitis in Commercial and Local Chickens based on Local Government in Oyo state

Local Government Area	Commercial Chickens		Local Chickens	
	No. Positive/Total Sample	**% Positive**	**No. Positive/Total Sample**	**% Positive**
Egbeda	17/30	56.7	11/20	55
Ibadan North	18/30	60	19/20	95
Akinyele	28/30	93.3	20/20	100
Lagelu	22/30	73.3	14/20	70
Ona- Ara	29/30	96.6	18/20	90
Total	114/150	**76**	82/100	**82**

Generally, there is high antibody titre against the virus in Lagos although it is lowest in Badagry probably because it had least concentration of chickens. It is note worthly that the antibody titre in commercial chicken is higher than in local chicken unlike in Ogun State where there is little difference (88% in commercial and 85% in Local chicken).However in Oyo State the antibody titre in local chicken is higher than in commercial chicken (76% in commercial and 82% in local chicken).

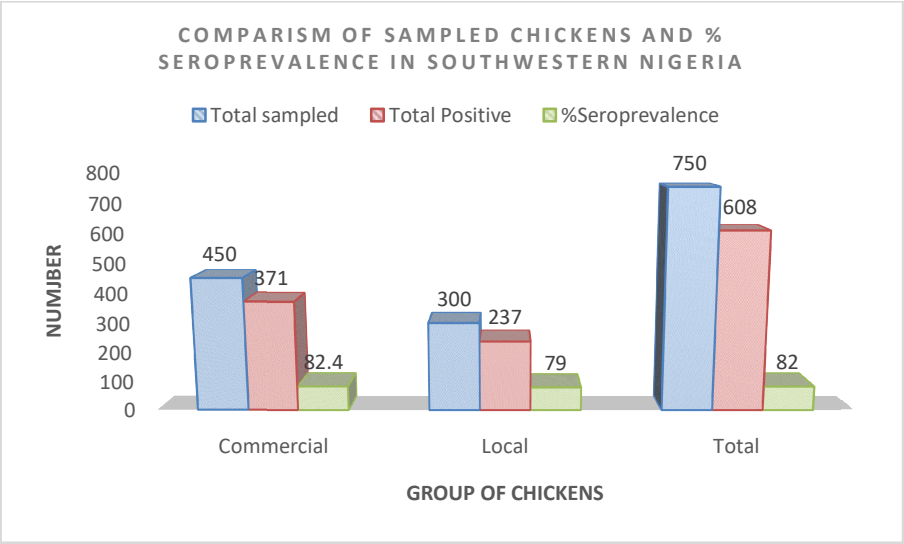


Figure 4.2.1: Seroprevalence of Infectious Bronchitis virus in commercial and local chickens in Southwestern Nigeria

4.2.1 Distribution of Infectious Bronchitis Virus Antibody titers in Chickens in study area

Mean infectious bronchitis virus antibody titers obtained for Lagos, Ogun and Oyo states i.e.39.73 ± 1.87, 44.44 ± 2.15 and 38.69 ±2.94 were not significantly different. However, mean antibody titer in commercial chickens in Lagos and Oyo states (49.74 ±2.50 and 43.25 ± 4.64, respectively) were significantly higher ($p<0.05$) than those of local chickens (24.71 ±2.02 and 31.85 ±2.24, respectively) as presented on Figure 4.2.2. With regards to age, result showed that chickens in age groups 21-30 weeks-old and 51-60 weeks-old had significantly higher ($p<0.05$) mean antibody titers i.e. 53.00 ±6.42 and 57.88 ± 5.36, respectively, than other age groups (Figure 4.2.3). Also, flocks with 4,000 or more chickens generally had significantly higher ($p<0.05$) antibody titers than those will smaller number of chickens (Figure 4.2.4). In addition, a significant correlation ($p<0.001$) was found between type of chicken and IB virus antibody titer.

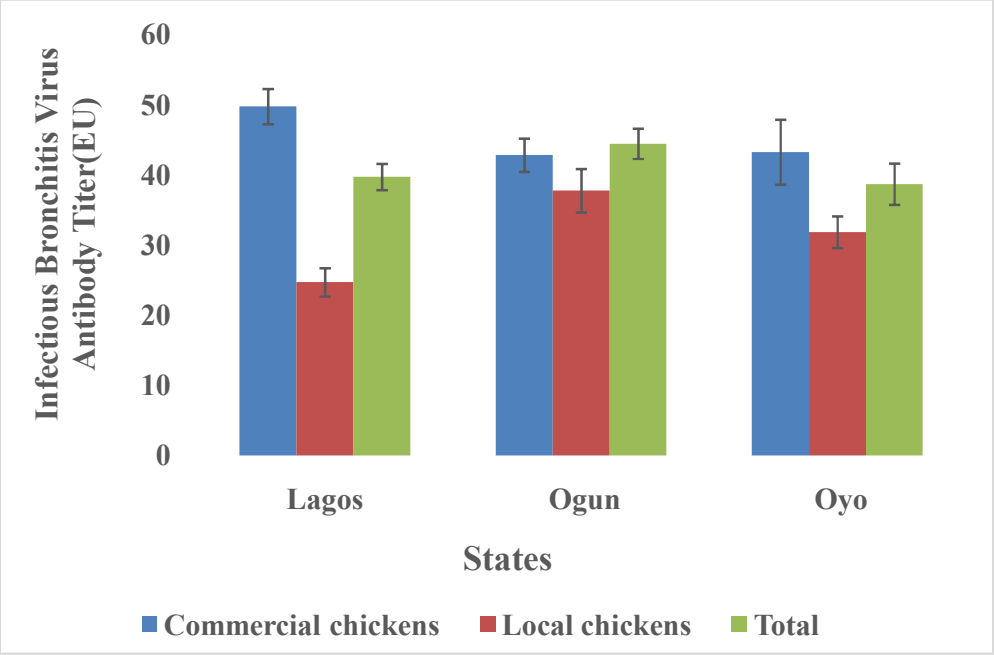


Figure 4.2.2: Mean ± SEM of infectious bronchitis virus antibody titers (ELISA Units) in commercial and local chickens in Lagos, Ogun and Oyo states

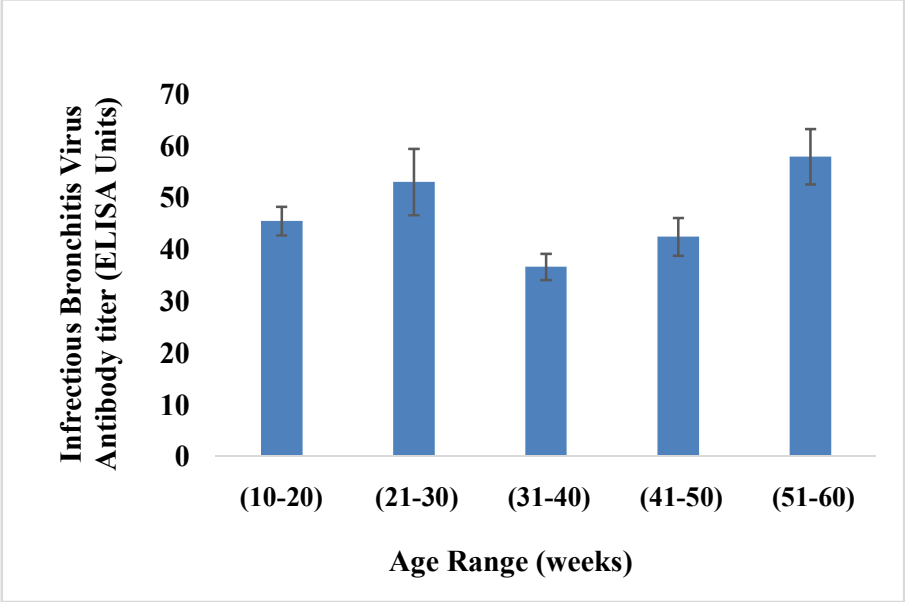


Figure 4.2.3: Mean ± SEM of infectious bronchitis virus antibody titers (ELISA Units) in different age groups of commercial chickens in Lagos, Ogun and Oyo states

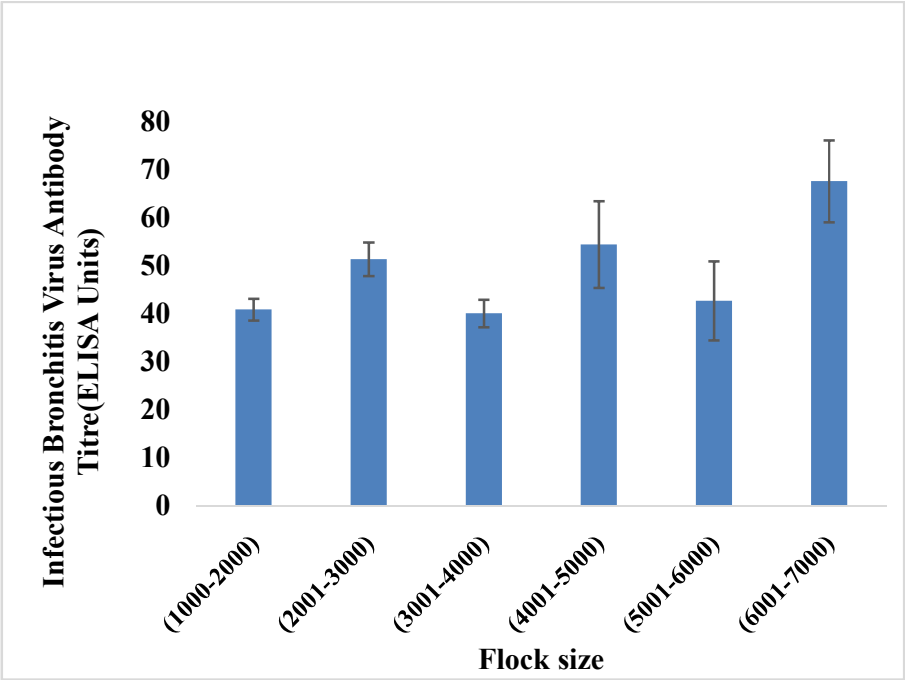


Figure 4.2.4: Mean ± SEM of infectious bronchitis virus antibody titers (ELISA Units) in different flock sizes of commercial chickens in Lagos, Ogun and Oyo states.

4.3 Molecular Detection and Prevalence of Infectious Bronchitis Virus

The amplification of an expected band (380bp) from positive control as well as IBV positive swab samples indicates that the RT-PCR was performed correctly (Figure 4.3.2). Few positives were observed with the first round RT- PCR (Figure 4.3.1) with more expected bands observed after specific Nested PCR were performed on RT-PCR positive samples (Figure 4.3.3).Thirty – two pooled samples were positive out of three hundred pooled samples that were subjected to molecular analysis. In Lagos state, five (5) pooled samples out of sixty (60) pooled samples from commercial chickens were positive while none from local sample was positive for IBV. In Ogun, twenty (20) pooled samples out of sixty (60) pooled samples from commercial and none from local sample was positive. In Oyo state, two (2) pooled samples out of sixty (60) pooled samples from commercial chickens were positive and five (5) pooled samples out of forty (40) from local chickens were positive (Table 5.7).Among the Local government areas of study, Ijebu North East had 21.9%, Obafemi Owode and Ade – Odo/Ota each, 15.3% Ikorodu had 12.5% of the virus detection. Ewekoro had 6.3% while Igbogbo/Bayeku, Abeokuta North and Egbeda had 3.1% of the samples with Infectious bronchitis virus detected. Akinyele and Ibadan North had 12.5 and 3.1% from local chickens. The prevalence in Lagos, Ogun and Oyo states were 8.3%, 33.3% and 3.3% respectively, in commercial chickens while 12.5% prevalence was observed in local chickens in Oyo state. Overall prevalence was 10.7% with more positives obtained in cloaca than oropharyngeal samples.

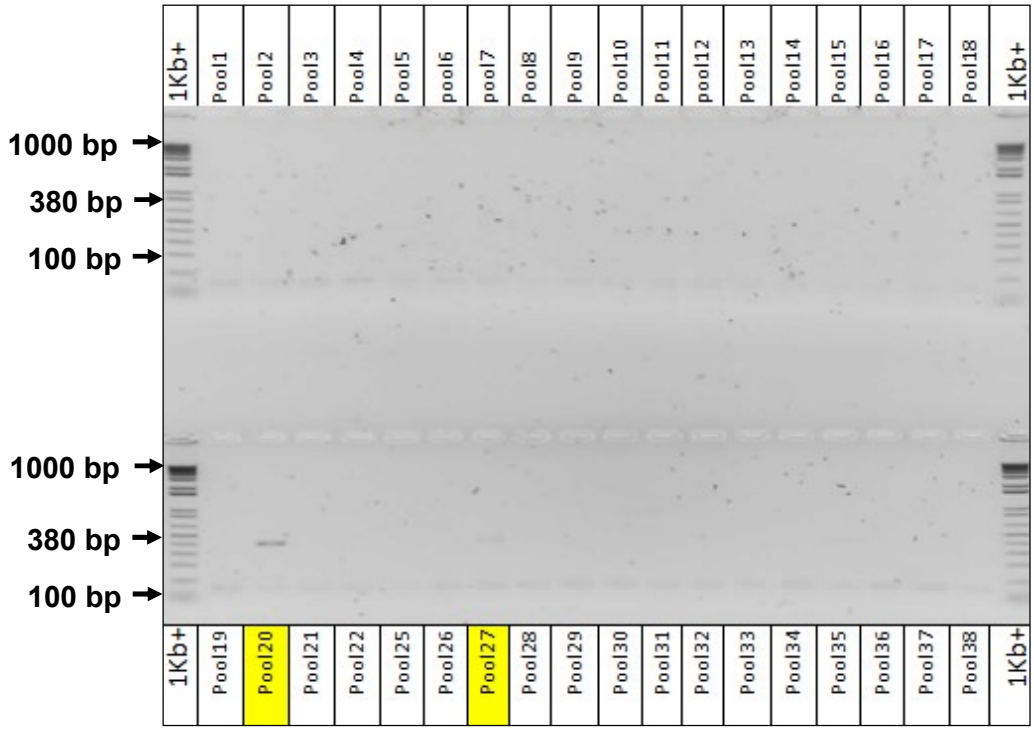


Figure 4.3.1: Agarose gel electrophoresis of 380 bp of IBV genes, weak positives of pool 20 and pool 27 after first round RT- PCR

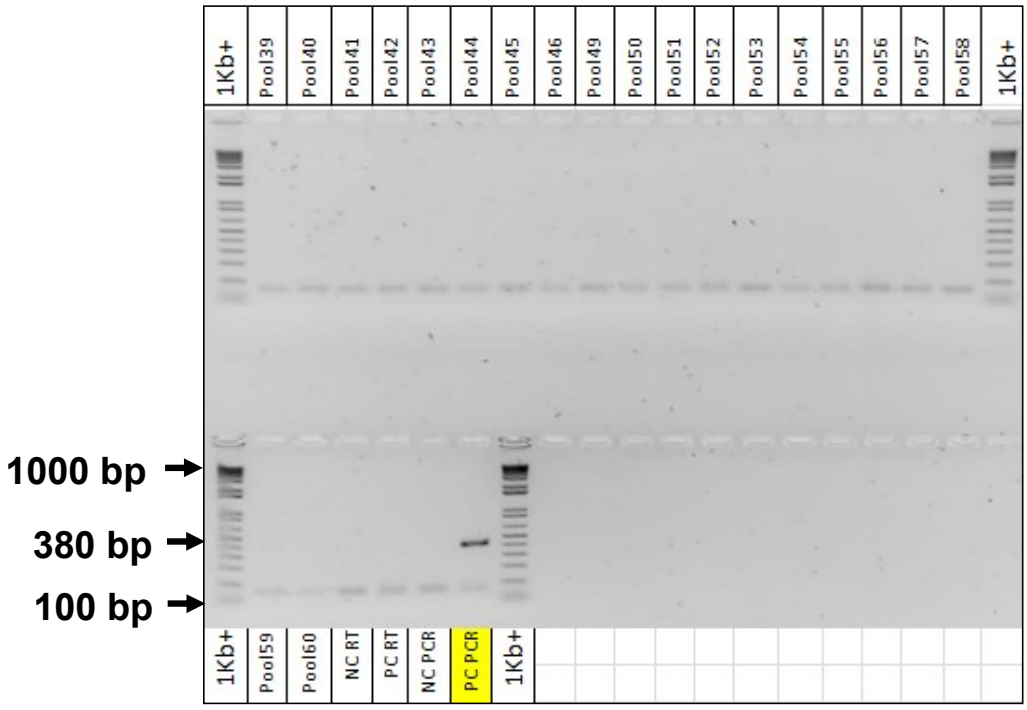


Figure 4.3.2: Agarose gel electrophoresis of 380 bp of IBV genes, negative and positive control

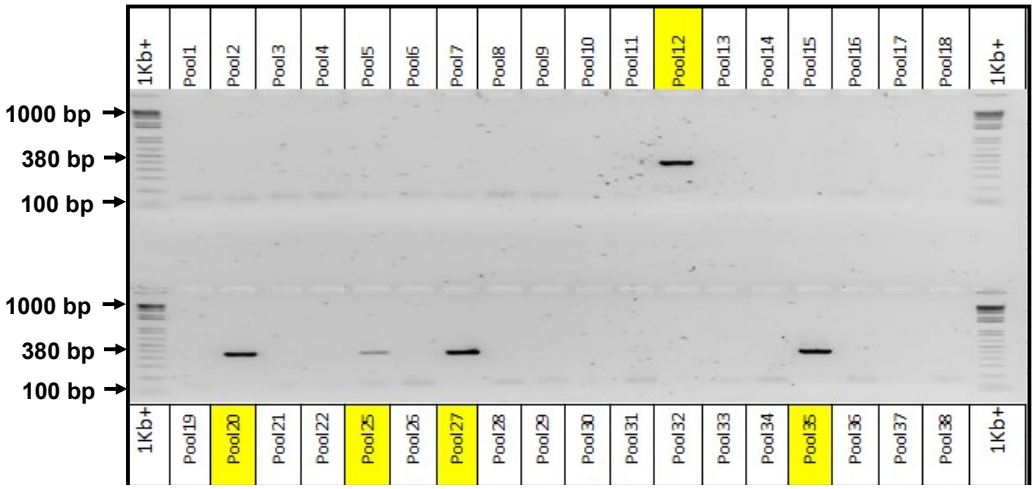


Figure 4.3.3: Agarose gel electrophoresis of 380 bp of IBV genes showing positive(yellow boxes) and negative(white boxes) results.

In figure 4.3.1 the positive band shown on Agarose gel electrophoresis was faint at the first round of RT-PCR. However with Nested PCR the band became clearer and thicker as shown in figure 4.3.3

Table 4.3.1: Identification and distribution of pooled samples positive for IBV in unvaccinated commercial and local chickens in Lagos, Ogun and OyoStates

Pool ID	State	Location	LGA	Type of swab	Chicken status
20	Lagos	Ikorodu	Ikorodu Central	Cloaca	Commercial
25	Lagos	Igbogbo	Igbogbo/ Bayeku	Cloaca	Commercial
130	Lagos	Ikorodu	Ikorodu Central	Cloaca	Commercial
132	Lagos	Ikorodu	Ikorodu Central	Cloaca	Commercial
163	Lagos	Ikorodu	Ikorodu Central	Cloaca	Commercial
27	Ogun	Idomila	Ijebu North East	Cloaca	Commercial
35	Ogun	Mowe	Obafemi/ Owode	Cloaca	Commercial
51	Ogun	Ado/odo	Ade/Odo/Ota	Cloaca	Commercial
59	Ogun	Ade-odo	Ade-Odo/Ota	Cloaca	Commercial
97	Ogun	Mowe	Obafemi/ Owode	Orpharayngeal	Commercial
133	Ogun	Idomila	Ijebu North East	Cloaca	Commercial
135	Ogun	Idomila	Ijebu North East	Cloaca	Commercial
136	Ogun	Alemafon	Ijebu North East	Cloaca	Commercial
139	Ogun	Mowe	Obafemi/ Owode	Cloaca	Commercial
142	Ogun	Mowe	Obafemi/ Owode	Cloaca	Commercial
151	Ogun	Ado-Odo	Ade Odo/Ota	Cloaca	Commercial
152	Ogun	Ado-Odo	Ade Odo/Ota	Cloaca	Commercial
156	Ogun	Ado-Odo	Ade Odo/Ota	Cloaca	Commercial
159	Ogun	Oke-Ata	Abeokuta North	Cloaca	Commercial
160	Ogun	Obada-Oko	Ewekoro	Cloaca	Commercial
161	Ogun	Ewekoro	Ewekoro	Cloaca	Commercial
180	Ogun	Idomila	Ijebu North East	Orpharayngeal	Commercial

183	Ogun	Alemafon	Ijebu North East	Orpharayngeal	Commercial
188	Ogun	Mowe	Obafemi/ Owode	Orpharayngeal	Commercial
213	Ogun	Ijebu-North East		Orpharayngeal	Commercial
12	Oyo	Abadina	Ibadan	Cloaca	Local
65	Oyo	Odo-Erimi	Egbeda	Orpharayngeal	Commercial
70	Oyo	Shasha	Ibadan	Orpharayngeal	Local
121	Oyo	Alabuke	Egbeda	Cloaca	Commercial
126	Oyo	Shasha	Ibadan	Cloaca	Local
127	Oyo	Shasha	Ibadan	Cloaca	Local
128	Oyo	Shasha	Ibadan	Cloaca	Local

Table 4.3.2 : Positive cloaca and oropharyngeal samples in local government of study.

STATE	LOCAL GOVERNMENT AREA	POSITIVE CLOACA SAMPLE	POSITIVE OROPHARYN-GEAL SAMPLE	TOTAL	% POSITIVE PER LOCAL GOVERNMENT
Lagos	Ikorodu	4	0	4	12.5
Lagos	Igbogbo/bayeku	1	0	1	3.1
Lagos	Epe	0	0	0	0
Lagos	Badagry	0	0	0	0
Ogun	Ado-odo/ota	5	0	5	15.6
Ogun	Ewekoro	2	0	2	6.3
Ogun	Abeokuta north	1	0	1	3.1
Ogun	Ijebu north east	4	3	7	21.9
Ogun	Obafemi/owode	4	1	5	15.6
Oyo	Egbeda	1	1	2	3.1
Oyo	Ibadan north	1	0	1	3.1
Oyo	Akinyele	3	1	4	12.5
Oyo	Lagelu	0	0	0	0
Oyo	Ona – ara	0	0	0	0

Table 4.3.1 showed that more positive were recorded in cloaca than orpharayngeal samples collected in the three States. Also the highest number of positive samples were in Ogun State probably because Ogun State has the highest number of chicken population. However in Oyo State more positive samples were recorded in local than commercial chicken(Table 4.3.3).

Table 4.3.3: Prevalence of IBV in commercial and local chickens in Lagos, Ogun and Oyo States

STATES	Prevalence (%)
Lagos (commercial)	8.3
Lagos (local)	0
Ogun (commercial)	33.3
Ogun (local)	0
Oyo (commercial)	3.3
Oyo (local)	12.5

Table 4.3.4: Prevalence of IBV in cloaca and oropharyngeal samplesin commercial and local chickens in Lagos, Ogun and Oyo states

	CLOACA (%)	OROPHARYNGEAL (%)
Lagos (commercial)	8.3	0
Lagos (Iocal)	0	0
Ogun (commercial)	26.7	6.7
Ogun (Iocal)	0	0
Oyo (commercial)	1.7	1.7
Oyo (local)	10	2.5

4.4 Characterisation of infectious bronchitis virus in unvaccinated commercial and local chickens

4.4.1 Evolutionary divergence sequences

The sequences of the isolates were compared for similarities or differences.It was discovered that isolates 127 and 128, 121 and 180 and 133 and 213 are similar with no nucleotide difference. Isolates 121 when compared to 161,180, 35 and 59 has six nucleotide different from others mentioned.The difference between 35 and 20 and 70 and 128 are 20 and 27 nucleotides respectively (Table 5.7)

4.4.2 Multiple alignments of nucleotide and deduced amino acid sequences of infectious bronchitis virus

Multiple alignments of nucleotide and amino acid was done using CLC Main workbench.The result of nucleotide alignment showed point mutation of the nucleotide in most of the pools i.e A to T, T to C and A to G (Figure 5.4 and 5.4a). There were also deletion on pool 127 and 161. On multiple amino acid alignments, it was shown that the alteration does not change the amino acid and that all the isolate are related because of the conserved area of similarities (figure 5.5).

4.4.3 Homology or blast result

The blast result showed all the pooled samples belonged to the same family with the range of 96% to 99% homology to the IBV NGA/A116E7/2006 (the novel genotype described by Ducatez *et al.,* 2006) with Accession number FN430415.1 except pooled sample 20 which has 96% homology with European Turkey Coronavirus reported in France with Accession number KR822424.1 (Table 5.7). All thesequences except the sequence related to turkey were deposited and given the following accession numbers: The accession numbers are between MK886445 and MK 886459

Table 4.4.1: Accesion numbers of sequences of infectious bronchitis virus detected in Lagos, Ogun and Oyo states.

BANKIT NUMBER	NAME	SEQUENCE NUMBER	ACCESSION NUMBER
2221051	NGA1	Seq1	MK886445
2221051	NGA2	Seq2	MK886446
2221051	NGA3	Seq3	MK886447
2221051	NGA4	Seq4	MK886448
2221051	NGA5	Seq5	MK886449
2221051	NGA6	Seq6	MK886450
2221051	NGA7	Seq7	MK886451
2221051	NGA8	Seq8	MK886452
2221051	NGA9	Seq9	MK886453
2221051	NGA10	Seq10	MK886454
2221051	NGA11	Seq11	MK886455
2221051	NGA12	Seq12	MK886456
2221051	NGA13	Seq13	MK886457
2221051	NGA14	Seq14	MK886458
2221051	NGA15	Seq15	MK886459

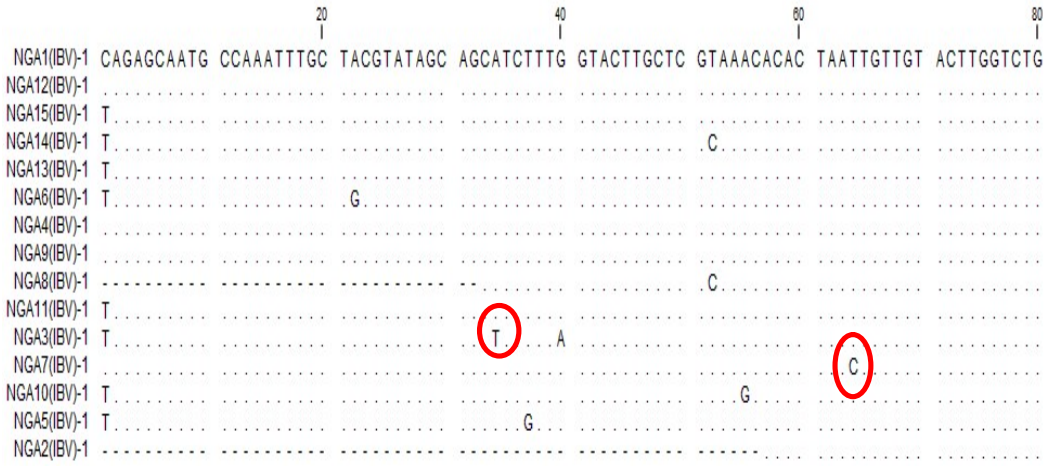


Figure 4.4.1: Multiple alignments of 1b gene nucleotides showing conserved regions. Dots(.) showing areas of similarities, dash (-) and point mutations G-A and T-C.

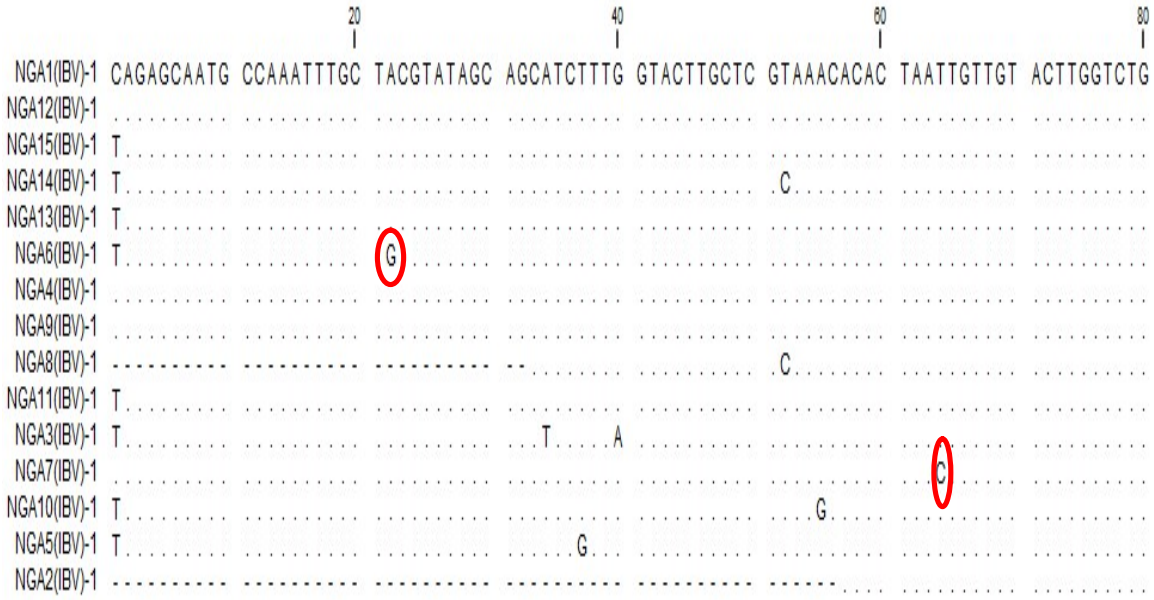


Figure 4.4.1a: Multiple alignments of 1b gene nucleotides showing conserved regions.Dot (.) showed areas of similarities, dash (--) showed areas of deletion and A – G, G – A and T – C showed areas of point mutations.

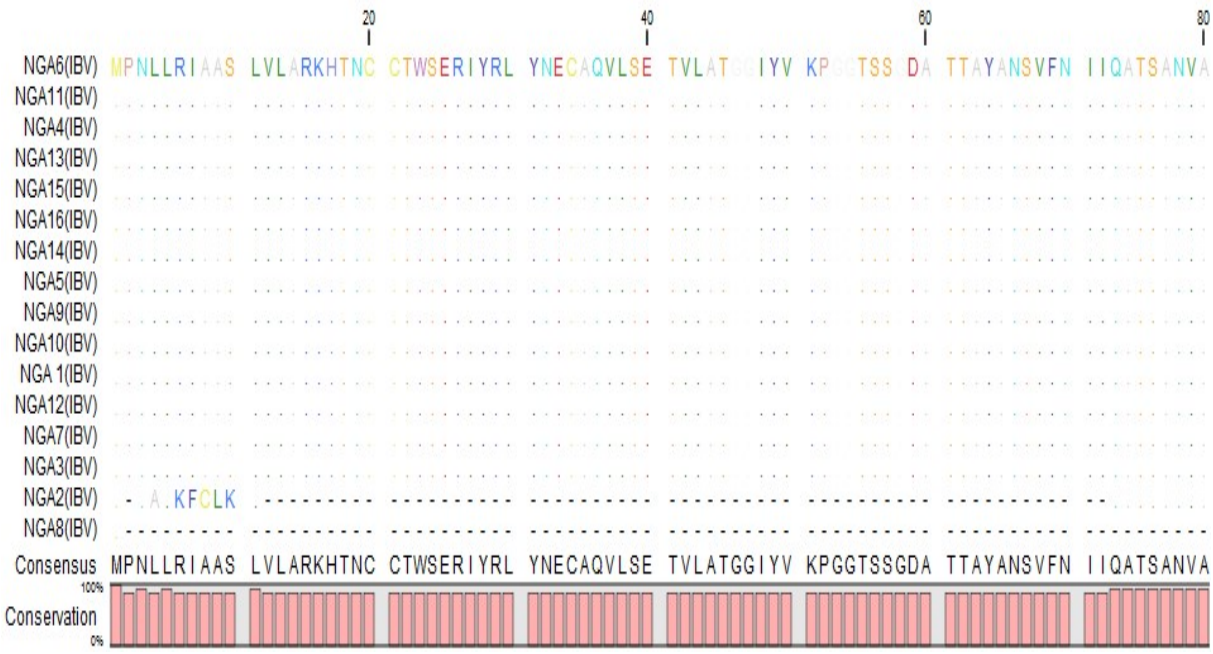


Figure 4.4.2: Multiple alignment of 1b gene deduced amino acid sequences of IB

Dot (.) showed areas of similarities, dash (--) showed areas of deletion

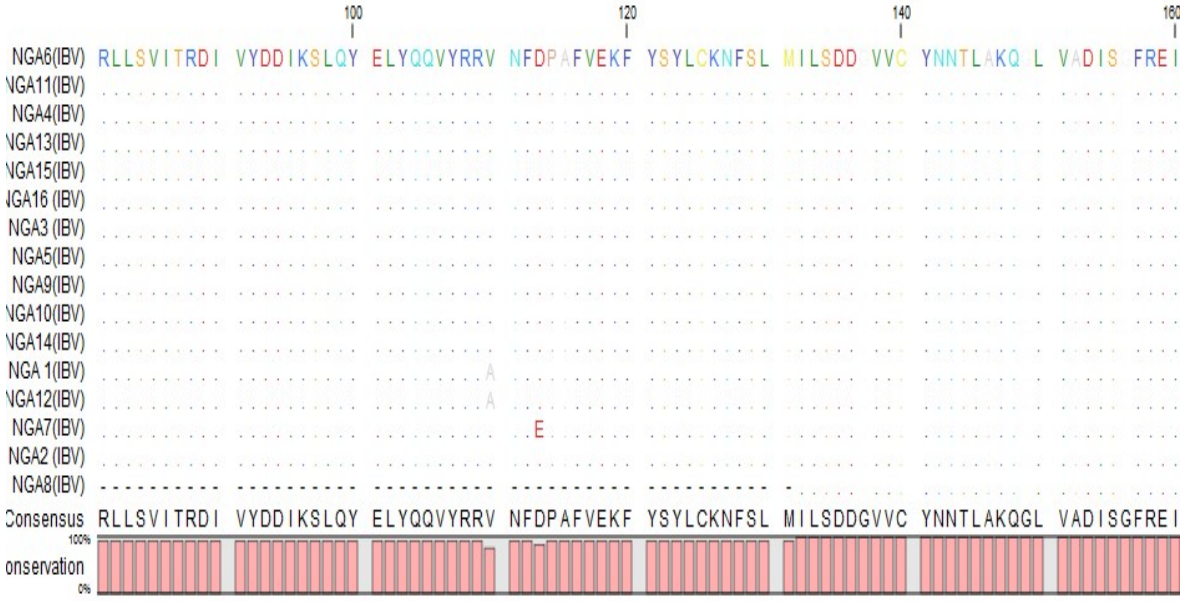


Figure 4.4.2a: Multiple alignments of 1b gene amino acid sequences of infectious bronchitis virus**.**Dots (.) showing areas of similarities, except NGA 2 which has arginine(R) replaced by lysine (k), Isoleucine (I) by Phenylalanine (F), Alanine(A) by Cysteine(C), Alanine (L) by Leucine (L) and NGA 7 that has Aspartic acid (D) by Glutamic acid (E)

Table 4.4.2: Blast result of sequences of 1b gene of infectious bronchitis virus

SAMPLE Pool	STRAIN IN THE GEN BANK.	MAX SCORE	TOTAL SCORE	QUERY SCORE	COUNTRY	IDENTITY (%)	ACCESSION
20	IBV NGR/A116E7/2006	826	826	100%	NIGERIA	96%	FN430415.1
27	1BV NGR/116E7/2006	907	907	99%	NIGERIA	99%	FN430415.1
35	IBV NGR/116E7/2006	905	905	100%	NIGERIA	99%	FN430415.1
59	IBVNGR/116E7/2006	894	894	100%	NIGERIA	99%	FN430415.1
65	IBV NGR/116E7/2006	924	924	100%	NIGERIA	99%	FN430415.1
70	EUROPEAN TURKEY CORONAVIRUS 080385d	830	830	100%	FRANCE	96%	KR822424.1
121	IBV NGR/A116E7/2006	907	907	99%	NIGERIA	99%	FN430415.1
127	IBV NGR/A116E7/2006	466	600	100%	NIGERIA	96%	FN430415.1
128	IBV NGR/A116E7/2006	821	821	100%	NIGERIA	96%	FN430415.1
133	IBV NGR/A116E7/2006	972	972	99%	NIGERIA	98%	FN430415.1
135	IBV NGR/A116E7/2006	859	859	100%	NIGERIA	97%	FN430415.1
159	IBV NGR/A116E7/2006	989	989	100%	NIGERIA	99%	FN430415.1
160	IBV NGR/A116E7/2006	828	828	99%	NIGERIA	96%	FN430415.1
161	IBV NGR/A116E7/2006	828	828	100%	NIGERIA	98 %	FN430415.1
180	IBV NGR/A116E7/2007	885	885	99%	NIGERIA	98%	FN430415.1
213	IBV NGR/A116E7/2007	922	922	100%	NIGERIA	97%	FN430415.1

*pool 20 – NGA1 * Pool 27 –NGA2 *Pool 35 – NGA 3 *pool 59 – NGA4 *Pool 65 – NGA5 *pool 121 –NGA6 *Pool 127 – NGA7 * Pool 128 – NGA8*Pool 133 – NGA9 * Pool 135 – NGA10 *Pool 159 – NGA11 *Pool 160 – NGA12 *Pool 161 – NGA13 *Pool 180 – NGA14 *Pool 15 Pool 70

Table 4.4.3: Estimates of evolutionary divergence sequences (The number of base differences from between sequences

	P. 121	P. 127	P. 128	P. 133	P. 135	P. 159	P. 160	P. 161	P. 180	P. 20	P. 213	P. 27	P. 35	P. 59	P. 65	P. 70
Pool 121																
Pool 127	11															
Pool 128	20	0														
Pool 133	6	9	18													
Pool 135	16	11	19	18												
Pool 159	7	12	20	11	19											
Pool 160	16	19	28	16	23	18										
Pool 161	6	10	19	6	17	9	16									
Pool 180	6	9	18	0	18	10	16	6								
Pool 20	22	14	23	20	24	22	24	19	20							
Pool 213	17	5	10	23	20	24	22	24	19							
Pool 27	0	11	20	6	16	7	16	6	6	17						
Pool 35	6	11	20	8	18	6	14	9	8	19	6					
Pool 59	6	9	18	6	16	9	16	1	6	17	6	8				
Pool 65	4	12	20	8	16	7	16	5	8	17	4	6	4			
Pool 70	26	18	27	24	18	26	26	23	24	27	26	26	22	24		

Table 4.4.4: Blast result of sequences of 1b gene (protein) of infectious bronchitis virus

SAMPLE ID	PROTEIN IN GEN BANK	MAX SCORE	TOTAL SCORE	QUERY SCORE	% IDENTITY	ACCESSION
20	RNA dependent polymerase	338	338	100	98.79	AOR52338
27	RNA dependent polymerase	336	336	100	98.18	AOR52338
35	RNA dependent polymerase	343	343	100	98.81	AOR52338
59	RNA dependent polymerase	340	340	100	98.8	AOR52338
65	RNA dependent polymerase	345	345	100	98.8	AOR52338
70	RNA dependent polymerase	340	340	100	98.8	AOR52338
121	RNA dependent polymerase	336	336	100	98.18	AOR52338
127	Polyprotein (IBV)	187	187	91%	96.81	AKP63364
128	RNA dependent polymerase(IBV)	334	334	100	98.18	AOR52338
133	RNA dependent polymerase(IBV)	375	375	100	98.9	AOR52338
135	RNA dependent polymerase(IBV)	378	378	100	98.79	AOR52338
159	RNA dependent polymerase(IBV)	380	380	100	98.91	AOR52338
160	RNA dependent polymerase(IBV	336	336	100	98.18	AOR52338
161	Polyprotein (IBV)	75.1	75.1	100	100	AKP63375
213	RNA dependent polymerase(IBV)	377	377	100	98.91	AOR52338

Table 4.4.5: G – C% content of sequences of 1b gene of infectious bronchitis virus

	Total Count	Adenine	Thymine	Guanine	Cytosine	G – C %
Pool 20	504	140	175	104	85	37.5
Pool 27	504	137	176	105	86	37.9
Pool 35	511	139	182	106	84	37.2
Pool 59	505	138	178	104	86	37.4
Pool 65	515	139	183	109	84	37.5
Pool 70	504	136	177	106	85	37.9
Pool 121	504	137	176	105	86	37.9
Pool 127	351	95	124	73	59	37.6
Pool 128	502	139	170	104	86	38.4
Pool 133	554	155	188	117	94	38.1
Pool 135	504	137	169	107	91	39.9
Pool 159	559	152	194	121	92	38.1
Pool 160	503	139	175	105	84	37.6
Pool 161	472	127	169	98	78	37.3
Pool 180	504	140	175	104	85	37.5
Pool 213	556	151	188	121	96	39.0

4.5Characterisation of S1 Gene of Infectious Bronchitis Virus

The BLAST result of S1 gene showed that isolates 20 (Ikorodu, Lagos state) and 35 (Mowe, Ogun state) were 96% and 97% homologous to Nigerian strain IBV NGA/A116E7/2006 while isolates 126 (Shasha, Oyo state), 127 (Shasha, Oyo state) and 160 (Ewekoro, Ogun state) were 95%, 95% and 96% homologous to Variant 2 strain from Israel. Isolates 132(Ikorodu, Lagos state) and 161 (Ewekoro, Ogun state) were 93% and 92% respectively homologous to AIBV strain IS/585/98 from Israel. Isolates 135 (Ijebu, North East) and 139 (Mowe, Ogun state) were also 93% and 95% homologous to IBV NGA/A176/2006, strain from Nigeria.

Also isolate 213 (Ijebu North East), was 93% homologous to AIBV strain IS/572/98 from Israel while Isolate 163 (Ikorodu, Lagos state) had 99% homology to AIBV isolate CK/CH/HUN/NTP strain from China.

Table 4.5.1: Accesion numbers of sequences of infectious bronchitis virus of S1 gene detected in Lagos, Ogun and Oyo states

BANKIT NUMBER	NAME	SEQUENCES NUMBER	ACCESSION NUMBER
BANKIT2235575	NGA 1	Seq1	MN082397
BANKIT2235575	NGA2	Seq 2	MN082398
BANKIT2235575	NGA3	Seq3	MN082399
BANKIT2235575	NGA4	Seq4	MN082400
BANKIT2235575	NGA5	Seq 5	MN082401
BANKIT2235575	NGA6	Seq 6	MN082402
BANKIT2235575	NGA7	Seq 7	MN082403
BANKIT2235575	NGA8	Seq 8	MN082404

4.5.1 Multiple nucleotide and amino acid alignment

The multiple nucleotide alignment showed areas of insertions and point mutations at NGA3, NGA4, NGA6, NGA7 and NGA8 at residue number 344 while at 374 (Figure 5.8), there are point mutations and insertions in all the isolates suggesting detection of four different genotypes at residue number 344 (CAG,CAT, TCT and TTT)and 374 (ATT,AGT,GGG and AGG) however at the amino acid level, three genotypes were observed at the hypervariable region 2 between 97 and 141.At residue number 114 (Figure 5.9),insertion of three amino acids were seen; threonine,aspergine and lysine while at 139,glutamic,aspartic and alanine. When the amino acid sequences were compared with the sequences of Variant 2, H120 and Nigerian strain from the gen bank, it was observed that one of the isolates belonged to each of the H120 (NGA7) and Nigerian strain (NGA4) while the remaining six isolates belonged to Variant 2.

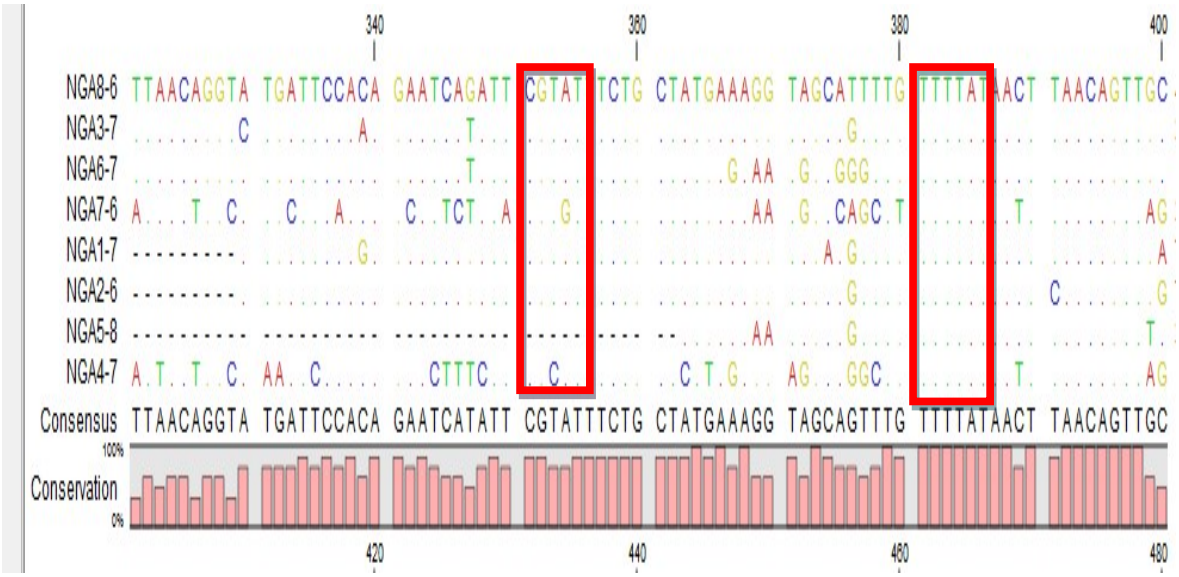
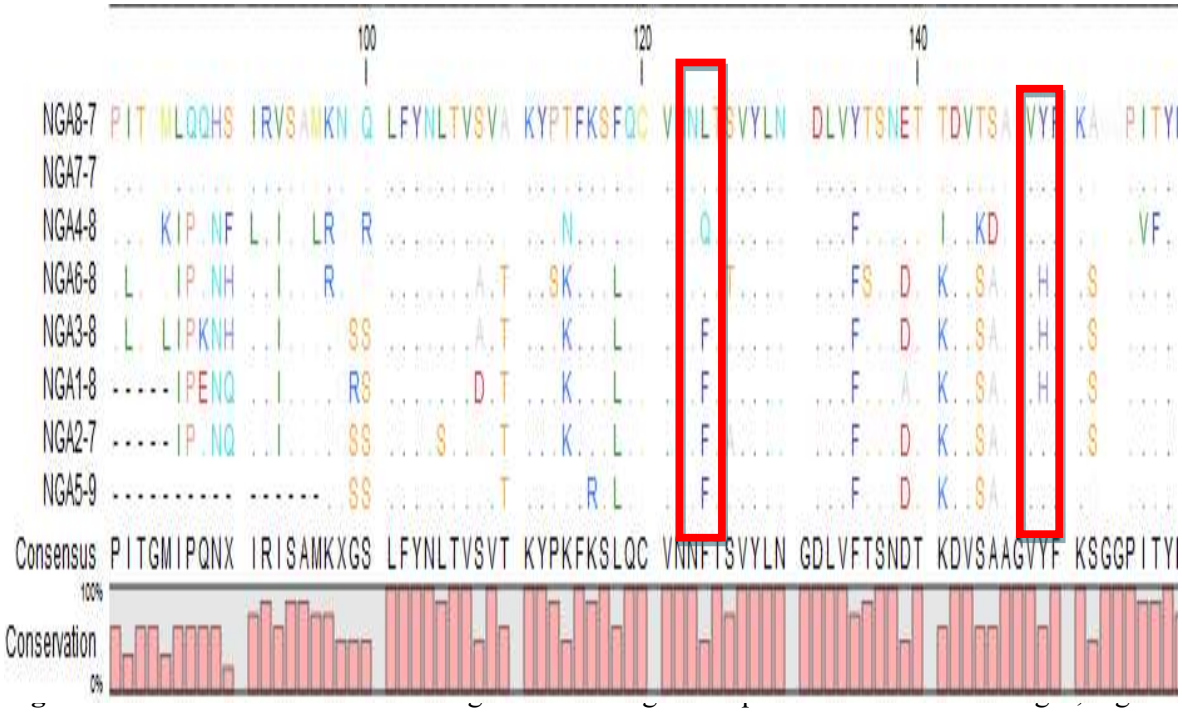


Figure 4.5.1: Multiple alignments of S1 gene nucleotide sequences of Infectious bronchitis virus.Dot (.) showed areas of similarities, dash (--) showed areas of deletion and A – C, G – A ,A – T and T – C showed areas of point mutations.



and Oyo states. Red boxes showing different serotypes at the hypervariable region.

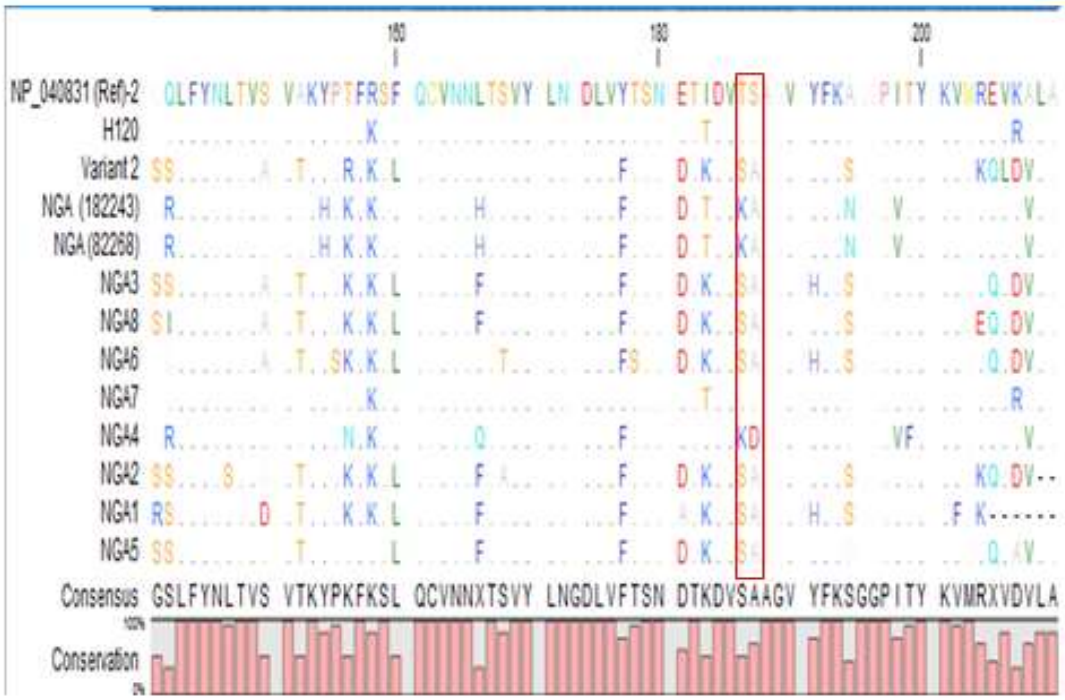


Figure 4.5.3: Amino acid alignment of S1 gene sequences of IBV from Lagos, Ogun and Oyo states compared with full length sequences of protein of S1 gene, variant 2, H120 and Nigerian strain from Gen Bank.Dot (.) showed areas of similarities, dash (--) showed areas of deletion and A – C, G – A ,A - Tand T – C showed areas of point mutations.

Table 4.5.2: BLAST result of sequences of S1 gene of infectious bronchitis virus detected in Lagos, Ogun and Oyo States

SAMPLE POOLED ID	STRAIN IDENTIFIED	COUNTRY	% DENTITY	ACCESSION
20	IBV NGA/A116E7/2006	NIGERIA	96%	FN430415.1
35	IBV NGA/A116E7/2006	NIGERIA	97%	FN430415.1
126	IBV Isolate IB variant 2	ISRAEL	95%	JX027069.1
127	IBV isolate IB variant 2	ISRAEL	95%	JX027069.1
132	AIBV strain IS/585/98	ISRAEL	93%	AY789962.1
135	IBV NGA/A176/2006	NIGERIA	93%	FN182262.1
139	IBV NGA/A176/2006	NIGERIA	95%	FN182262.1
160	IBV Isolate IB variant 2	ISRAEL	96%	JX027069.1
161	IBV isolate IS/585/98	ISRAEL	92%	AY789962.1
I63	AIBV isolate CK/CH/HUN/NTP	CHINA	99%	KX107793.1
213	AIBV strain IS/572/98	ISRAEL	93%	AY789996.1

*Pool 126 – NGA1 *Pool 127 –NGA2 *Pool 132 – NGA3 *Pool 139 – NGA4

*Pool 160 – NGA5 *Pool 161 – NGA6 *Pool 163 – NGA7 *Pool 213 – NGA8

*Pool 20 – NGA9 *Pool 35 – NGA10 *Pool 135 – NGA11

Table4.5.3: Blast result of sequences of S1 gene (protein) of infectious bronchitis virus detected in Lagos, Ogun and Oyo States

SAMPLE ID	PROTEIN IN THE GEN BANK	MAX SCORE	QUERY	EVALUE	% Identity	ACCESSION
20	S1 glycoprotein(IBV)	201	89%	1e-59	95%	CAX52753
35	S1 glycoprotein(IBV)	234	100%	3e-72	96.61%	CAX52641
126	S1 glycoprotein(IBV)	147	98%	2e-43	83.95%	ADV74899
127	S1 glycoprotein(IBV)	170	100%	1e-52	89.23%	QAY29979
132	S1 glycoprotein(IBV)	381	100%	7e-133	87.86%	AAV83685
139	S1 glycoprotein(IBV)	398	100%	7e-135	93.66%	CAX52729
160	S1 glycoprotein(IBV)	155	100%	4e-46	91.57%	AAV83687
161	S1 glycoprotein(IBV)	372	100%	4e-129	87.32%	AAV83680
163	S1 glycoprotein(IBV)	416	100%	1e-146	98.53%	AAV83690
213	S1 glycoprotein(IBV)	387	100%	3e-72	96.61%	CAX52741

TABLE 4.5.4: G – C content of the nucleotide sequences of S1 gene of infectious bronchitis virus detected in Lagos,Ogun and Oyo states

	Total Count	Adenine	Thymine	Guanine	Cytosine	%G-C
Pool 20	655	183	253	116	98	32.7
Pool 35	356	97	144	61	54	32.3
Pool 126	262	79	101	49	33	31.3
Pool 127	286	86	102	57	41	34.3
Pool 132	620	176	222	122	100	35.8
Pool 135	538	148	191	106	93	37
Pool 139	610	162	222	130	96	37
Pool 160	251	69	100	48	34	32.7
Pool 161	612	167	216	129	100	37.4
Pool 163	610	153	227	129	101	37.7
Pool 213	623	174	218	129	102	37.1

4.5.2 Amino acid identity result

Pairwise amino acids similarities and identity varies from 14.4% (comparing pool 20 and pool 135) and 55.07% (pool 35 and pool 126, 127, 20,160 and 161)

TABLE 4.5.5: Amino acids sequence identity values for the partial S1 sequences of the isolates

Pool 126	100										
Pool 127	53.4	100									
Pool 132	52.9	50%	100 %								
Pool 135	21.2 9	18.8 2	14.1 5	10 0							
Pool 139	53.0 1	49.3 9	49.3 9	36.14	100						
Pool 160	52	52	52	56	52	100					
Pool 161	52.2 5	52.3 5	52.2 3	15.42	53.0 1	48	100				
Pool 163	54.1 9	50.5 8	53.3	14.6	51.8	52	51.24	100			
Pool 20	50.9 6	53.5 2	53.3 9	14.47	50.6	52	52.23	52.03	100		
Pool 213	52.2 5	52.9 4	28.8 9	29.2	50.6	56	43.28	33.07	39.36	100	
Pool 35	55.0 7	50.7 2	50.7 2	47.82	49.7 2	52	52.72	55.07	55.07	50.72	100
	Pool 126	**Pool 127**	**Pool 132**	**Pool 135**	**Pool 139**	**Poo l60**	**Pool 161**	**Pool 163**	**Pool 20**	**Pool 213**	**Pool 35**

4.5.3 Phylogenetic analysis of 1b gene of IBV

Phylogenetic analysis of 1b gene in this study suggested that all strains were clustered into three distinct branches. Groups 1 and 11 have most of the isolates clustered around the Nigerian strain IBVNGA/A116E7/2006 with accession number FN430415 (Figure 5.11a. The third group had isolates 20 (Ikorodu, Lagos) and 70 (Shasha, Oyo) clustered around the Italian strain ITA/90254/2005Qx with accession number FN430414 as shown in

4.5.4 Phylogenetic analysis of the S1 gene of IBV

Phylogenetic analysis of S1 gene showed three major clusters, isolate 163 clustered around H120 Netherland vaccine strain Isolates 126 (Sasha), 127 (Sasha) and 132 (Ikorodu) and also 160 (Ewekoro), 161 (Ewekoro) and 213 (Ijebu-Ode) clustered around Variant 2 with accession number AF093796 while the last cluster comprises of isolate 20 (Ikorodu, Lagos), 135 (Ijebu –Ode) and 139 (Mowe) which clustered around Nigerian strain with accession number FN 1882266 as shown in Figure 4.5.4

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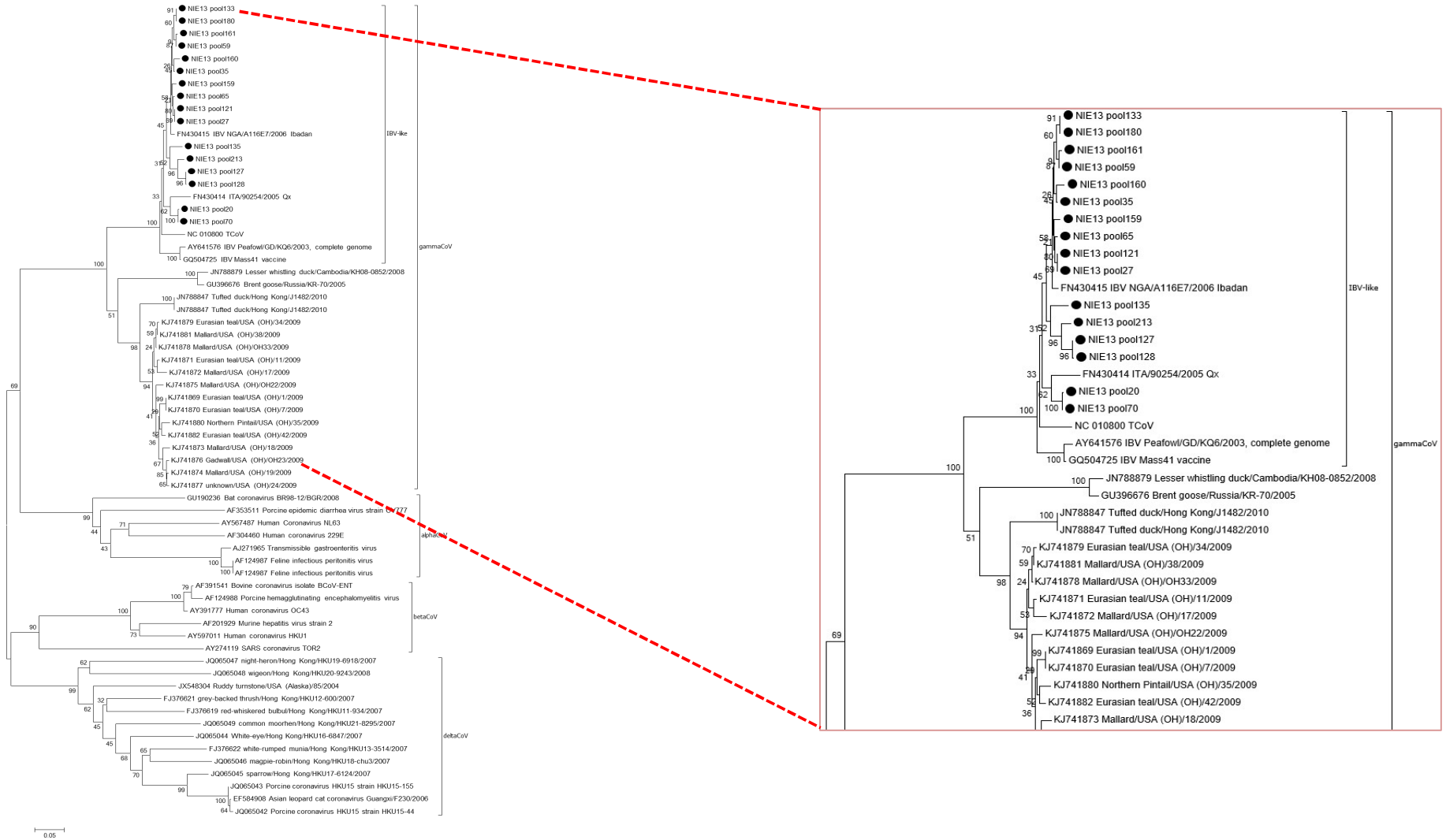

Figure 4.5.4: Genotype assignment using S1 partial sequences and compared to full S1 gene dataset from Valastro *et al*., (2016). MEGA 6, Kimura 2 method, partial deletion 500 bootsraps.

4.6: Detection and molecular characterization of infectious bronchitis virus in vaccinated chickens

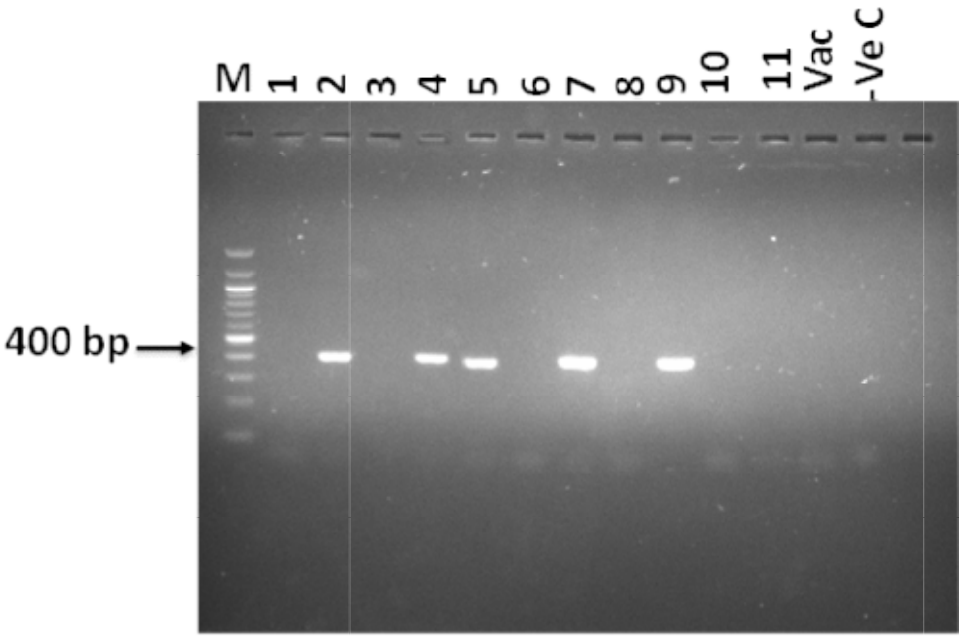

Figure 4.6.1:Agarose gel electrophoresis of 400bp of IBV genes.Lane **1:** Molecular marker(M),Lane 1 – 11 IB cloaca sampes,Lane 12:IB vaccine,Lane 13: Negative control.

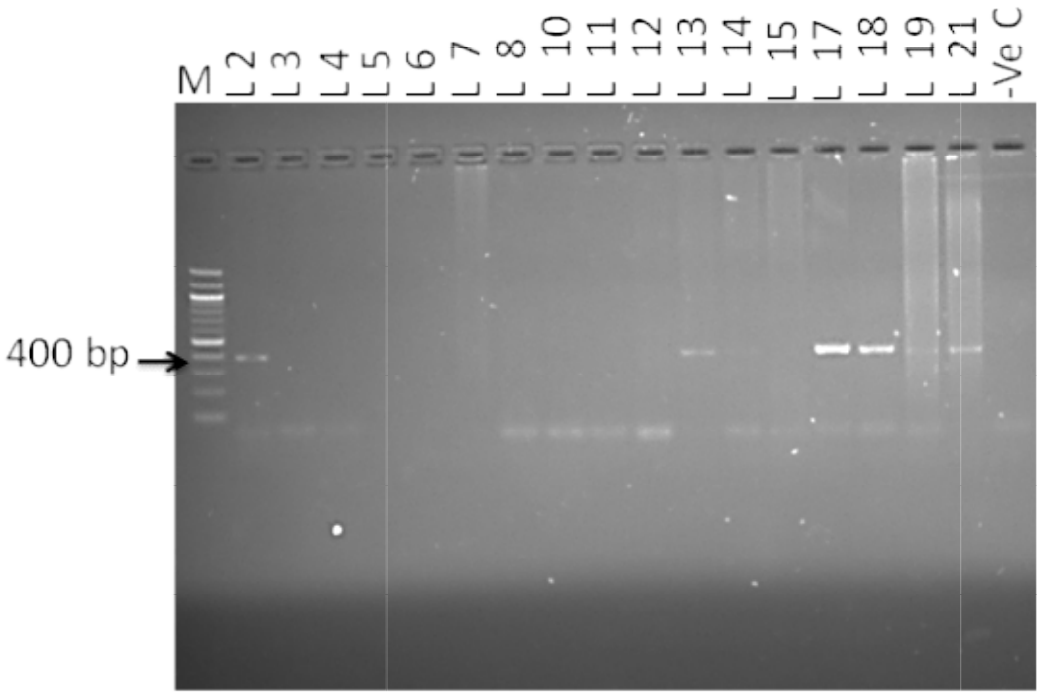

Figure 4.6.2:Agarose gel electrophoresis of 400bp of IBV genes.Lane 1: Molecular marker(M), Lane 2 – 21Infectious Bronchitis lung samples, Lane 22: Negative control.

Table 4.6.1: Summary of sample details and IBV detection status

NAME OF FARM	TYPE OF BIRDS	AGE OF BIRDS (weeks)	VACCINATION RECORDS.	HISTORY	SAMPLES TAKEN.	PCR RESULT
Farm1 (Sample)	Broiler Breeder	40	IB	Mortality	Congested Lung, Kidney, Tracheal tissue Cloaca	Negative
Farm (Sample 2)	Layer	68	LA SOTA + IB, IB + EDS + ND	Fall in Production Mortality	Congested Lung, Kidney, Tracheal tissue Cloaca	Positive (cloaca)
Farm 2 (Sample 3)	Layer	56	IB, IB + EDS + ND	Mortality	Congested Lung, Kidney, Tracheal tissue Cloaca	Negative
Farm 2 (Sample 4)	Layer	28	IB, IB + EDS + ND	Mortality	Congested Lung, Kidney, Tracheal Cloaca.	Positive (cloaca)
Farm 3 (Sample 5)	Layer	65	IB,IB + EDS + ND	Mortality	Congested Lung, Kidney, Trachel tissue Cloaca.	Positive (cloaca)

Farm 3 (Sample 6)	Broiler	4	IB	Mortality	Congested Lung, Kidney, Tracheal tissue Cloaca	Negative
Farm 4 (Sample 7)	Layer	36	IB + EDS +ND	Mortality	Congested lung, Kidney, Tracheal tissue Cloaca	Positive (cloaca)
Farm 4 (Sample 8)	Chicks	2	IB	Mortality	Congested Lung, Kidney, Tracheal Cloaca.	Negative
Farm 4 (Sample 9)	Chicks	6 Days	IB	Mortality	Congested Lung, Kidney, Tracheal tissue Cloaca	Positive (cloaca)
Farm 5 (Sample 10)	Layer	30	IB, IB+ND+EDS	Fall in Production Mortality	Congested Lung, Kidney, Tracheal tissue Cloaca.	Negative
Farm 5 (Sample 11)	Pullets	8	IB + La Sota	Mortality	Congested Lung, Kidney, Tracheal tissue	Negative

Farm 6 (Sample 12)	Layer	38	IB, IB+ND+EDS	Mortality	Cloaca Congested Lung, Kidney, Tracheal tissue	Negative
Farm7 (Sample 13)	Layer	46	IB + EDS + ND	Mortality	Cloaca Congested Lung, Kidney, Tracheal tissue	Negative
Farm 8 (Sample 14)	Pullets	13	NONE	Mortality	Cloaca Congested Lung, Kidney, Tracheal tissue	Negative
Farm 9 (Sample 15	Broiler	6	IB	Mortality	Cloaca Congested Lung, Kidney, Tracheal tissue	Negative
Farm 10 (Sample 16)	Chicks	7	IB	Mortality	Cloaca Congested Lung, Kidney, Tracheal tissue	Negative
Farm 11 (Sample 17)	Layer	30	LA SOTA + IB	Fallin Egg	Cloaca Congested Lung,	Positive (Lung)

				Production, Mortality	Kidney, Tracheal tissueCloaca.	
Farm 12 (Sample 18)	Layer	23	IB, IB +EDS+ND	Fall In Egg Production, Mortality	Congested Lung, Kidney,Tracheal tissue Cloaca	Positive (Lung)
Farm 13 (Sample 19)	Layer	27	IB + EDS + ND	Fall In Egg Production, Mortality.	Congested Lung, Kidney, Tracheal tissue Cloaca	Negative
Farm 14 (sample 20)	Pullets	18	IB	Mortality	Congested Lung, Kidney, Tracheal tissue Cloaca.	Negative
Farm 15 (sample 21)	Chicks	8	IB	Mortality	Congested Lung, Kidney, Tracheal tissue Cloaca.	Positive (lung)

Table 4.6.2: BLAST results of IBV from vaccinated commercial chickens compared with the sequences from the Gen Bank

ID	STRAIN FROM GEN BANK	MAX SCORE	TOTAL SCORE	QUERY	EVALUE	IDENTITY	ACCESSION	COUNTRY
Cloaca 2 NGA 1	AIBV Isolate 210-02	649	649	99%	0.0	98%	AY790350	SOUTH KOREA
Cloaca 7 NGA 2	AIBV Isolate 210-02	536	536	99%	1e-148	99%	AY790350	SOUTH KOREA
Cloaca 9 NGA 3	IBV NGA/A166E/2006	564	564	94%	7e-148	94%	FN430415	NIGERIA
Lung 18 NGAL1	IBV NGA/A166E/2006	588	588	94%	4e-164	97%	FN430415	NIGERIA
Lung 21 NGA L2	IBV NGA/A166E/2006	597	597	94%	7e-167	97%	FN430415	NIGERIA

The above results showed high percentage similarity to South Korea strain (AY790350) and Nigerian strain (FN182280) even though Massachussets strain H120 was the only strain available in the southwest. Furher classification was done by multiple alignments of nucleotides of the sequences from the field compared to strain from other countries including South Korea.

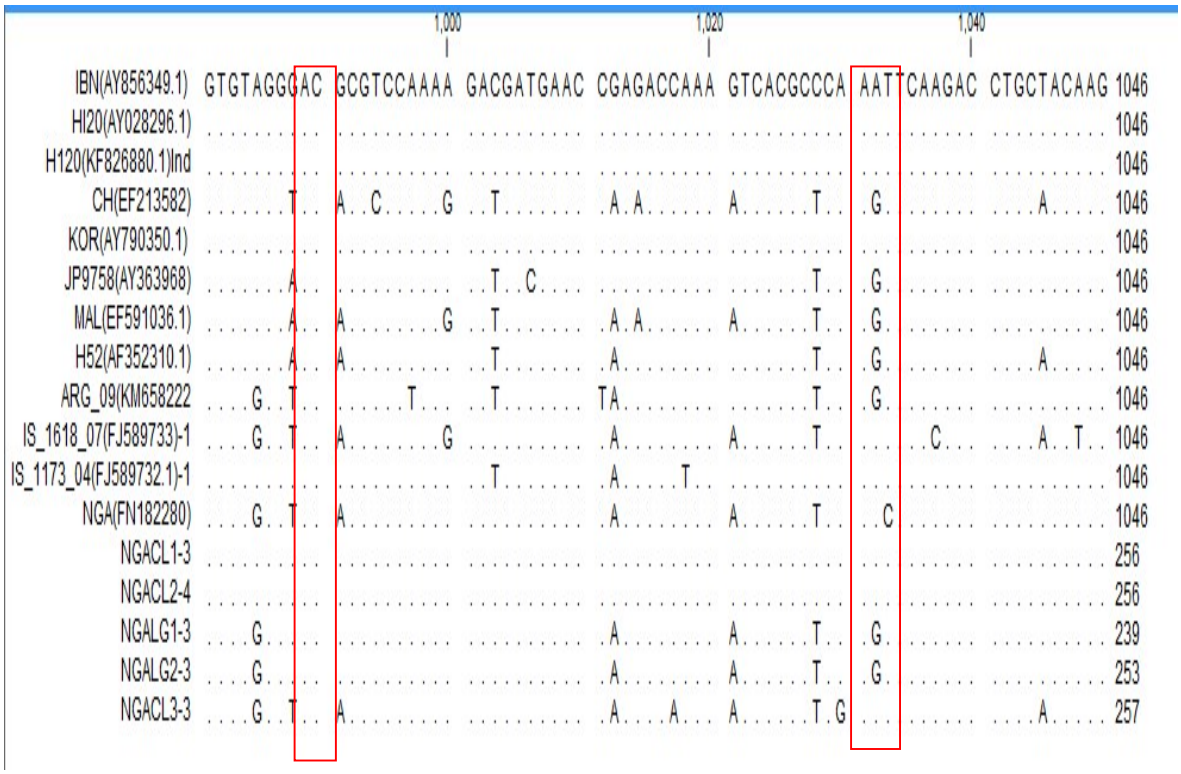

Figure 4.6.3: Multiple alignments of nucleotide sequences of positive Nigerian samples compared with sequences of other countries from the gene bank. Dot (.) showed areas of similarities, areas of point mutations G - A, A – G, C – T and the red boxes showing similarities of the detected serotypes with other vaccines strains from other countries at point 984 and 1028.

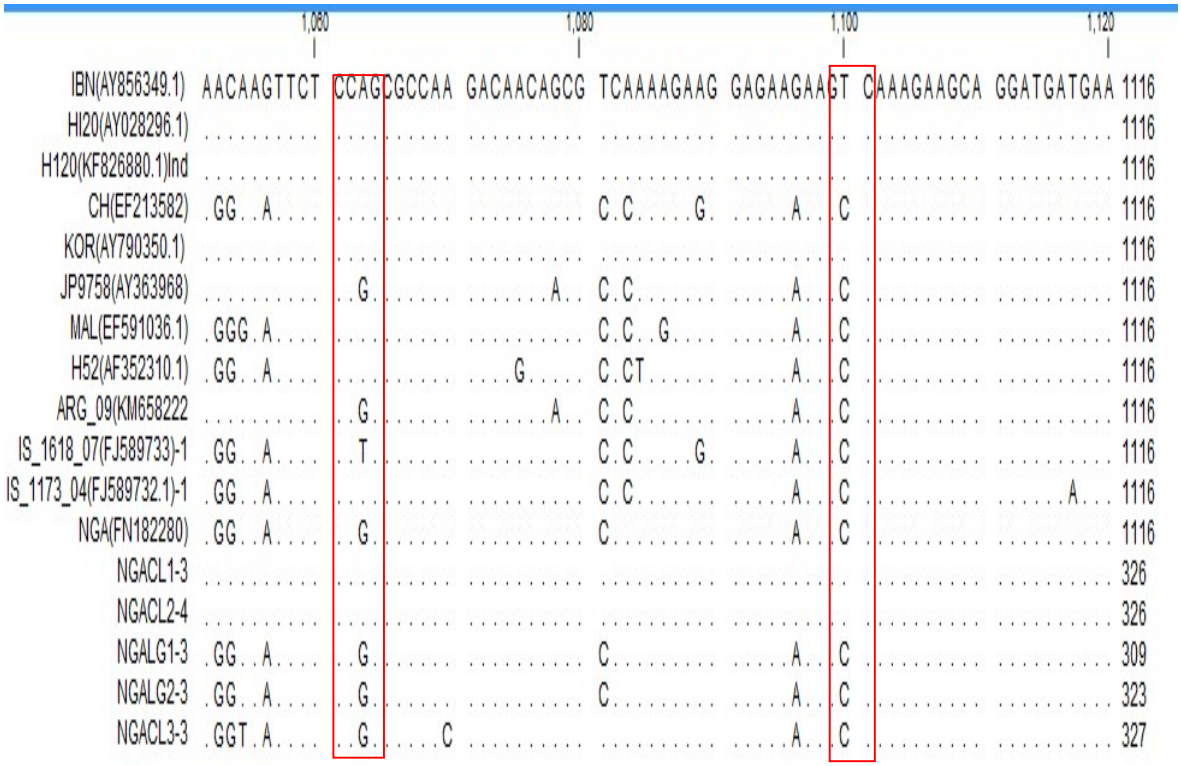

Figure 4.6.3a: Multiple aligments of nucleotide sequences of detected strains compared with H120 nucleotide sequences of H120 and other vaccine strains from other countries.Dot (.) showed areas of similarities, areas of point mutations G - A, A – G, T – C and the red boxes showing similarities of the detected serotypes with other vaccines strains from other countries at point 1,056 and 1,100.

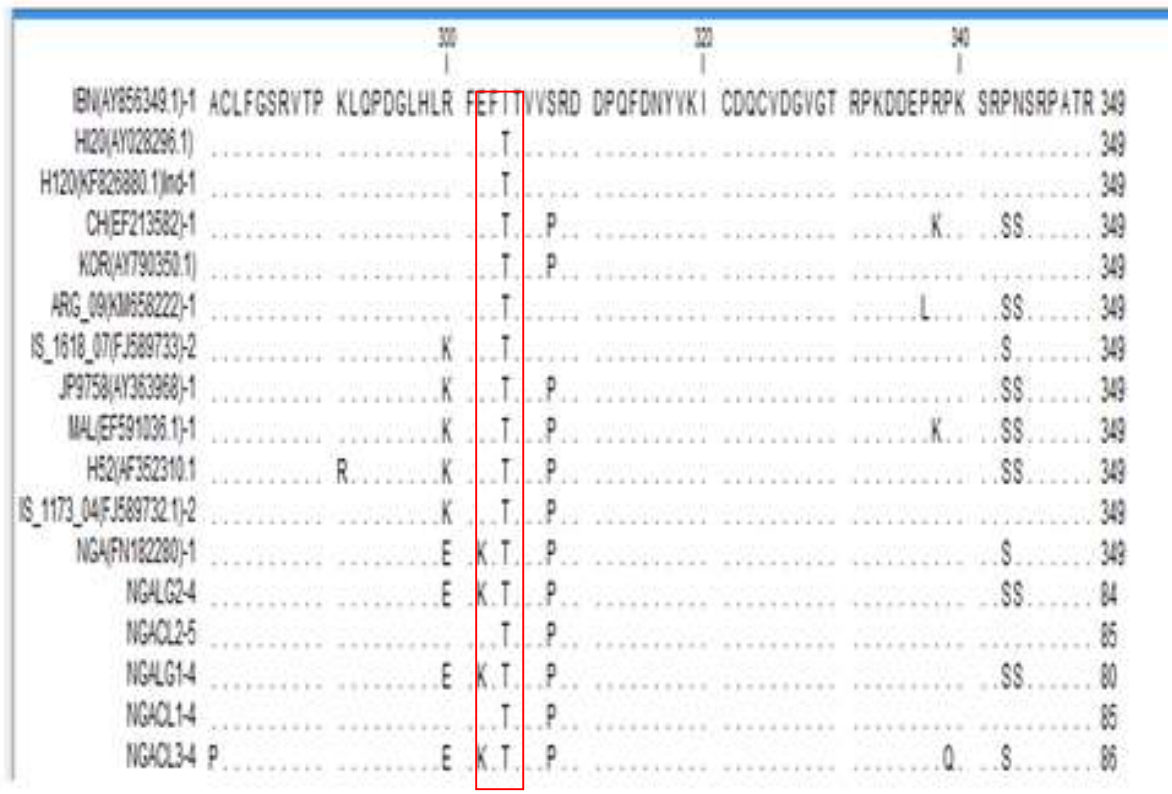

Figure 4.6.4: Multiple aligments of amino acid sequences of detected strains compared with amino acid sequences of H120 and other vaccine strains from other countries. Dot (.) showed areas of similarities, areas of mutation, K– R, I – P, S – P and the red box showing similarities of the detected serotypes with strains from other countries.

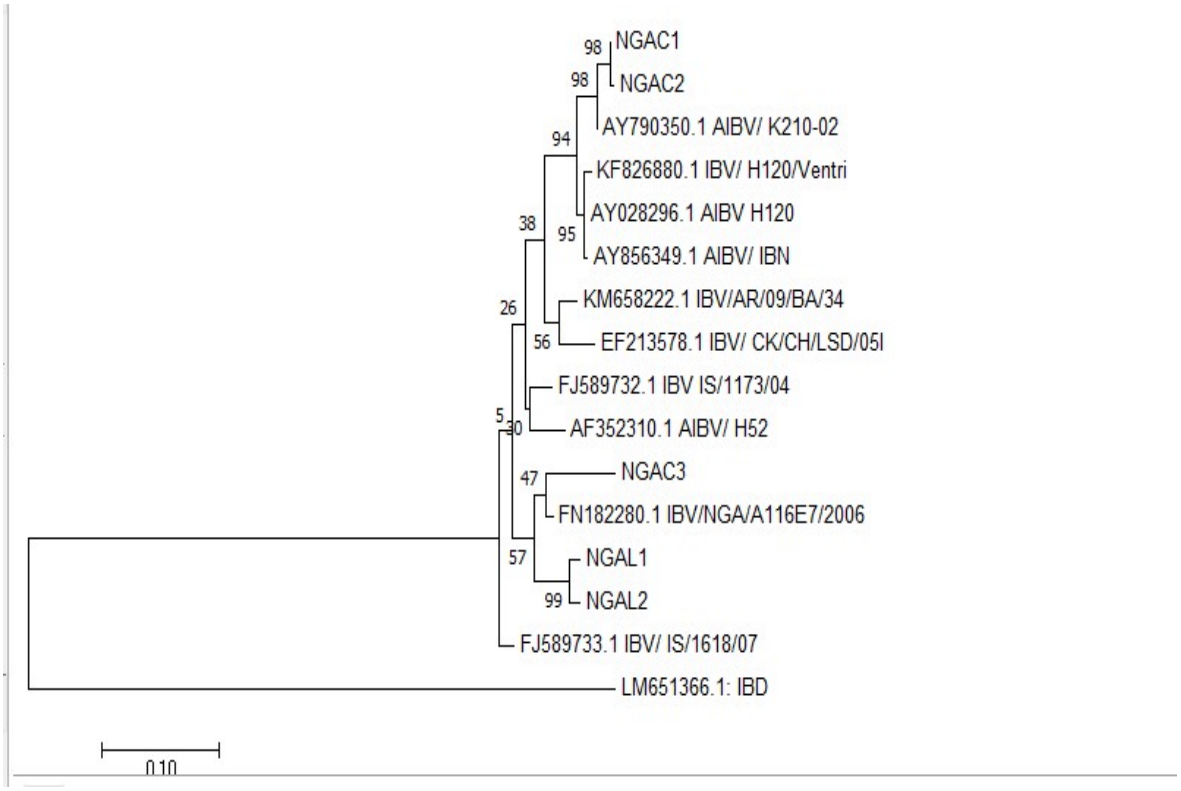

Figure 4.6.5: Phylogenetic analysis of the detected IBV sequences compared with vaccine sequences from the genbank using maximum likelihood

4.6.1 Available vaccines in the study area.

The survey of vaccines available to farmers from the veterinary stores in these three states showed that all infectious bronchitis vaccines available in the three states are Massachusettests strain from different countries of origin are diverse i.e Italy, India, Israel and Hungary as shown in Table 4.6.3.

Table4.6.3: Details of commercially available infectious bronchitis vaccines in Lagos, Ogun and Oyo States

Name	Vaccine Strain	Country
Indovax	H120	India
ABIC	H120	Israel
Isovac	H120	Italy
Isovac (La Sota + H120)	H120	Italy
Biomed	H120	India
Cevac	H120(ND/IB/EDSK	Hungary

The result of the survey showed that H120 strain was the only commercial vaccines available in Lagos, Ogun and Oyo states from Italy, India, Israel and Hungry (Table 4.6.4). For the detection of IBV using N-gene RT-PCR, eight out of twenty – One samples from fifteen farms were positive (Table 4.6.1). The eight positive samples are three lung samples and five cloaca samples while all the trachea and kidney samples were negative. The multiple alignments of both vaccine strains from the gene bank and samples' sequences using CLC Main Workbench 8 showed at point 984 single nucleotide alteration that involves change of A to G, and C to T at 1028 and at 1,056 and 1,100 G-A, T-C respectively, for isolates NGAL2, NGACL3 and NGAL1 and it is similar to Japan, Argentina and Nigerian strains. However, the remaining two detected serotypes have a nucleotide similar to H120, Korea and India also with Israel 1173 strain, H52 and Malaysia. Israel 1168 has T instead of A nucleotide (Table 4.6.3, 4.6.4).

The multiple amino acid sequence (Figure 6.5) showed three of the five isolates NGALG1(NGALG18),NGALG2(NGAL21),andNGACL3(NGACL9), had arginine(R) replaced by glutamic acid (E) similar to Nigerian strain while NGACL2(NGACL7) has arginine, NGACL1 (NGACL2) had arginine amino acid. H120 strain from Argentina, India, China, and South Korea strain also has arginine contrary to the three strains that had glutamic acid (E) similar to strains from Nigeria.It also shows that the two Israel strains (IS1173, IS1168), Malaysia strain, Japan strain and H52 srains were different from the isolates having Lysine (K) instead of R (Arginine). The BLAST result after comparing the sampled sequences with vaccine sequences from the Gen Bank showed that cloaca samples 2 & 7 (NGACL1 and NGACL2) from farm 2 and 4 are 98% and 99% homology to strain from Korea while cloaca samples from farm 4(NGACL3) and Lung samples from farm 18(NGALG1) and 21 (NGALG2) with 94%, 97% and 97% homology to Nigerian strain. Also from the BLAST, cloaca samples 7 and 9 from farm 4 (the same farm) are closely related to the two different strains which implies that chickens from the same farm could be infected by two different strains or serotypes (Table 4.6.2).

Phylogenetic analysis of the detected IBV and vaccine strains from other countries is in agreement with the BLAST result that NGAL1.NGAL2 and NGAC3 are identical to Nigerian strain and so are protected by it but NGAC1 and NGAC2 are

closely related to Korea strain and distantly related to H12O from other countries like India, China and others (Fig 4.6.5). This implies that H120 may not protect the chicken adequately in case of any challenge from the field from the detected serotypes.It is also important that three of the isolates are closely related to Nigerian strain and cannot be protected by H120.

CHAPTER FIVE
DISCUSSION

The research was carried out to accurately assess knowledge and experience of poultry farmers and veterinarians on infectious bronchitis in order to justify stakeholders' education in the southwestern part of Nigeria being the hub of poultry production. It was also to establish the endemicity of IB in Lagos, Ogun and Oyo states and to identify and characterize circulating IBV isolates in Lagos, Ogun and Oyo states. It was also to establish the cause of vaccine failure in the vaccinated flocks.

Commercial poultry business seems to be dominated by men as shown in Table 4.1 although women's involvement cannot be underated as they are involved in brooding, marketing, recording and so on. According to Ironkwe and Ajayi (2007), this domination by men is because poultry production is labour intensive, full of risks and uncertainties that can hardly be handled by women.This is in accordance with the report of Adisa and Akinkunmi, (2012) who also reported the dominance of men in commercial production of poultry.It has also shown that most respondents were married and Lagos had the least percentage (67.5%) of married and the highest percentage (25%) of unmarried farmers. This is probably due to high cost of living in Lagos as a result of the dense population that also makes accommodation difficult. The high unemployment rate which was 23.1% at the third quarter of 2018 with 55.4% being youth has discouraged marriage among youths (NBS, 2019). However, it is pertinent to say that marriage encourages productivity because of the opportunity of the couple to share knowledge and ideas that will improve the business. Married farmers are likely to be more committed and stable at work because of emotional support from their spouses.

Also, the study showed that most of the respondents underwent education above primary school level with over 60% being graduates in the three States.This confirms the report of Kolawole and Adepoju (2007) that the literacy level in the southwestern

States is higher than in other zones in Nigeria and also corroborated the assertion that most poultry farmers are civil servants, retirees and young graduates and are mostly into small commercial farming (Obi *et al*., 2008). This implies that the farmers will have positive attitude towards innovation and adoption of new techniques which is crucial to successful poultry production since it aids knowledge on identification of symptoms of diseases, medication and vaccination of chickens when necessary.

The majority of farmers in the three States have been in the business for more than five years which implies that they would have been conversant with symptoms of major diseases and probably have experienced outbreaks and consequently, management procedures. Thus the accuracy of information on infectious bronchitis from farmers is likely to be high as earlier opined by Akintunde *et al.* (2015).

With reference to flock number, 50%, 36.2% and 15% of the farmers in Lagos, Ogun and Oyo States respectively, had more than one flock as at the time of this research. Multiple flocks promote infection since there is chance of disease transfer from older chickens to younger chickens and also young chickens may also introduce disease into the flock. Introduction of pathogens is influenced by the density of farms especially for air-borne diseases like infectious bronchitis. (Trustcott *et al*., 2007; Ayim Akonor *et al*.,2018). As regards the farm size, most farmers in the three States operate small commercial poultry farms between 1,000 and 5,000 birds probably because of high cost of poultry production and inability to access credit.As such, most poultry farms grow from backyard poultry as opined by Obi *et al*.,(2008). Farmers in this category may not have access to loans and so are unable to purchase poultry inputs as well as veterinary services thus resorting to self- medication. They also operate under poor management and production techniques (Heise *et al*., 2015) which increase potential for infections and disease outbreaks in flocks. Although, it has been reported that the flock size had no influence on implementing measures of biosecurity but other reports have shown that farmers with large farm area and larger flock size seemed to ensure strict implementation and compliance of biosecurity on their farms (Dorea *et al.,* 2010; Akintunde and Adeoti, 2014).

As regards farmers' awareness of IB, 27.7%, 24.8% and 28.1% of farmers were aware of infectious bronchitis in the study area. This shows that the awareness is still low probably because it shares similar symptoms with other respiratory diseases especially Newcastle disease (Emikpe *et al.,*2010) which has the highest awareness

among farmers. However, the low awareness does not indicate absence or non-prevalence of the disease as Stachowiak *et al*., 2005 reported that 87% of the farmers that responded to questionnaires in Ontario claimed that they did not have continuous problem of the disease even though a prevalence of 14.2% was reported in layers in the province. This shows that, farmers in Nigeria seem to have more awareness of the disease than their counterpart in Canada. In West Africa, most work done on infectious bronchitis was on antibody detection and not much is known on isolation, characterization of IB and pathogenicity of the virus and consequently the economic impact. In Nigeria, Newcastle disease which has similarities with IB is well known to farmers (Aboe *et al*., 2006; Yakubu *et al*., 2014). Thus absence of laboratory confirmation of diseases most times due to poor laboratory facility and financial power might have led to misdiagnosis as there are other respiratory diseases with similar symptoms (Emikpe *et al*., 2010).

With reference to awareness of outbreak of infectious outbreak, 10.8%, 19.0% and 10.4% respondents have experienced outbreak of infectious bronchitis in their farms and the outbreak commonly occurred at 4-6 weeks in Ogun state and 7-8 weeks in Lagos and Oyo States.The outbreak occurring at 4-6 these times is likely due to prior vaccination of breeders at the hatchery which conferred protection on chicks for 3-4 weeks after hatching. Ogun state had the highest percentage of occurrence of outbreak probably because it had highest concentration of poultry farms and also the least percentage of vaccinated farms as shown in (Table 4.1.2). The duration of outbreak in the States is mostly between 3 and 4 weeks in Lagos state and 1 and 2 weeks in Ogun and Oyo States.This is probably because there was no complication due to application of antibiotics without thought of its consequences by poultry farmers in the Southwest (Oluwasile *et al*.,2014).

Confirmation of the IB in the laboratory was high as stated by the respondents that had experienced outbreak probably because farmers seek veterinary service after failed efforts to curtail an outbreak. It could also be because diagnostic services are privately driven and they create awareness of their services.

As regard consultancy services, most Veterinarians in the three States do not consult for poultry farmers, they preferred to own their farm or being into small animal practice probably because most farmers prefer medication without prescription which is considered to be cheaper while some other poultry farmers believe that they do not

need veterinary service having being in the business for some years.The sale of veterinary drugs and input are now privately driven unlike in 1980s when it was regulated by government thus providing unrestricted access to these poultry inputs by farmers (Fagbamila *et al*., 2010; Kingsley, 2015),

Most respondents had between 5 and 10 years of farming and practice experiences.As such it is expected they would have acquired skills for the disease management and control. Most farmers in Lagos and Ogun with few in Oyo had more than one flock of multiple ages on their farms. According to Ayim *et al*., 2018, the most important source of novel variants of IB virus is commercial layer with multiple flocks of different ages on the same farm as periodic introduction of pullets promotes continuous infection of IBV in layers thus escalating the incidence of the disease and a pointer to the possibility of detecting IB virusor its variants in these states.

With regards to vaccination, 22.9%, 19% and 24% of the farmers vaccinated their flocks while 72%, 55.6 % and 66.7% of Veterinarians advised their clients to vaccinate against the disease in the study area. It is noteworthy that vaccinated birds can shed the virus intermittently for up to 24 weeks especially under physical and environmental stress (Ignjatovic and Sapart, 2000, Stachowiak *et al*., 2005) and this may lead to field infection and presence of vaccine strains in unvaccinated chickens. It should be noted that the virus may be transferred horizontally from farm to farm and even through fomites. Live attenuated vaccine could also undergo reversal to virulence under field condition and can lead to outbreaks (Nix *et al*., 2001). The number of farmers that vaccinated their birds is highest in Oyo State probably because they have unrestricted access to vaccines since they can easily purchase vaccines from veterinary product outlets without professional input to the extent that some outlets sell vaccines in fractions contrary to the situation in Ogun and Lagos states where there is reasonable restriction.

With regards to infectious bronchitis outbreak, 10.8%, 19.0% and 10.4% of poultry farmers and 28%, 37% and 30% of veterinarians in Lagos, Ogun and Oyo States, respectively had encountered Infectious bronchitis outbreak on their farm or clients' chicken flocks. Ogun State had the highest number of farmers and veterinarians that had experienced IB outbreak probably because it has the highest concentration of farms and hosts the headquarters of an indigenous diagnostic laboratory with many veterinarians as staff that served as extension officers. The regular trainings

organized by this company for potential and practicing farmers as well as seminars for professionals emphasize the importance of laboratory diagnosis to farmers and professionals since most farmers do not seek veterinarians' advice until there is an outbreak (Isegbe *et al*., 2014) at which time the laboratory is their first point of call. Results also showed that most professionals were consulting for up to 5 or more farms and if care is not taken, could aid disease transmission from one farm to another which implies that veterinarians could also be agents of transmission of the virus within or among states through fomites such as contaminated operators, vehicles, boots or lab coats.

The awareness of infectious bronchitis among poultry farmers is 27.7%, 24.8% and 28.1% in Lagos, Ogun and Oyo States, respectively. It is highest in Oyo State probably because of the unrestricted access of farmers to vaccines through interactions with attendants at veterinary shops who are 'pseudo veterinarians' (Obi *et al*., 2008) followed by Lagos state probably because of the level of literacy in the state which is 92% compared to Ogun and Oyo states that are 62.9% and 62.8% respectively (UNESCO, 2012). Ogun had the lowest level of awareness, probably because it had the highest number of experienced farmers that might have mistaken it for related diseases with similar symptoms such as Newcastle disease, Infectious coryza and Egg drop syndromeand somight not be willing to seek veterinary service or attention.

In conclusion, low level of awareness of infectious bronchitis among poultry farmers could be due to similarities in symptoms of IB with other respiratory diseases especially Newcastle disease which could be confusing to them (Emikpe *et al*.,2010) especially if outbreak occurred at the laying stage. It could also be due to lack or poor disease reporting system and underreporting by animal health workers (Cattoli *et al*., 2010). Low level of awareness of IB among poultry farmers and veterinarians might be responsible for the non-listing of IB among important poultry diseases in Nigeria even though 42.5% seroprevalence was reported by Oyejide *et al.* in the southwest in 1988.

In respect of seroprevalence, the sample population was unvaccinated commercial and local chickens and Ogun State had the highest prevalence of 88% and 85% in both commercial and local chickens, respectively. Aside for the State having the highest concentration of poultry farms, it also has the highest number of households

involved in subsistence farming, while Oyo with the least concentration of poultry farms and number of households involved in subsistence poultry farming. Oyo state had the lowest seroprevalence of 76% and 82% in commercial and local chickens, respectively (Omodele and Okere, 2014; Obi *et al.,* 2008). Backyard poultry subsistence farming has been reported to be sources of infection due to low biosecurity and contact with other chickens especially freshly purchased from markets and wild birds (Whiteford and Shere 2004; Wang *et al*.,2013). Within States and Local governments, varying percentage seroprevalence of IBV was observed. This was directly related to population of poultry farms in sampled areas, age of farms and closeness of farms where samples were obtained to other farms with vaccinated flocks. It was observed that flocks that were isolated and far away from other farms had low antibody titre compared to flocks that were within vaccinated flocks. This was observed at various locations of sample collection especially Idi Omo in Egbeda Local Government, Oyo State where IB virus antibody was not detected in the flock despite the age of the chickens (34 weeks), probably because it was a newly established solitary farm in comparison toforty-seven weeks' flockof chickens with 70% seroprevalence in the same Local Government within a farm settlement. At Aradagun in Badagry Local Government in Lagos state poultry population was low and seroprevalence was lower compared to other locations in the state. This is similar to the report of varying seroprevalence of IB in commercial chickens in four different locations in Pakistan (Kanwal *et al*., 2018). Therefore, high seroprevalence obtained in some of these farms might be due to exposure to the virus shed by chickens from vaccinated or infected flocks and not necessarily as a result of clinical infection.

The high seroprevalence in local chickens suggests the endemicity of the disease since they move from one location to another; get infected or exposed through contact with the fomites or even poultry dungs. It should be noted that most poultry farmers practise open air dumping of farm wastes which may be JUST about 100 metres from the farm (Ogundiran, 2015).

Both commercial and local chickens in the three States had high titers of antibody against IBV which suggests an exposure to the virus either through field infection or shedding of the virus by vaccinated chickens from other farms (Lucio and Fabricant 1990).The results of this study showed that IB virus antibody titer was significantly higher in commercial than local chickens in Lagos and Ogun states compared to titers

in Oyo State, probably because Oyo State has the highest concentration of hatcheries and grandparents farms in the country (Oloso *et al.,* 2019) and so local chickens are likely to be more exposed to vaccine strains than in other states since IB vaccination is routinely carried out in hatcheries.

As regards the age of flocks, age ranges 21-30 and 51-60 weeks old had significantly higher ($p<0.05$) mean antibody titers than the other age groups. Mean antibody titre was highest in the age range 51 – 60. This findings agrees with the report of Javed *et al*.(1991), Bhuiyan *et al*. (2018) and Ayim-Akonor *et al*. (2018) that the prevalence of IB increases with age because of long period of exposure to field virus. The significantly higher ($p<0.05$) mean antibody titer recorded for age range 21-30 weeks old could be due to increase in virus shedding as a result of increase in physical and reproductive activities which could induce immunosuppression (Stoichwaik *et al*., 2005). At this age range, chickens undergo a lot of stress due to transfer from litter to battery cages, vaccination and egg laying.

Concerning the flock size, antibody titres recorded in this study varied across various flock sizes contrary to the report in Austria that respiratory diseases such as IB are not affected by flock size (Yunus *et al*., 2008). However, variations in antibody titre based on flock sizes within states may result from varying adherence of poultry farmers to biosecurity measures.

With reference to the states, there is no significant difference in the antibody titre of IBV (P value ≥ 0.05) in the three states, this is in accordance with several literatures that stated that IBV is prevalent where poultry is intensively reared and so non – significance is probably because poultry production in the country is highly concentrated in the three States (Witt *et al*., 2010; Obi *et al.,* 2008)

In Lagos and Ogun States, seroprevalence obtained in commercial chickens was higher than in local chickens. This agrees with the findings of Shettima *et al*. (2016) in Maiduguri. However, it contradicts result from Oyo State in which the seroprevalence was higher in local than commercial chickens. This is probably due to its sharing border with the Northern part of Nigeria through Kwara state. A high prevalence of 91.3% in indigenous chickens was previously reported in the city of Kano which happens to be a commercial center for local chickens in the North (Oyejide *et al*., 1988). These chickens and other wild birds could aid in IBV

transmission through trans-boundary businesses since most northerners including cattle dealers bring indigenous birds for sale in the southern part of the country. Consequently, indigenes of Oyo state have more access to indigenous chickens from the North than those of Ogun and Lagos states.

The overall seroprevalence of 81% obtained in this study is lower than 91.67% reported by Emikpe *et al*. (2010) and 84% reported by Ducatez *et al.* (2004), probably because this survey was limited to unvaccinated chickens. This overall prevalence of 81% is also lower than 85.5% reported in Ghana (Ayim Akonor, 2018), 99.02% and 98.85% in unvaccinated layers in Trinidad and Tobago, respectively.

As regards to molecular detection, Lucio and Fabicant (1990) and Ignjatovic and Sapats (2000) reported that acute phase infection can be detected using oropharyngeal swabs while the cloaca swab is useful for detection at the chronic stage.The virus is detected between third and fifth day post-infection in the respiratory tract but could be detected in the cloaca for up to twenty–one days post-infection which explains the widespread of the disease and difficulty in its control.

Thus the detection of the virus from the oropharyngeal swab indicated recent or field infection while detection from cloaca swab showed previous infection that led to the shedding of the virus.The overall prevalence of infectious bronchitis virus in the three states was 8.3%, 33.3% and 3.3% of both oropharyngeal and cloaca samples (Table 4.3.3). The percentage of virus detected in cloaca samples is higher than in oropharyngeal samples (Table 4.3.4) probably because of the short duration of detection in the respiratory tract as compared to detection in cloaca samples which could be for months (Ignjatovic and Sapats 2000; de Witt *et al.*,2010). The Positive cloaca results also suggest that the chickens were shedding the virus after an acute infection or could also as a result of environmental stress on laying chickens that had the infection at early stage (Ignjatovic and Sapats 2000; Stoichwoch *et al.,* 2005).The positive results have established the presence of infectious bronchitis in the three states that happens to be the hub of poultry farming in the country.

Generally, the prevalence in commercial chickens was 8.3%, 33.3% and 3.3% in Lagos, Ogun and Oyo States and it is proportional to the number of farms and intensity of production in the States. However, no positive was recorded in local chickens sampled in Lagos and Ogun States but 12.5% of local samples from Oyo

State were positive. Overall prevalence in each state was 5%, 20% and 7% in Lagos, Ogun and Oyo States respectively. The overall prevalence for the three States was 10.7%. It is pertinent to emphasize that Ogun State has the highest poultry farms because of its closeness to Lagos State thus having marketing advantage and availability of large expanse of land unlike Lagos State. Consequently, most farmers in Lagos state actually have their farms in Ogun State. Thus prevalenceof infectious bronchitis is basd on intensive poultry production as stated above. Thus low prevalence in Lagos must have been due to low farming activities due to non – availability of land. Among local governments, Ijebu North has the highest percentage of detection probably because it seems to be the poultry hub of Ijebu- land and it is dominated by medium scale commercial category with equal number of the backyard and large scale poultry farms (Omodele and Okere, 2014). The sharing of boundary with Ibadan could also be a factor since the novel IBV was detected there and this study also confirmed many positive cases from local chickens indicating that the disease existed among local chickens and could easily be transmitted by them because of their high activity and their scavenging habit (Ohore *et al.,* 2007). The high prevalence recorded in Ado – Odo/Ota and Obafemi/Owode is probably due to their proximity to Lagos and so they have highest number of performing farms. The closeness of Ade –Odo/Ota to Republic of Benin could be a source infection due to unrestricted movement of poultry and poultry products into the country (Obi *et al*., 2008; Omodele *et al*., 2014). The prevalence for the three states was 10.7 %, this is lower than 26% prevalence reported earlier in Nigeria (Ducatez *et al*., 2006). This is probably because the research was on unvaccinated chickens and did not include other types of chickens.It is lower than 64% prevalence reported in Ghana in unvaccinated flocks probably because the samples screened for IBV in Ghana were from farms where chickens were manifesting respiratory symptoms . The 10.7% recorded in this research seems high because the samples were obtained from unvaccinated flocks and it becomes complicated with the 15.6% detected in local birds which portends a very high prevalence in future because of the mode of spread of the disease especiallythrough fomites.

For local chickens, 12.5% was recorded in Oyo State which suggests a clinical disease and potential source of spread of the disease. ‘Ibadan genotype was discovered in Ibadan thus probably suggests that the IB virus is indigenous and probably spread to other States even among commercial chickens. None of the local

samples from Ogun and Lagos was positive. This is similar to the report in Ghana in local chickens although the sample size was smaller (Anyim-Akonor *et al*., 2018). Generally, most commercial layers are raised on a farm with many flocks of different ages and types and this has been suggested to be a source of IBV outbreak and variant. The introduction of new pullets at intervals and the continual re- infection and recycling of IBV in layers results in a greater chance for infection and spread because it does not allow complete and total disinfection of farms after sales. The poor or no biosecurity of most farms could also be the cause and spread of infection since the susceptibility of the virus increase with bacterial infections, immunosuppressive infections and management problems.

The disease could also be imported into the country through the purchase of Grandparent and Parent stocks since all grandparent and Parent stocks used in Nigeria are sourced from Europe especially Holland, Belgium, UK, Israel and recently Egypt (Adene and Oguntade, 2006) Lack of policy or strict compliance to the policy or enforcement of policy enable poultry farmers to import chickens indiscriminately including infected or IBV vaccinated Grandparents or Parent stocks to the country. There is also no restriction to importation of poultry vaccines in the country and consequently no regulation on poultry vaccination or strict vaccination regime based on the common diseases detected in the country. Lack of knowledge of the disease or control measures by Government or its agencies promote the spread of the disease and adversely affect poultry industry. It is therefore imperative to infer that infection in the area of study is due to clinical infection or exposure to vaccine strain resulting from reversal to virulence. However, the detection of the virus in both cloaca and oropharyngeal samples has confirmed presence of the disease.

Mutation is a change in the genetic material that can be passed to the next generation and it occurs as a result of substitution, insertion and deletion. It could be neutral, advantageous or deleterious depending on its impact on the organism. Substitution involves exchange of single base for the other. Insertion is a mutation with addition of at least one extra in the sequence and deletion is the removal of at least one base from the sequence and it has similar consequences as of insertion. In multiple sequence alignment, a given sequence is compared to a group of other sequences from related sequences thus in this wise, sixteen sequences of the isolates were compared with one another.In the multiple alignment of 1b gene, areas of point mutations were seen, C toT, A to T and G to A and also deletion in pools 127 and

161 between 0 – 56 and 0 – 30 respectively which do not affect the multiple alignment of protein (Figure 4.4.1 and 4.4.2) which suggests that the mutation is silent. However, there are conserved areas of the sequences which show similarities and show they are related. It is important to know that insertions and deletions are common in sequences belonging to the same family and often occur at the loop regions.

The BLAST result showed that all the samples were between 96% and 100% homologous to the Nigerian strain IBVNGR/AE116E7/2006 except pool 70 which was 96% homologous to European turkey coronavirus. This is in agreement with the report of a Nigerian strain described by Ducatez *et al*., 2009 and indicates the uniqueness of the strain to Nigeria which will help in the control of the disease (Callison *et al.,* 2001; Mo *et al*., 2013)

The % G – C content varies from 37.2 and 39.0(for 1b gene) and 37.2 and 37.7 (S1 gene) G – C (Guanine- Cytosine) content is the percentage of nitrogeneous bases on a DNA or RNA molecule that are either guanine or cytosine (from a possibility of four different ones, also including adenine and thymine in DNA and adenine and uracil in RNA. Importance of the G – C base pair is its higher thermal stability compared with AT base pair, a feature that arises from stacking interaction between GC bases and the presence of triple compound with hydrogen bond between the paired bases (Yakovchuk *et al*., 2006). Two additional features of G-C base pair are its higher mutability related to frequent cytosine methylation and the high cost of its synthesis compared with AT base pair In PCR experiment, G – C content of primers are used to predict their annealing temperature. Consequently, weak % G – C indicates weak hydrogen bond, resulting in low thermal stability of the isolates and subsequently high rate of mutation. This implies that the higher the G-C percentage content, the more stable the isolate therefore isolate with 37.3% is less stable than isolate with 39.3% isolate. Since the GC content of infectious bronchitis is 38% (Woo *et al*.,2010), it is therefore imperative that the isolates of 1b are more stable than those of S1gene . Most of the isolates of S1 gene have GC content of less than 35% this is probably because it is a hypervarible region and it implies that it is proned to mutation. Consequently, the serotypes obtained in a region can continuously change and so molecular characterization needs to be done regularly at least at five year interval for effective control of the disease with the right choice of vaccine.

To identify the serotypes, sequences of eleven positive samples were randomly picked, blasted and compare with the strains deposited in Gen Bank, the result showed that all the sequences showed different percentage homology to five different strains that are independent of location, local government or state of sample collection. These are: IBV/NGA/A176/2006 from Nigeria, AIBV strain IS/585/98 and AIBV strain IS/572/98 from Israel. It also includes Variant 2 strain Israel and CK/CH/HUN/NTP strain form China.The predominance of strains from Israel might be as a result of importation of Grandparents, parents and even chicks from Israel. Variant 2 strains are predominant in Middle East and Israel is in the Middle East and so the likelihood of vaccinating these chickens with the strains in that country before importation and subsequent shedding of the virus due to stress in the country might be a means of introducing IBV into the country. Isolate (sample 20 taken at Ikorodu) in Lagos state and isolates from samples from Mowe, Idomila (Ijebu North East) were closely related to Nigerian strain. Six of the sequences of the isolates were related to two strains from Israel, IS/885/98 and Variant 2. BLAST results also showed that samples from Sasha, Oyo State and Obada, Ogun state were related to variant 2 from Israel which means the strain is not limited to commercial birds and the local chickens must have been exposed to the strain. The last pooled positive sample, 163 from Ikorodu has 99% homology to a strain AIBV isolate/CK/CH/HUN/NTP with accession number KX107793 from China and the only isolate and it is likely to be a vaccine strain.

Sequence identity was done to know the level of cross-protection of the isolates and it is the amount of characters which match exactly between two different sequences. Sequence identity showed relatedness and those that have antigenic related value (ARV) between 50 and 100 are said to be related and those below 50 are said to be unrelated. Generally, different serotypes of the virus do not confer cross protection against each other as cross protection decreases as the degree of amino acid identity between S1 protein of 2 IBV strain decreases thus Isolates with very high S1 sequence identities induced consistently higher levels of cross protection than isolates with lower sequence identities (Ignjatovic and sapart, 2000, Gelb *et al*., 2005) Consequently, those that have sequence identity of 50 and above are related and may show a very low cross protection while those below 50 are not related and will not have any cross protection against each other. It thus implies that pool 35 is related to pool 126,127 and 132. Also 160, 161 and 163. Pools 20 and 213 are also related to

pool 35. Therefore there is likelihood of weak cross-protection among them because the maximum relationship is 55%.

Phylogenetic analysis of sequencing partial 1b gene to know the genus indicates two distinct clusters, 14 (87.5%) out of 16 were closely related to the strain from Nigeria, NGA/A116E7/2006 while 2 (12.5%) were related to Italy 02 genotype ITA/90254/2005. This is in agreement with the report that Italy 02 shared 71% nucleotide identity with NGA/A116E7/2007(Ducatez *et al.,* 2009). The Italy 02 genotype is very predominant in Europe. It has been reported in France, Spain, United Kingdom and Gemany (Worthington *et al*., 2004; Jones *et al*., 2004). It has also been reported for the first time in Africa from Morrocco in poultry farms between 2010 and 2014 (Fellahi *et al.,* 2015). It therefore implies that Italy 02 must have been imported into Nigeria through day old chicks or breeders since Nigerian poultry farms sourced their breeders from these countries (Adene and Oguntade, 2006). The samples are 20 and 70, pool 20 was from commercial chicken in Ikorodu, Lagos and pool 70 was from local chickens in Oyo State. This suggests that two prevalent strains of IB coronavirus are available in the southwest, Nigeria. On partial analysis of S1 sequences, three distinct clusters were also identified. One, Pool 163, closely related to H120, vaccine strain from Netherland. Two, those related to Variant 2, these include Pools 160, 161, 132, 126, 127 and 213. Three, these are closely related to novel Nigeria genotype, (FN182269 NGA/N544/2006) and they are Pools 20,135 and 139. This implies that 10%, 60% and 30 % of the positive samples analysed are closely related to Massachusetts, Variant 2 and Nigerian strains respectively. While the only closely related to Massachusetts sample was from Ikorodu in Lagos State, those that were related to Variant 2 were found in all the three states including local birds in Sasha, Oyo State. Nigerian strains related sample were from Lagos and Ogun State. It is noteworthy that sample from Ikorodu related to Massachussets is the only sample that blast result showed close relationship to China strain (CK/CH/HUN/NTP) which is likely to be a vaccine strain. Nigerian strain was first described by Ducatez *et al*., 2009 and it has been found once in breeders in Belgium (De Herdt *et al.,* 2016).

Generally, variant strains emerge due to changes in the IBV genome through point mutations, deletions, insertion or RNA recombinations and these variants are responsible for outbreak in vaccinated flock (Liu *et al.,*2007) and multiple IBV variant strains are circulating in the poultry flocks in many countries (de Wit *et*

al.,2011). Variant 2 has been reported in the Middle East and North Africa (Meir, 2004), Iraq (Mahmood *et al*., 2011), Egypt, Turkey (Kahya *et al.,* 2013) and Libya (Awad *et al.,* 2014a). Presently IBV variant 2 is the predominant serotype in Egypt causing losses in chickens (Susan *et al*., 2011) and the chickens in the southwest are sourced from most of these countries. It is important to state that most of these serotypes might likely be due to mutations as seen in the multiple alignments even though the possibility of importation cannot be over emphasized.

Presently, H120 strain vaccine has been used successfully for many years to prevent IB globally and this would have informed importation of the vaccine from different countries indiscriminately into Nigeria (Callison *et al*., 2006; Lin & Chen, 2017). Thus, continuous shedding of the virus by chickens or reversal to virulence causing clinical diseases and also recombination between vaccine and field strain could be responsible for the emergence of serotypes and variants obtained in the study (Zhang *et al*., 2010: Bande *et al*., 2015).

In vaccinated flocks, the emergence of two serotypes that were identical to South Korea and Nigerian strains has shown again circulation of multiple serotypes in the poultry industry and thus suggests why outbreaks occurred in flocks vaccinated against IB. It has once again been established that H120 could not confer immunity on the Nigerian strain which is another reason for outbreaks in vaccinated flocks (Kahya *et al*., 2013) as shown by multiple protein alignment. The BLAST results and the phylogenetic analysis have also queried the complete protection of H120 vaccines available in the states and suggested that South Korea strain is better preferred to offer adequate protection against some isolates in the states. The detection of different serotypes in the same farm indicates multiple infections in the farm and is probably the cause of vaccine failure. It is noteworthy that none of the serotypes could be protected by H120 as shown by the blast result and phylogenetic analysis, which implies that sometimes vaccination with H120 is not effective. This result has confirmed the insinuation by Ducatez *et al* 2009 and Valestro *et al*., 2016 that H120 might not protect against Nigerian strain.

Multiple nucleotide alignment indicated the replacement of double ring guanine (purines) in most of the vaccines notably H120 with single ring thymine (pyrimidines). This implies that transversion and not transition mutation has taken place. Transition mutation is exchanging the same number of ring in the nucleotide

base, that is, a one ring- pyrimidine with another pyrimidine, or a two ring purine for another purine while transversion mutation is the change in the nucleotide from a purine to a pyrimidine or vice versa. Thus, transversion is more likely to result in an amino acid substitution because the third nucleotide codon position of the DNA that is responsible for the degeneracy of the code is less tolerant of transversion. However, the amino acid multiple alignments have negated the observation because of the similarities of arginine amino acid. The strains from Argentina, China, India and the South Korea are to be related to the strain which implies that they are all Massachusettes vaccine type serotypes.It can therefore be emphasized that Massachusettses vaccine cannot protect against the Nigerian strain. Also IS1173(FJ589732),IS1618(FJ589733)JPN(AY36398) and MYL(EF591036) from Isreal,Japan and Malaysia cannot protect against both the Nigerian and Massachussetes strains which are present in Nigeria. It is therefore advantageous that vaccines should be produced from Nigerian strain for effective protection against the disease and heterogenous vaccination regime that will accommodate all the strains should be adopted. It is pertinent to say that two groups of IBV exist in South Korea, South Korean group 1 and 2, South Korean group 1 is closely related to Massachussets strain and its emergence is due to mutation of H120 strain while group 2 has three subgroups, some of which are nephropathogenic (Lim *et al.,* 2012). It can therefore be inferred that South Korean strain preferable for control of IBV must have resulted mutation of H120 strain. The study has also shown nucleotide sequencing and identification of amino acids substitution involving N gene that involves change of arginine to lysine or glutamine and this is suggestive of vaccine failures due to antigenic variation (Kuo *et al.,* 2013). Thus, it is important to state that live vaccinations are used globally for the control of IB and can result in over throwing pathogenicity and genetic modification which may cause mutation rate of up to 1.5%. The appearance of mutations in the vaccine viruses after their passage of field population is considered as one of the reasons for vaccine failure (Abdelheq *et al.,* 2015)

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 SUMMARY

Poultry production is intensively practiced in the studied three States and is dominated by male, educated and experienced farmers. Most experienced farmers were in Ogun state because it had the highest concentration of farms, large expanse of land and it is close to Lagos where there is high demand for eggs and poultry meat. The availability of land made it easier for undisrupted poultry business. Most farmers engaged in small commercial poultry farming with the flock size ranging between 1,000 and 5,000. The farmers kept multiple flocks which encouraged diseases including infectious bronchitis to spread from one flock to another flock. Although the awareness of infectious bronchitis is low in these three states, some farmers had experienced outbreak and so vaccinations against the disease were carried out in some farms as recommended by veterinarians or other farmers. This confirms the presence of the virus in circulation.

Shedding of the virus during vaccination and outbreaks contributed to the seroprevalence of the virus which suggest that the disease is endemic and the constant exposure to the virus explains why most chickens had high level of antibodies against the disease in the three States. The seroprevalence as had been established is not dependent on the flock size, age of the birds, state of collection but location which supports the fact that the disease is prevalent where intensive poultry production is practised and confirms that Lagos, Ogun and Oyo states are the hub of poultry production. The seroprevalence was highest in Ogun state almost with both local and commercial poultry having the same percentage that is, 88% and 85% respectively, Lagos State had 83% and 76% in commercial and local chickens respectively but for Oyo State, it was more prevalent in local than commercial

chickens and this translated to more infectious bronchitis virus detection in local birds. It is worth mentioning that Oyo State is the closest to the Northern States and since one of the mode of transmission is through live bird markets, it is thus possible that the virus is transmitted to southwest from the North. The prevalent is not high compared to Newcastle disease but 10.7% prevalence in unvaccinated and one type of chicken is significant. The potential spread by local chickens because of their scavenging nature portends danger for the poultry industry and an exponential increase in the prevalence of the disease in the future. It is noteworthy that the disease might also be prevalent in cities and villages with high population of local chickens in the North as it might have influenced the number of positive result in local chickens in Oyo State moreso the disease is said to be spread by migratory birds.

Sequencing of the 1b gene of the virus has revealed a major strain which is Nigerian strain thus suggesting that the strain is peculiar to the region and so makes control of the disease easy by vaccination. However, sequencing of S1 gene has established that the Nigerian strain is specific to the region and five serotypes are circulating in the southwest which suggests the possibility of vaccine failures due to multiple serotypes and so difficulty in control. It has been shown that most of the isolated serotypes cannot protect against each other as this is collaborated by the emergence of five different serotypes. It has also been shown that most of the serotypes belonged to Nigerian strain and Variant 2 which implies that for effective control, vaccine must be produced from Nigerian strain and the vaccination regime that will be heterogeneous in nature incorporating the three strains should be considered having established possibility of multiple infection and non – protectiveness of H120 against Nigerian strain.

H120 is the only vaccine strain available for vaccination in the studied States and the research has shown that it cannot protect against the Nigerians strains and most of the isolated strains. Findings have shown that South Korea 210 is preferable and will protect some isolates adequately. The H120 Netherland isolate has confirmed vaccination against infectious bronchitis as stated by farmers and veterinarians and with the detection of the virus especially in the oropharyngeal swab has confirmed field infection which suggests possibility of recombination. Also point mutations, nucleotide insertion and deletions caused evolution of the genome and so difficulty in control of the disease. The outbreak of infectious bronchitis as a result of two

different serotypes has also confirmed the complicity in the control of the disease thus for effective control, vaccines and vaccination must include all the serotypes and characterization must be carried out often to detect emergence of new variants

6.2 CONCLUSION

1. Awareness of IB in southwestern Nigeria is low even though farmers vaccinate against it.

2. There is shedding of the virus during outbreaks and vaccinations resulting in high seroprevalence observed in both local and commercial chickens.

3. The detection of IBV in local chickens portends danger as they contribute to the spread while scanvenging.

4. Nigerian strains and Italy O2 are the genotypes available in Nigeria

5. Two main serotypes (Nigeria and variant 2) are circulating in southwestern Nigeria and so farmers will experience outbreaks because available vaccine are not produced from the strains.

6.3 RECOMMENDATIONS

1. Veterinary structure must be strengthened so as to ensure testing and quarantine of poultry genetic material being imported into the country.

2. Importation of vaccines should be strictly monitored to prevent introduction of new strains of the virus into the country.

3. Handling and administration of vaccines and vaccination should be strictly by veterinary officers to prevent mishandling and subsequent introduction of IB.

4. National Veterinary Research Institute should be empowered to produce local IB vaccines as most imported vaccines cannot protect against our local diseases.

5. The country or each State should have its own vaccination regime based on available strains to prevent indiscriminate use of vaccine and spread of the disease.

6. Monitoring activities of hatcheries to ensure strict compliance to standard rule of operation.

7. Characterization of the virus should be consistently and regularly done for prevailing strains since the virus has tendency of continuous mutation. This will ensure effective control through vaccines and vaccination.

8. There should be constant and regular seminars for poultry farmers to intimate them of emerging diseases and the best way of controlling them.

9. In case of outbreaks, the virus is susceptible to common disinfectants like virkons, ethers, sodium hypochlorite.

6.4 CONTRIBUTIONS TO KNOWLEDGE

This study has been able to contribute to knowledge through the following:

i. The establishment of low level of awareness of infectious bronchitis and vaccination against it by farmers.

ii. The establishment of moderately high prevalence of IB in commercial and local chickens in south western Nigeria.

iii. The establishment of presence of infectious bronchitis in the south western Nigeria.

iv. That Nigeria and Italy 02 strains of IB virus are the genotypes distinct and perculiar tothe south western Nigeria.

v. Five infectious bronchitis virus serotypes are circulating in south western Nigeria and they are not cross-protective.

vi. This is the first time variant 2 serotype will be discovered in Nigeria.

vii. That H120 vaccine currently in use in Nigeria does not adequately protect against the available strains of the virus.

6.5 FURTHER STUDIES

There is need to establish the biological characteristics of the serotypes in relation to virulence.

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APPENDIX 1:

QUESTIONAIRE ON THE PREVALENCE OF INFECTIOUS BRONCHITIS

Demographic information

Sex …….. ..Male ( ) Female ( )

Marital status ……Single ( ) Married ( ) Widowed ( )

Level of Education …… No formal education ( ) Primary education ( ) Secondary education Tertiary education ( )

1. Location of farms……,…. ……
   GPS……
2. Location Government Area…….
3. When did you start the farm ……
4. How many flocks do you have in your farm? ……
5. List the flocks and size of each ……
6. Vaccination record Mareks ( ) Gumboro ( ) La Sota ( ) NDVK ( ) IB ( ) ND/IB/EDS ( )
7. Have you heard of infectious bronchitis disease Yes ( ) NO ( )
8. Have you ever had an outbreak of infectious Bronchitis? Yes ( ) No ( )
9. If yes, at what age did you have it?
10. What signs did you observe? A. Fall in egg production Yes ( ) No ( )

B. Misshaped or malformed egg Yes ( ) No ( )

C. Watery albumen Yes No ( )

D. Coughing and sneezing.

e. Mortality.

11. What was the duration of the outbreak?

12. Was the outbreak confirmed?

13. Was the diagnosis confirmed? Yes ( ) No ( )

14. If yes, how was it confirmed?

15. Have you ever vaccinated your birds against the disease? Yes ( ) No ( )

16. Do you still vaccinate? Yes ( ) No ( )

17. If yes, when do you vaccinate? A. 1-2weeks B. 3 – 6weeks C. 15 – 18weeks.

18. Have you ever observed the following symptoms?

a. Sharp and sudden drop in production. Yes ( ) No ( )

b. Malformed or misshaped egg Yes ( ) No ( )

c. Watery albumen Yes ( ) No ( )

d. Coughing and sneezing especially in chicks Yes ( ) No ( )

19. if yes, what did you do ?

20. What was the outcome?

APPENDIX 11

QUESTIONAIRES FOR POULTRY CONSULTANTS

1. Do you work for Government or Private (1) Government ( ) (2) Private ()
2. What is your designation?
3. How long have you been into veterinary practice < 5years ( ) > 5 years ( ) < 10 years > 10 years.
4. Do you consult for poultry farmers? Yes ( ) No ( )
5. If yes, how many farms? (1) 1-5 farms (2) 6- 10 farms (3) 11- 15 farms
6. If No, do you have your own farm? Yes ( ) No ( )
7. Do you advise poultry farmers to vaccinate against IB. (1) Yes (2) No
8. If yes, why (1) prevention ( ) (2) After outbreak in a farm (3) After outbreak in an area.
9. Have you ever suspected IB? (1) Yes ( ) (2) No ( )
10. How many cases? (1) 1-5 cases (2) 6- 10 cases (3) 11- 15 cases
11. How? Clinical signs ( ) Post mortem lesions ( ) Clinical and post mortem lesions ( )
12. What were the symptoms observed? (1) Fall in egg production (2) Watery albumen (3) Misshaped or malformed eggs
13. Did you confirm it? Yes ( ) No ( )

Appendix 111: Distribution of serum samples collected from Lagos and their infectious bronchitis antibody status

FARM	LOCATION	LOCAL GOVERNMENT AREA	AGE (weeks)	FLOCK SIZE	NO OF SAMPLE TAKEN	POSITIVE SAMPLE (%)
1	Odo-Ngunyan	Ikorodu	38	2,200	10	10(100)
2	Odo – Ngunyan	Ikorodu	36	1,000	10	10(100)
3	Odo – Ngunyan	Ikorodu	52	2,200	10	10(100)
4	Igbogbo	Ikorodu	37	2,000	10	10(100)
5	Igbogbo	Igbogbo/Bayeku	25	1,000	10	10(100)
6	Igbogbo	Igbogbo/Bayeku	32	4,059	10	9(100)
7	Poka	Epe	42	1,500	10	10(100)
8	Araga	Epe	20	3,000	10	10(100)
9	Araga	Epe	32	4,000	10	10(100)
10	Eleko	Ibeju/Lekki	35	2,000	10	10(100)
11	Eleko	Ibeju/Lekki	40	1,000	10	10(100)
12	Eleko	Ibeju/Lekki	30	2,000	10	4(40)
13	Aradagun	Badagry	40	4,000	10	2(20)
14	Aradagun	Badagry	22	3,859	10	8(80)
15	Aradagun	Badagry	32	2,800	10	2(20)

Appendix IV: Distribution of serum samples collected from Ogun and their infectious bronchitis antibody status

Farm	Location	LGA	Age (weeks)	Flock Size	No Of Samples Taken	No Positive (%)
1	Ade-Odo	Ade/Ota	48	2,700	10	10(100)
2	Ade-Odo	Ade - Odo/Ota	24	6,652	10	10(100)
3	Ade-Odo	Ade-Odo/Ota	23	3,500	10	10(100)
4	Obada-Oko	Ewekoro	39	3,800	10	10(80)
5	Obada-Oko	Ewekoro	45	4,300	10	10(80)
6	Obada-Oko	Ewekoro	18	2,000	10	10(90)
7	Oke Ata	Abeokuta North	27	2,500	10	10(90)
8	Oke –Ata	Abeokuta North	43	6,500	10	10(80)
9	Oke – Ata	Abeokuta North	15	1,100	10	10(70)
10	Mowe	Obafemi/ Owode	10	1,020	10	10(60)
11	Mowe	Obafemi/ Owode	38	2,653	10	10(80)
12	Mowe	Obafemi/ Owode	29	1,350	10	10(90)
13	Idomila	Ijebu North East	32	4,560	10	10(100)
14	Idomila	Ijebu North East	15	3,670	10	10(100)
15	Idomila	Ijebu North East	46	4,456	10	10(100)

Appendix V: Distribution of serum samples collected from Oyo and their infectious bronchitis antibody status

Farm	Location	Local Government Area	Age (weeks)	Flock Size	No of Sample Taken	No of Sample (%)
1	Idi –Omo	Egbeda	34	1,500	10	0
2	Idi – Omo	Egbeda	47	3,600	10	7(70)
3	Erinmi	Egbeda	48	1,800	10	10(100)
4	Abadina	Ibadan North	46	4,850	10	9(90)
5	Abadina	Ibadan North	13	1,750	10	1(10)
6	Abadina	Ibadan North	59	3,950	10	8(80)
7	Sasha	Akinyele	29	4,890	10	9(90)
8	Sasha	Akinyele	19	3,335	10	9(90)
9	Sasha	Akinyele	26	4,952	10	10(100)
10	Apatere	Lagelu	45	5,890	10	6(60)
11	Ejioku	Lagelu	60	1,020	10	6(60)
12	Ilegbon	Lagelu	33	1,780	10	10(100)
13	Badeku	Ona – Ara	35	1,000	10	9(90)
14	Badeku	Ona – Ara	20	3.959	10	10(100)
15	Jago	Ona – Ara	30	1,876	10	10(100)

Appendix VI:

Mean ± SEM of infectious bronchitis virus antibody titers (ELISA Units) in commercial and local chickens in Lagos, Ogun and Oyo States

State	Type of birds	N	Mean±SEM
Lagos	Commercial	150	49.74 ±2.50[a]
	Local	100	24.71 ±2.02[b]
	Total	**250**	**39.73 ± 1.87[a]**
Ogun	Commercial	150	42.81 ±2.38[a]
	Local	100	37.75 ± 3.10[a]
	Total	**250**	**44.44 ± 2.15[a]**
Oyo	Commercial	150	43.25 ± 4.64[a]
	Local	100	31.85 ±2.24[b]
	Total	**250**	**38.69 ±2.94[a]**

Appendix V11:

Mean ± SEM of infectious bronchitis virus antibody titers (ELISA Units) in different age groups of commercial chickens in Lagos, Ogun and Oyo States

Age of flocks	N	Mean±SEM
10-20 weeks	100	45.46 ± 2.77^{b}
21-30 weeks	100	53.00 ± 6.42^{a}
31-40 weeks	120	36.60 ± 2.55^{b}
41-50 weeks	90	42.41 ± 3.66^{b}
51-60 weeks	40	57.88 ± 5.36^{a}
Total	**450**	**45.27 ± 1.93**

Appendix VI11: Mean ± SEM of infectious bronchitis virus antibody titers (ELISA Units) in different flock sizes of commercial chickens in Lagos, Ogun and Oyo States

Flock size	N	Mean ± SEM
1000-2000	170	40.79 ±2.28 [b]
2001-3000	80	51.29 ±3.48[ab]
3001-4000	100	40.00 ± 2.85 [b]
4001-5000	70	54.36 ±9.02[a]
5001-6000	20	42.65 ±8.23 [ab]
6001-7000	10	67.50 ± 8.54[a]
Total	**450**	**45.27 ±1.93**

Appendix IX: Analysis of data on antibodies titre against IB in commercial and local chickens in Lagos, Ogun and Oyo states

FARM 1		OGUN					STD/ERROR
	AGE(WKS	POPULATION	S/P RATIO	ELISA UNIT	STD DEV.	MEAN	STD/ERROR
1	48	2,700	0.5561	56			
2	48	2,700	0.1725	17			
3	48	2,700	0.5821	58			
4	48	2,700	0.1213	12			
5	48	2,700	0.44	44	0.235014074	0.48342	0.074
6	48	2,700	0.3349	33			
7	48	2,700	0.7686	77			
8	48	2,700	0.8656	87			
9	48	2,700	0.5325	53			
10	48	3	0.4606	46	483		
FARM 2							
1	24	6,654	0.6039	60			
2	24	6,654	0.3888	39			
3	24	6,654	0.9795	98			
4	24	6,654	0.114	11	0.268908832	0.67573	0.085
5	24	6,654	0.5867	59			
6	24	6,654	0.9306	93			
7	24	6,654	0.8297	83			
8	24	6,654	0.6536	65			
9	24	6,654	0.7739	77			
10	24	6,654	0.8966	90			
FARM 3					675		
1	23	3,500	0.449	45			
2	23	3,500	0.485	49			
3	23	3,500	0.1129	11			
4	23	3,500	0.683	68			
5	23	3,500	0.0907	9.1	0.312245969	0.40944	0.098
6	23	3,500	0.1725	17			
7	23	3,500	0.0422	4.2			
8	23	3,500	0.951	95			
9	23	3,500	0.3544	35			
10	23	3,500	0.7537	75	408.3		
FARM 4							

1	39	3,800	0.7002	70			
2	39	3,800	0.226	23			
3	39	3,800	0.6257	63			
4	39	3,800	0.5431	54			
5	39	3,800	0.078	8	0.337014401	0.43997	0.1065
6	39	3,800	0.1665	17			
7	39	3,800	0.8664	86	33.47967875		
8	39	3,800	0.179	18			
9	39	3,800	0.0609	6			
10	39	3,800	0.9539	95			
FARM 5							
					440	38.5	
1	45	4,300	0.1419	14			
2	45	4,300	0.6458	65			
3	45	4,300	0.3593	36			
4	45	4,300	0.8909	89			
5	45	4,300	0.756	75	0.288314019	0.55177	0.091
6	45	4,300	0.1676	17			
7	45	4,300	0.5512	55			
8	45	4,300	0.9677	97			
9	45	4,300	0.6841	68			
10	45	4,300	0.3532	35			
					551		
FARM 6							
1	18	2,000	0.871	87			
2	18	2,000	0.1228	12			
3	18	2,000	0.0907	9			
4	18	2,000	0.3991	40	0.298638837	0.38858	0.091
5	18	2,000	0.6801	68			
6	18	2,000	0.1866	19			
7	18	2,000	0.1006	10			
8	18	2,000	0.2382	24		29.82467	
9	18	2,000	0.3849	38			
10	18	2,000	0.8118	81			
					388		
FARM 7							
1	27	2,500	1.0613	106			
2	27	2,500	0.3376	34			
3	27	2,500	0.4243	42			
4	27	2,500	0.0368	4			
5	27	2,500	0.145	15			

6	27	2,500	0.5672	57	0.324647405		0.38098
7	27	2,500	0.0426	4			
8	27	2,500	0.6303	63			
9	27	2,500	0.0861	9			
10	27	2,500	0.4786	48			
					382		
FARM 8							
1	43	6,500	0.0617	6			
2	43	6,500	0.8205	82			
3	43	6,500	0.6555	66			
4	43	6,500	0.054	5			
5	43	6,500	0.0257	2	0.346545688		0.42215
6	43	6,500	0.5607	56			
7	43	6,500	0.923	92			
8	43	6,500	0.0536	5		419	
9	43	6,500	0.4484	44			
10	43	6,500	0.6184	61			
FARM 9							
1	15	1,100	0.0869	9			
2	15	1,100	0.3945	39			
3	15	1,100	0.5752	58			
4	15	1,100	0.1709	17			
5	15	1,100	0.0196	2	0.244005802		0.37124
6	15	1,100	0.393	39			
7	15	1,100	0.7197	72			
8	15	1,100	0.187	19			
9	15	1,100	0.6259	63			
10	15	1,100	0.5397	54			
					372		
FARM 10							
1	10	1,020	0.29	29			
2	10	1,020	0.17	17			
3	10	1,020	0.53	53			
4	10	1,020	0.06	6			
5	10	1,020	0.12	12			
6	10	1,020	0.11	11	0.38484629		0.362
7	10	1,020	0.27	27			
8	10	1,020	0.3	30			
9	10	1,020	0.39	39			
10	10	1,020	1.38	138		362	
FARM 11							
1	38	2,653	0.19	19			

2	38	2,653	1.25	125			
3	38	2,653	0.39	39			
4	38	2,653	0.65	65			
5	38	2,653	0.27	27	0.355135342		0.591
6	38	2,653	0.25	25			
7	38	2,653	1.02	102			
8	38	2,653	0.87	87			
9	38	2,653	0.48	48			
10	38	2,653	0.54	54			
						591	
FARM 12							
1	29	1,350	0.19	19			
2	29	1,350	0.59	59			
3	29	1,350	0.62	62			
4	29	1,350	1.15	115			
5	29	1,350	0.73	73	0.268222544		0.539
6	29	1,350	0.28	28			
7	29	1,350	0.53	53			
8	29	1,350	0.38	38			
9	29	1,350	0.43	43			
10	29	1,350	0.49	49			
					539		
FARM 13							
1	32	4,560	0.38	38			
2	32	4,560	0.3	30			
3	32	4,560	0.64	64			
4	32	4,560	0.78	78			
5	32	4,560	0.03	3			34.9
6	32	4,560	0.31	31	21.9465		
7	32	4,560	0.32	32			
8	32	4,560	0.35	35			
9	32	4,560	0.24	24			
10	32	4,560	0.14	14			
FARM 14						349	
1	15	3,670	0.12	12			
2	15	3,670	0.15	15			
3	15	3,670	0.79	79			
4	15	3,670	0.29	29	0.228512095		
5	15	3,670	0.11	11			0.332
6	15	3,670	0.29	29			
7	15	3,670	0.28	28			
8	15	3,670	0.6	60			
9	15	3,670	0.51	51			
10	15	3,670	0.18	18			

							332	
FARM								
15						22.85120955		
1	46	4,456	0.45	45				
2	46	4,456	0.27	27				
3	46	4,456	0.06	6				
4	46	4,456	0.22	22				
5	46	4,456	0.27	27	0.122583305			0.246
6	46	4,456	0.14	14				
7	46	4,456	0.34	34				
8	46	4,456	0.31	31				
9	46	4,456	0.32	32				
10	46	4,456	0.08	8				
				246	0.296499146	0.436152		
LAGOS	LOCAL							
FARM								
1				65.4228				
1	0.57	57		6537.3				
2	0.42	42		39				
3	0.34	34		43.582				
4	0.31	31		29.64033183				
5	0.35	35						
6	0.14	14						
7	0.25	25	0.155817032	0.235				
8	0.01	1						
9	0.09	9						
10	0.2	20						
11	0.46	46						
12	0.1	10						
13	0.18	18						
14	0.28	28						
15	0.15	15						
16	0.16	16						
17	0	0						
18	0.43	43						
19	0.15	15	470					
20	0.11	11	23.5					
21	0.4	40	19					
22	0.16	16						
23	0.53	53	15.58170317					
24	0.72	72	470					
25	0.107	11	20					
26	0.32	32	23.5					
27	0.4	40						
28	0.035	4	0.206968463	0.29995				
29	0.05	5						
30	0.02	2						
31	0.15	15	0.206968463	0.29995	0.0462			

32	0.197	20			
33	0.41	41			
34	0.38	38			
35	0.69	69			
36	0.45	45			
37	0.25	25			
38	0.07	7	20.64097866		
39	0.25	25			
40	0.41	41	30.05	601	
41	0.53	53			
42	0.24	24			
43	0.52	52			
44	0.47	47			
45	0.05	5			
46	0.07	7	601		
47	0.3	30	5.999		
48	0.28	28	25		
49	0.73	73	30.05		
50	0.31	31	20.64097866	0.20474567	0.3255
51	0.2	20			
52	0.25	25	6.51		
53	0.05	5	651		
54	0	0	30.5		
55	0.19	19	32.55		
56	0.61	61	20.47456702		
57	0.57	57			
58	0.34	34			
59	0.46	46			
60	0.34	34			
61	0.24	24	651		
62	0.23	23			
63	0.14	14			
64	0	0			
65	0.44	44			
66	0.16	16			
67	0.4	40			
68	0.17	17			
69	0.23	23			
70	0	0		0.23265205	
71	0	0		0.23265205	0.25135
72	0.25	25			
73	0.33	33	5.027		
74	0.66	66	503		
75	0.39	39	23		
76	0.905	91	25.15		
77	0.05	5	23.3357079		
78	0.332	33			
79	0.1	10			

80	0	0			
81	0.08	8			
82	0.03	3			
83	0.42	42			
84	0.19	19			
85	0.07	7			
86	0	0			
87	0.14	14			
88	0	0			
89	0.29	29		0.137140305	0.1119
90	0.12	12			
91	0.04	4		0.137140305	0.1119
92	0.042	4.2			
93	0	0			
94	0	0			
95	0.04	4	2.238	24.474	
96	0.47	47	224.2	2449.2	
97	0.07	7	5.6	0.24474	
98	0.18	18	11.21	0.2	
99	0.039	4	13.70047637	0.201121583	
100	0.017	2	224.2		
				0.201121583	0.24474

OGUN LOCAL

1	0.59	59				
2	0.44	44				
3	0.24	24				
4	0.29	29				
5	0.28	28				
6	0.49	49				
7	0.26	26				
8	0.98	98				
9	0.22	22				
10	0.006	0.6		0.333415751	0.4648	0.074
11	0.45	45				
12	0.85	85				
13	0.93	93		0.333415751	0.4648	
14	0.28	28				
15	1.07	107				
16	0.25	25				
17	0.29	29				
18	0.06	6				
19	0.26	26				
20	1.06	106				
21	1.12	112	929.6			
22	0.17	17	29			
23	0.67	67				
24	0.16	16				
25	0.38	38				
26	0.07	7				
27	0.14	14				
28	0.42	42				
29	1.04	104				
30	0.24	24				
31	0.39	39				
32	1.04	104				
33	0.34	34		0.381035984	0.481	0.085
34	0.24	24				
35	0.14	14		38.10359839	48.1	
36	0.96	96				
37	0.97	97				
38	0.02	2				
39	0.9	90				
40	0.21	21				
41	0.23	23	962			
42	1.1	110				
43	0.35	35				
44	0.11	11				
45	0.23	23				

46	0.27	27					
47	0.09	9					
48	0.13	13					
49	1.05	105		0.330341131			
50	0.37	37		0.330341131	0.411		
51	0	0			0.411	0.51	
52	0.31	31		0.330341131	41.1		
53	0.96	96					
54	0.33	33					
55	0.78	78		0.330341131	0.411		
56	0.29	29	822				
57	0.24	24	33.03411315				
58	0.75	75	41.1				
59	0.43	43	41.1				
60	0.2	20	822				
61	0.12	12					
62	0.1	10					
63	0.44	44					
64	0.09	9					
65	0.09	9					
66	0.15	15					
67	0.53	53					
68	0.2	20					
69	0.2	20					
70	0.1	10		0.189636772		0.0424	
71	0.32	32			0.246		
72	0.08	8					
73	0.34	34					
74	0.84	84					
75	0.1	10					
76	0.3	30					
77	0.22	22					
78	0.36	36		492			
79	0.18	18		18.96367719			
80	0.16	16					
81	0.62	62					
82	0.64	64					
83	0.15	15					
84	0.04	4					
85	0.48	48					
86	0.34	34					
87	0.13	13					
88	0.11	11					
89	0.72	72		0.214555163	0.2845	0.047	
90	0.39	39					
91	0.12	12					
92	0.16	16					
93	0.18	18		22.3264653			

94	0.14	14				
95	0.65	65				
96	0.12	12				
97	0.1	10		37.746		
98	0.36	36		12.73516022		
99	0.07	7		0.275		
100	0.17	17		0.309486881		
				0.37746		
			0.309486881		0.37746	0.086
OYO						
1	0.22	22				
2	0.33	33				
3	0.07	7				
4	0.09	9				
5	0.13	13				
6	0.19	19				
7	0.07	7				
8	0.019	2				
9	0.07	7				
10	0.15	15				
11	0.156	16		0.106144328	0.15125	0.023
12	0.08	8				
13	0.12	12				
14	0.21	21				
15	0.06	6				
16	0.17	17				
17	0.07	7				
18	0.09	9				
19	0.28	28				
20	0.45	45	303			
21	0.41	41				
22	0.09	9				
23	0.1	10				
24	0.12	12				
25	0.03	3				
26	0.25	25				
27	0.21	21				
28	0.64	64				
29	0.26	26				
30	0.31	31		0.159191212	0.2345	0.0355
31	0.26	26				
32	0.23	23				
33	0.43	43				
34	0.04	4				
35	0.35	35				
36	0.05	5				
37	0.02	2				
38	0.25	25	469			

39	0.31	31				
40	0.33	33				
41	0.27	27				
42	0.58	58				
43	0.13	13				
44	0.26	26				
45	0.11	11				
46	0.16	16				
47	0.24	24				
48	0.32	32				
49	0.2	20				
50	0.55	55		0.129015503	0.2665	0.028
51	0.36	36				
52	0.35	35				
53	0.16	16				
54	0.3	30				
55	0.32	32				
56	0.14	14				
57	0.11	11				
58	0.22	22				
59	0.25	25				
60	0.3	30				
61	0.28	28	533			
62	0.19	19				
63	0.54	54				
64	0.09	9				
65	0.49	49				
66	0.56	56				
67	0.78	78				
68	0.21	21				
69	0.59	59				
70	0.57	57		0.243082724	0.4495	0.054
71	0.24	24				
72	0.94	94		0.243082724	0.4495	
73	0.68	68				
74	0.44	44				
75	0.74	74				
76	0.7	70				
77	0.19	19				
78	0.21	21				
79	0.23	23				
80	0.32	32	899			
81	0.36	36	24.30827236			
82	0.39	39				
83	0.69	69				
84	0.07	7				
85	0.11	11				
86	0.45	45				

87	0.65	65					
88	0.41	41					
89	0.13	13		0.225495945	0.352	0.0504	
90	0.39	39		0.225495945	0.352		
91	0.52	52					
92	0.74	74					
93	0.13	13					
94	0.06	6					
95	0.24	24					
96	0.27	27	20.45526284	29.08			
97	0.77	77					
98	0.21	21		29.075			
99	0.23	23		2908			
100	0.22	22	704	24			
				0.204592247	0.29075	0.016	

LAGOS STATE

FARMI	AGE	POPULATION	S/P RATIO	EU			
FARM 1	38	2,200	0.791	79			
FARM 2	38	2,200	0.873	87			
FARM 3	38	2,200	0.58	58			
FARM 4	38	2,200	0.595	60			
FARM 5	38	2,200	0.84	84	16.071		79.
FARM 6	38	2,200	0.8	80			
FARM 7	38	2,200	0.75	75			
FARM 8	38	2,200	0.96	96			
FARM 9	38	2,200	0.66	66			
FARM 10	38	2,200	1.1	110			
FARM 2							
FARM 1	36	1000	0.815	81			
FARM 2	36	1000	0.857	86			
FARM 3	36	1000	0.58	58			
FARM 4	36	1000	0.982	98			
FARM 5	36	1000	0.87	87			
FARM 6	36	1000	0.74	74	15.2694		79.
FARM 7	36	1000	0.568	57			
FARM 8	36	1000	0.716	72			
FARM 9	36	1000	1.043	104			
FARM 10	36	1000	0.788	79			
FARM 3							
FARM 1	52	2,200	0.52	52			
FARM 2	52	2,200	0.82	82			
FARM 3	52	2,200	0.846	85			
FARM 4	52	2,200	0.58	58			
FARM 5	52	2,200	0.576	58			
FARM 6	52	2,200	0.655	66	15.52775293		7
FARM 7	52	2,200	0.856	86			
FARM 8	52	2,200	0.87	87			

FARM 9	52	2,200	0.98	98		
FARM 10	52	2,200	0.68	68		
FARM 4						
FARM 1	37	2000	0.708	71		
FARM 2	37	2000	0.56	56		
FARM 3	37	2000	0.772	77		
FARM 4	37	2000	0.497	50		
FARM 5	37	2000	0.452	45	17.82663426	64.
FARM 6	37	2000	0.76	76		
FARM 7	37	2000	0.58	58		
FARM 8	37	2000	0.46	46		
FARM 9	37	2000	1.03	103		
FARM 10	37	2000	0.646	65		
FARM 5						
FARM 1	25	1,000	0.8432	84		
FARM 2	25	1,000	0.423	42		
FARM 3	25	1,000	0.536	54		
FARM 4	25	1,000	0.22	22		
FARM 5	25	1,000	0.13	13	25.32916632	51.
FARM6	25	1,000	0.66	66		
FARM 7	25	1,000	0.85	85		
FARM 8	25	1,000	0.73	73		
FARM 9	25	1,000	0.304	30		
FARM 10	25	1,000	0.48	48		
FARM 6						
FARM 1	32	4,059	0.339	34		
FARM 2	32	4,059	0.4	40		
FARM 3	32	4,509	0.29	29		
FARM 4	32	4,509	0.72	72		
FARM 5	32	4,509	0.86	86	26.72410481	60.
FARM 6	32	4,509	0.83	83		
FARM 7	32	4,509	0.94	94		
FARM 8	32	4,509	0.25	25		
FARM 9	32	4,509	0.85	85		
FARM 10	32	4,509	0.535	54		
FARM 7	32					
FARM 1	42	1,500	0.22	22		
FARM 2	42	1,500	0.69	69		
FARM 3	42	1,500	0.71	71		
FARM 4	42	1,500	0.804	80		
FARM 5	42	1,500	1.03	103	33.58306452	64.
FARM 6	42	1,500	0.5	50		
FARM 7	42	1,500	1	100		
FARM 8	42	1,500	0.47	47		
FARM 9	42	1,500	0.04	4		
FARM 10	42	1,500	0.98	98		
FARM8						
FARM 1	20	3,000	0.26	26		

FARM 2	20	3,000	0.73	73		
FARM 3	20	3,000	0.304	30		
FARM 4	20	3,000	0.58	58		
FARM 5	20	3,000	0.63	63		
FARM 6	20	3,000	0.26	26	25.6334997	48.34
FARM 7	20	3,000	0.76	76		
FARM 8	20	3,000	0.419	0.42		
FARM 9	20	3,000	0.63	63		
FARM 10	20	3,000	0.68	68		
FARM 9						
FARM 1	32	4,000	0.76	76		
FARM 2	32	4,000	0.92	92		
FARM 3	32	4,000	0.44	44		
FARM 4	32	4,000	0.546	55		
FARM 5	32	4,000	0.34	34	17.3400628	62.
FARM 6	32	4,000	0.57	57		
FAAM 7	32	4,000	0.52	52		
FARM 8	32	4,000	0.64	64		
FARM 9	32	4,000	0.77	77		
FARM 10	32	4,000	0.72	72		
FARM 10						
FARM 1	35	2000	0.98	98		
FARM 2	35	2000	0.37	37		
FARM 3	3	2000	0.79	79		
FARM 4	5	2000	0.81	81		
FARM 5	35	2000	0.48	48	23.72902583	55.
FARM 6	35	2000	0.3	30		
FARM 7	35	2000	0.27	27		
FARM 8	35	2000	0.43	43		
FARM 9	35	2000	0.5	50		
FARM 10	35	2000	0.589	59		
FARM 11	AGE	POPULATION	S/P	EU		
FARM 1	40	1,000	0.23	23		
FARM 2	40	1,000	0.12	12		
FARM 3	40	1,000	0.19	19		
FARM 4	40	1,000	0.39	39		
FARM 5	40	1,000	0.62	62		
FARM 6	40	1,000	0.43	43	17.42922195	3
FARM 7	40	1,000	0.61	61		
FARM 8	40	1,000	0.401	40		
FARM 9	40	1,000	0.418	42		
FARM 10	40	1,000	0.19	19		
FARM12						
FARM 1	30	2,000	0.07	7		
FARM 2	30	2,000	0.25	25		
FARM 3	30	2,000	0.008	0.8		
FARM 4	30	2,000	0.401	40		

FARM 5	30	2,000	0.006	0.6	12.84646082	11.5
FARM 6	30	2,000	0.14	14		
FARM 7	30	2,000	0.018	2		
FARM 8	30	2,000	0.17	17		
FARM 9	30	2,000	0.07	7		
FARM 10	30	2,000	0.019	2		
FARM 13						
FARM 1	40	4000	0.04	4		
FARM 2	40	4000	0.07	7		
FARM 3	40	4000	0.018	2		
FARM 4	40	4000	0.068	7		
FARM 5	40	4000	0.079	8		
FARM 6	40	4000	0.084	8	6.899275324	8.
FARM 7	40	4000	0.02	2		
FARM 8	40	4000	0.12	12		
FARM 9	40	4000	0.269	26		
FARM 10	40	4000	0.08	8		
FARM 14						
FARM 1	22	3,859	0.53	53		
FARM 2	22	3,859	0.33	33		
FARM3	22	3,859	0.33	33		
FARM 4	22	3,859	0.05	5		
FARM 5	22	3,859	0.83	83		
FARM 6	22	3,859	0.69	69	27.33821095	48.2222
FARM 7	22	3,859	0.7	70		
FARM 8	22	3,859	0.36	36		
FARM 9	22	3,859	0.52	52		
FARM 10	22	3,859	0	0		
FARM 15						
FARM 1	32	4,800	0.23	23		
FARM 2	32	4,800	0.048	4.8		
FARM 3	32	4,800	0.03	3		
FARM4	32	4,800	0.008	0.8	7.540181253	5.9
FARM5	32	4,800	0.047	4.7		
FARM 6	32	4,800	0	0		
FARM 7	32	4,800	0.016	1.6		
FARM8	32	4,800	0.042	4.2		
FARM 9	32	4,800	0.01	1		
FARM10	32	4,800	0.16	16		

OYO				
FARM 1				
FARM 1	AGE	POPULATION	S/P RATIO	EU
FARM 1	34	1,500	0.1	10
FARM 2	34	1,500	0.07	7
FARM 3	34	1,500	0.03	3
FARM 4	34	1,500	0.04	4

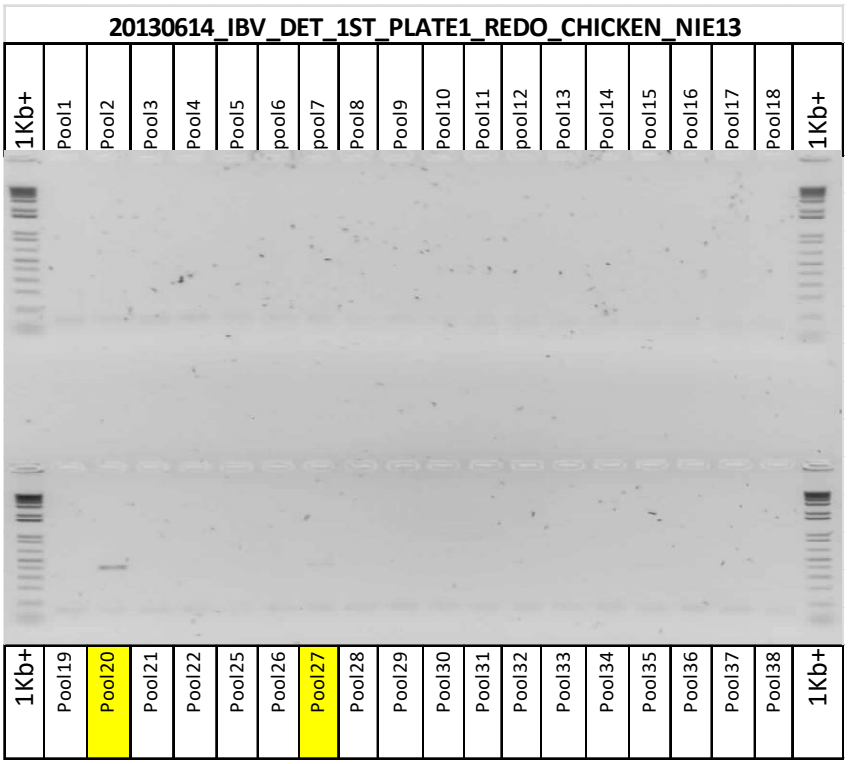
FARM 5	34	1,500	0.08	8		
FARM 6	34	1,500	0.04	4	2.359378449	6.
FARM 7	34	1,500	0.08	8		
FARM 8	34	1,500	0.09	9		
FARM 9	34	1,500	0.06	6		
FARM 10	34	1,500	0.08	8		
FARM 2						
FARM 1	47	3,600	0.03	3		
FARM 2	47	3,600	0.04	4		
FARM 3	47	3,600	0.04	4		
FARM 4	47	3,600	0.01	1		
FARM 5	47	3,600	0.52	52	28.94515887	30.
FARM 6	47	3,600	0.27	27		
FARM 7	47	3,600	0.64	64		
FARM 8	47	3,600	0.21	21		
FARM 9	47	3,600	0.47	47		
FARM 10	47	3,600	0.81	81		
FARM 3						
FARM I	48	1,800	0.21	21		
FARM 2	48	1,800	0.57	57		
FARM 3	48	1,800	0.12	12		
FARM 4	48	1,800	0.92	92		
FARM 5	48	1,800	0.18	18	45.86077966	45.
FARM 6	48	1,800	0.31	31		
FARM 7	48	1,800	0.12	12		
FARM 8	48	1,800	0.19	19		
FARM 9	48	1,800	1.55	155		
FARM 10	48	1,800	0.34	34		
FARM 4						
FARM 1	46	4,850	0.2	20		
FARM 2	46	4,850	0.56	56		
FARM 3	46	4,850	0.45	45		
FARM 4	46	4,850	1.33	133		
FARM 5	46	4,850	0.5	50	38.17634521	58.
FARM 6	46	4,850	0.09	9		38.1763
FARM 7	46	4,850	0.81	81		
FARM 8	46	4,850	0.82	82		
FARM 9	46	4,850	0.86	86		
FARM 10	46	4,850	0.19	19		
FARM 5						
FARM 1	13	1,750	0.13	13		
FARM 2	13	1,750	0.33	33		
FARM 3	13	1,750	0.09	9		
FARM 4	13	1,750	1.02	102		
FARM 5	13	1,750	0.2	20		
FARM 6	13	1,750	0.71	71	30.95588259	34.
FARM 7	13	1,750	0.5	50		
FARM 8	13	1,750	0.22	22		

FARM 9	13	1,750	0.13	13			
FARM 10	13	1,750	0.11	11			
FARM 6							
FARM 1	59	3,950	0.98	98			
FARM 2	59	3,950	0.12	12			
FARM 3	59	3,950	0.53	53			
FARM 4	59	3,950	0.73	73			
FARM 5	59	3,950	0.09	9	37.69482723		52.
FARM 6	59	3,950	0.2	20			
FARM 7	59	3,950	0.36	36			
FARM 8	59	3,950	1.15	115			
FARM 9	59	3,950	0.28	28			
FARM 10	59	3,950	0.83	83			
FARM 7							
FARM 1	29	4,890	0.93	93			
FARM 2	29	4,890	0.3	30			
FARM 3	29	4,890	1.24	124			
FARM 4	29	4,890	0.07	7			
FARM 5	29	4,890	0.75	75	38.33608686	62.1	
FARM 6	29	4,890	0.15	15			
FARM 7	29	4,890	1.02	102			
FARM 8	29	4,890	0.67	67			
FARM 9	29	4,890	0.43	43			
FARM 10	29	4,890	0.65	65			
FARM 8							
FARM 1	19	3,335	0.7	70			
FARM 2	19	3,335	0.02	2			
FARM 3	19	3,335	0.47	47			
FARM 4	19	3,335	0.5	50			
FARM 5	19	3,335	0.61	61	20.36991245		46.
FARM 6	19	3,335	0.48	48			
FARM 7	19	3,335	0.25	25			
FARM 8	19	3,335	0.69	69			
FARM 9	19	3,335	0.48	48			
EARM 10	19	3,335	0.44	44			
FARM 9							
FARM 1	26	4,952	0.05	5			
FARM 2	26	4,952	0.76	76			
FARM 3	26	4,952	0.52	52			
FARM 4	26	4,952	0.69	69	24.90002231		41.
FARM 5	26	4,952	0.55	55			
FARM 6	26	4,952	0.34	34			
FARM 7	26	4,952	0.62	62			
FARM 8	26	4,952	0.12	12			
FARM 9	26	4,952	0.29	29			
FARM 10	26	4,952	0.19	19			
FARM 10							

FARM 1	45	5,890	1.03	103		
FARM 2	45	5,890	0.72	72		
FARM 3	45	5,890	0.76	76		
FARM 4	45	5,890	0.04	4		
FARM 5	45	5,890	0.78	78	38.51925925	43.
FARM 6	45	5,890	0.03	3		
FARM 7	45	5,890	0.1	10		
FARM 8	45	5,890	0.63	63		
FARM 9	45	5,890	0.13	13		
FARM 10	45	5,890	0.1	10		
FARM 11						
FARM 1	60	1,020	0.4	40		
FARM 2	60	1,020	0.15	15		
FARM 3	60	1,020	0.25	25		
FARM 4	60	1,020	0.5	50	13.89684217	32.
FARM 5	60	1,020	0.56	56		
FARM 6	60	1,020	0.2	20		
FARM 7	60	1,020	0.23	23		
FARM 8	60	1,020	0.4	40		
FARM 9	60	1,020	0.2	20		
FARM 10	60	1,020	0.34	34		
FARM 12						
FARM 1	33	1,780	0.35	35		
FARM 2	33	1,780	0.58	58		
FARM 3	33	1,780	0.31	31		
FARM 4	33	1,780	0.57	57		
FARM 5	33	1,780	0.46	46		
FARM 6	33	1,780	0.21	21	15.08825886	38.
FARM 7	33	1,780	0.6	60		
FARM 8	33	1,780	0.29	29		
FARM 9	33	1,780	0.3	30		
FARM 10	33	1,780	0.22	22		
FARM 13						
FARM 1	35	1,000	0.43	43		
FARM 2	35	1,000	0.46	46		
FARM 3	35	1,000	0.24	24		
FARM 4	35	1,000	0.17	17		
FARM 5	35	1,000	0.1	10	12.18423389	26.
FARM 6	35	1,000	0.26	26		
FARM 7	35	1,000	0.11	11		
FARM 8	35	1,000	0.34	34		
FARM 9	35	1,000	0.29	29		
FARM 10	35	1,000	0.23	23		
FARM 14						

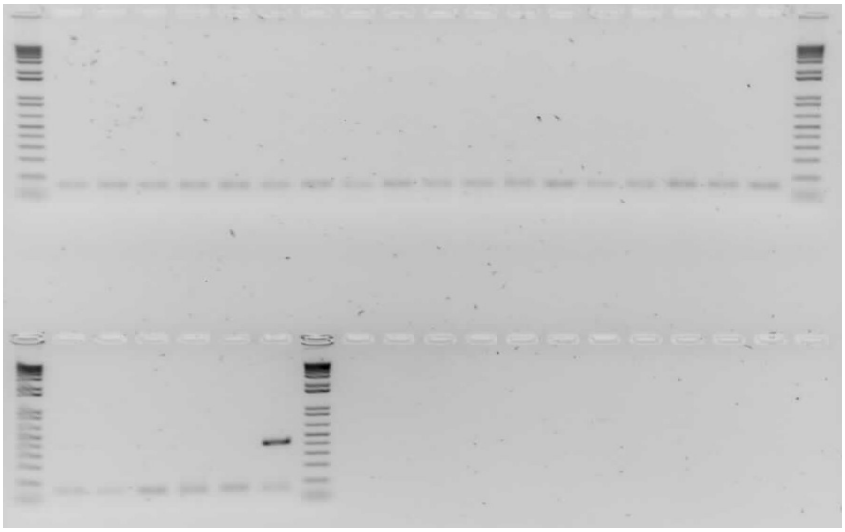
FARM 1	20	3,959	0.17	17		
FARM 2	20	3,959	0.25	25		
FARM 3	20	3,959	0.17	17		
FARM 4	20	3,959	0.41	41	24.43494765	42.
FARM 5	20	3,959	0.95	95		
FARM 6	20	3,959	0.48	48		
FARM 7	20	3,959	0.39	39		
FARM 8	20	3,959	0.58	58		
FARM 9	20	3,959	0.63	63		
FARM 10	20	3,959	0.25	25		
FARM 15						
FARM 1	30	1,876	0.78	78		
FARM 2	30	1,876	0.57	57		
FARM 3	30	1,876	0.4	40		
FARM 4	30	1,876	0.42	42		
FARM 5	30	1,876	0.49	49	15.87590767	57.
FARM 6	30	1,876	0.43	43		
FARM 7	30	1,876	0.51	51		
FARM 8	30	1,876	0.84	84		
FARM 9	30	1,876	0.56	56		
FARM 10	30	1,876	0.74	74		

Appendix X: Polymerase Chain Reaction of cloaca and trachea in commercial and local chickens in Lagos, Ogun and Oyo states

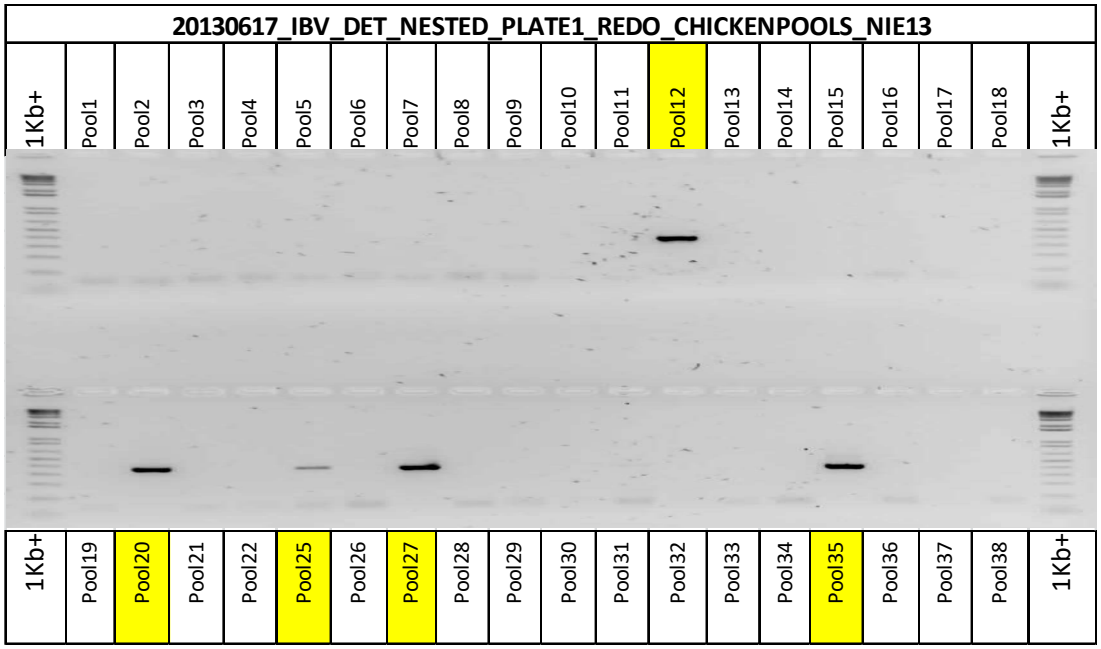


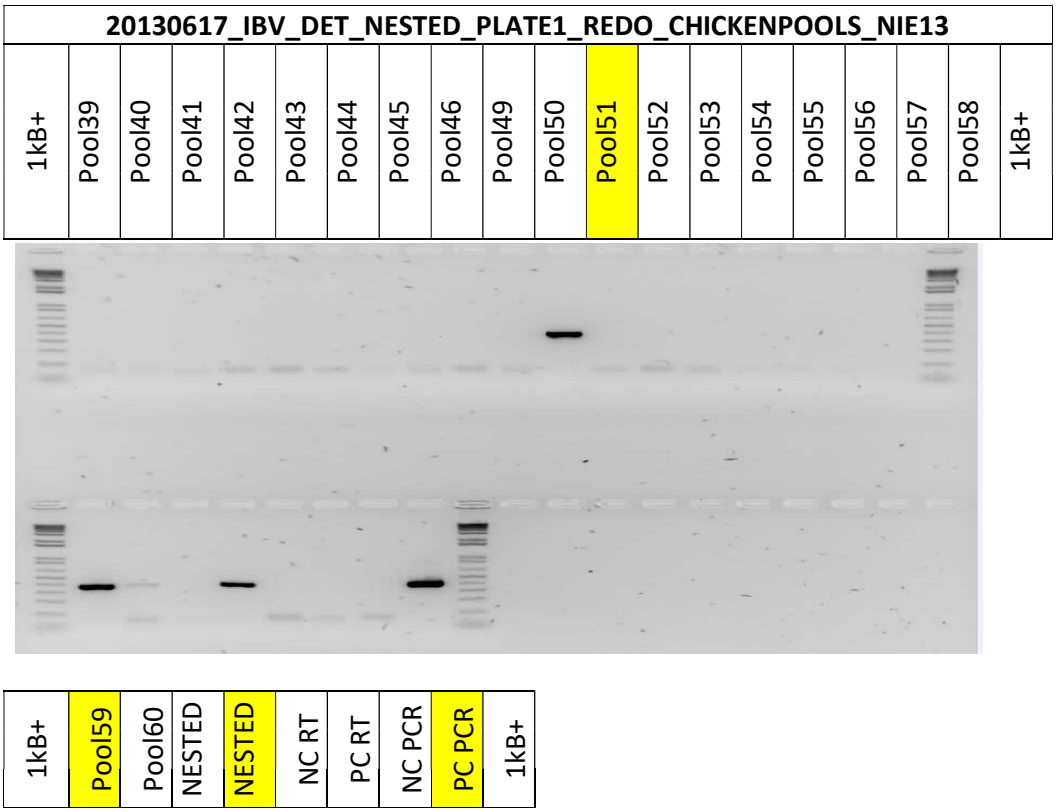
20130614_IBV_DET_1ST_PLATE1_REDO_CHICKEN_NIE13

1Kb+	Pool39	Pool40	Pool41	Pool42	Pool43	Pool44	Pool45	Pool46	Pool49	Pool50	Pool51	Pool52	Pool53	Pool54	Pool55	Pool56	Pool57	Pool58	1Kb+



1Kb+	Pool59	Pool60	NC RT	PC RT	NC PCR	PC PCR	1Kb+

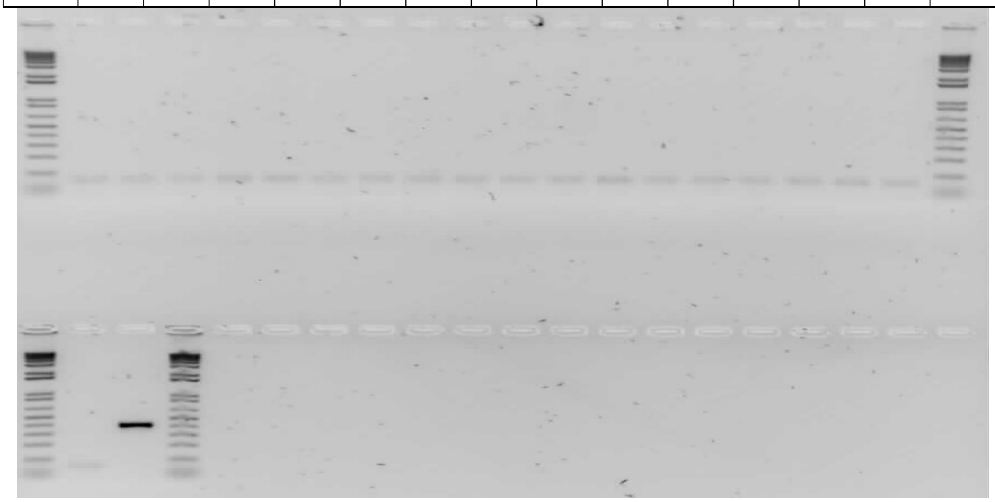

20130617_IBV_DET_NESTED_PLATE1_REDO_CHICKENPOOLS_NIE13
1Kb+
Pool1
Pool2
Pool3
Pool4
Pool5
Pool6
Pool7
Pool8
Pool9
Pool10
Pool11
Pool12
Pool13
Pool14
Pool15
Pool16
Pool17
Pool18
1Kb+
1Kb+
Pool19
Pool20
Pool21
Pool22
Pool25
Pool26
Pool27
Pool28
Pool29
Pool30
Pool31
Pool32
Pool33
Pool34
Pool35
Pool36
Pool37
Pool38
1Kb+


20130617_IBV_DET_NESTED_PLATE1_REDO_CHICKENPOOLS_NIE13
1kB+
Pool39
Pool40
Pool41
Pool42
Pool43
Pool44
Pool45
Pool46
Pool49
Pool50
Pool51
Pool52
Pool53
Pool54
Pool55
Pool56
Pool57
Pool58
1kB+
1kB+
Pool59
Pool60
NESTED
NESTED
NC RT
PC RT
NC PCR
PC PCR
1kB+

20130617_IBV_DET_1ST_PLATE2_REDO_CHICKENPOOLS_NIE13																			
1Kb+	Pool61	Pool62	Pool63	Pool64	Pool65	Pool66	Pool67	Pool68	Pool69	Pool70	Pool73	Pool74	Pool75	Pool76	Pool77	Pool78	pool79	Pool80	1Kb+

1Kb+	Pool81	Pool82	Pool83	Pool84	Pool85	Pool86	Pool87	Pool88	Pool89	Pool90	Pool91	Pool92	Pool93	Pool94	Pool97	Pool98	Pool99	Pool100	1Kb+

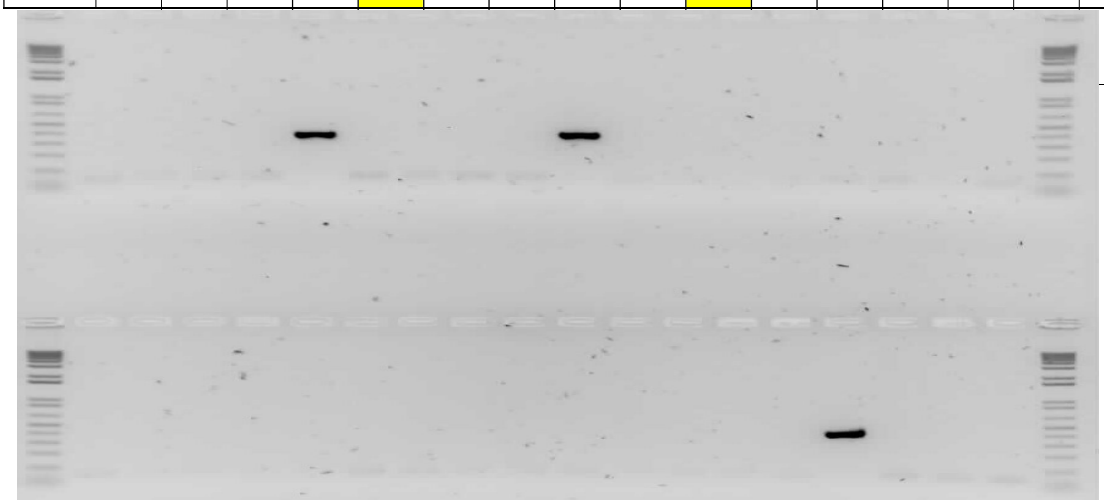
20130617_IBV_DET_1ST_PLATE2_REDO_CHICKENPOOLS_NIE13																			
1Kb+	Pool101	Pool102	Pool103	Pool104	Pool105	Pool106	Pool107	Pool108	Pool109	Pool110	Pool111	Pool112	Pool113	Pool114	Pool115	pool116	Pool117	Pool118	1Kb+



1Kb+	NC PCR	PC PCR	1Kb+

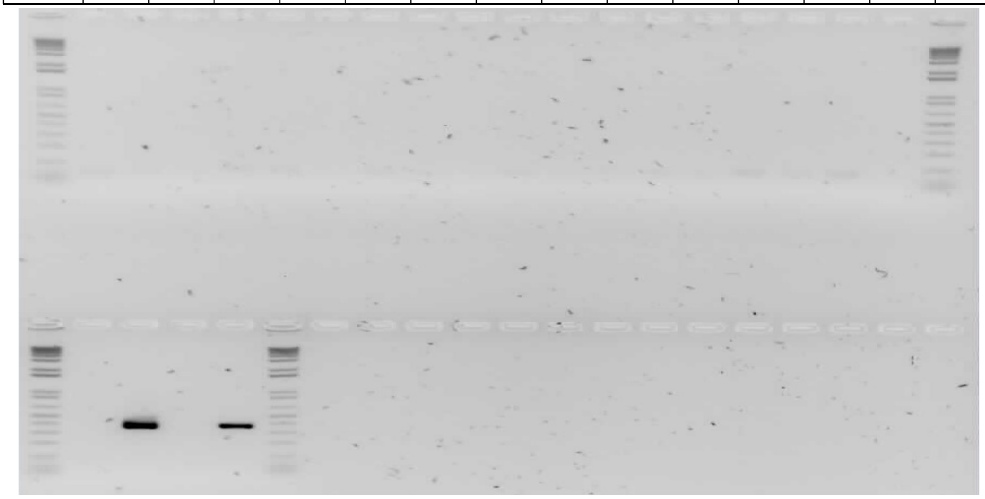
20130617_IBV_DET_NESTED_PLATE2_REDO_CHICKENPOOLS_NIE13

1Kb+	Pool61	Pool62	Pool63	Pool64	Pool65	Pool66	Pool67	Pool68	Pool69	Pool70	pool73	Pool74	Pool75	Pool76	Pool77	Pool78	Pool79	Pool80	1Kb+

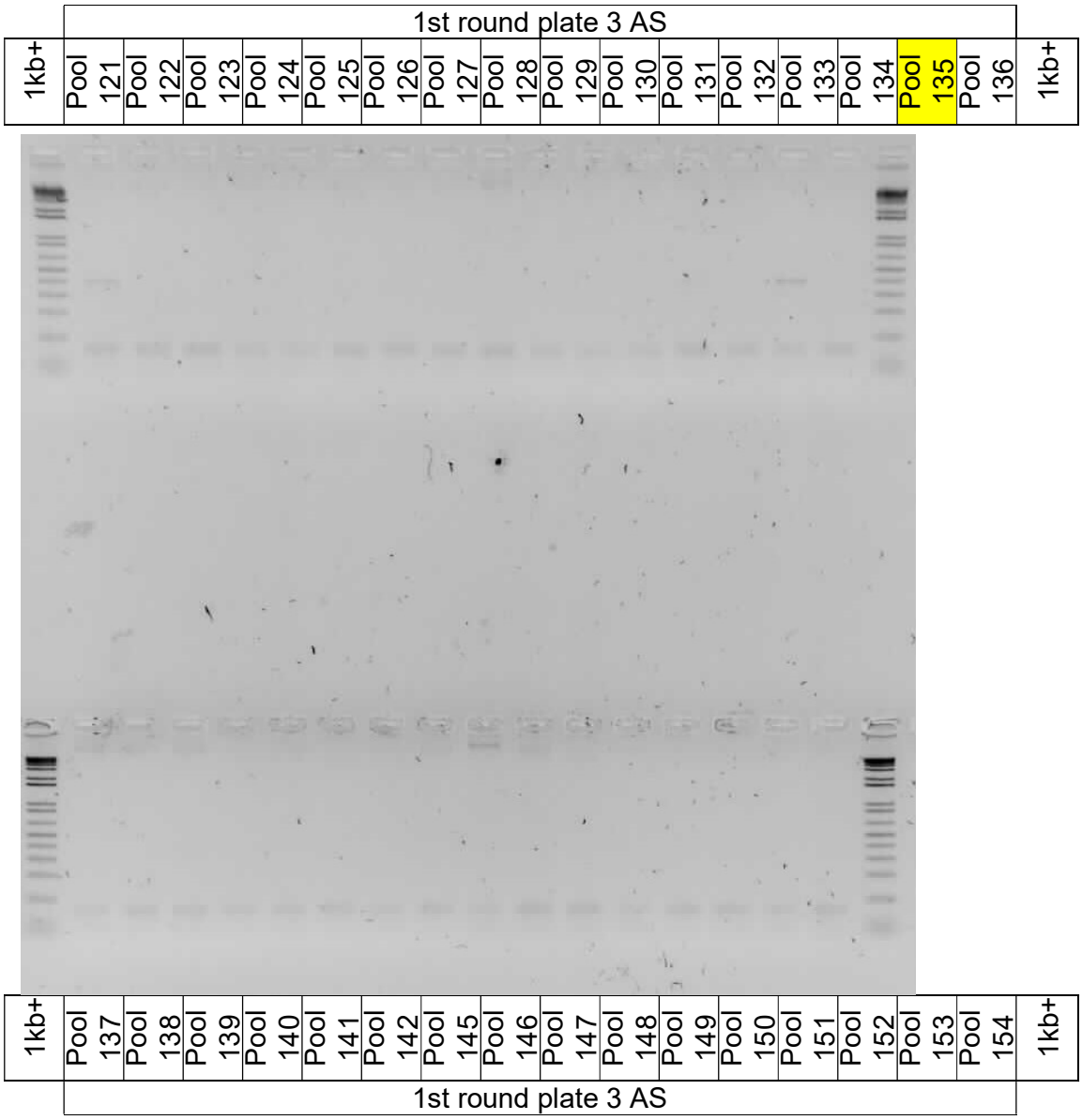


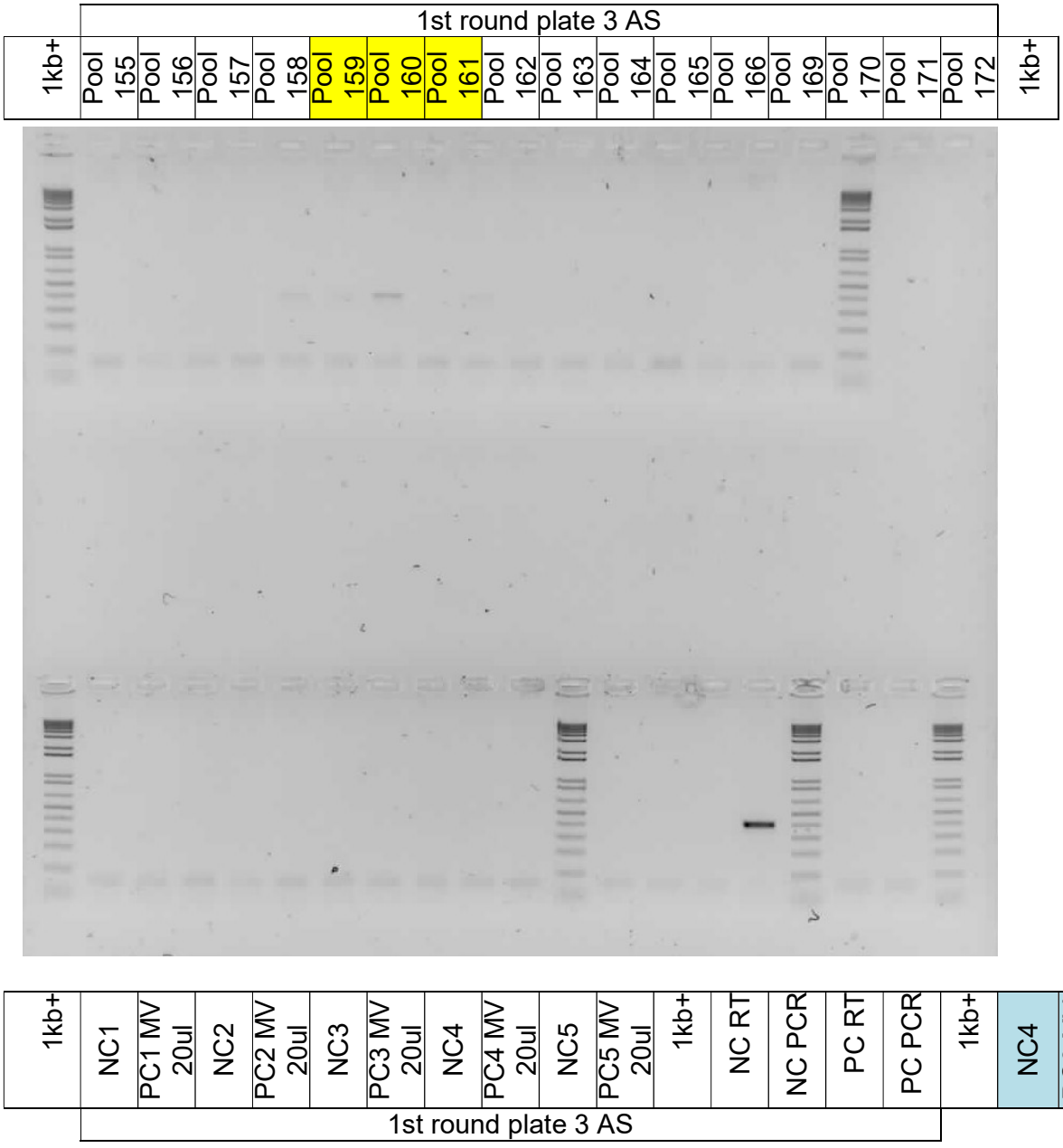
1Kb+	Pool81	Pool82	Pool83	Pool84	Pool85	Pool86	Pool87	Pool88	Pool89	Pool90	Pool91	Pool92	Pool93	Pool94	Pool97	Pool98	Pool99	Pool100	1Kb+

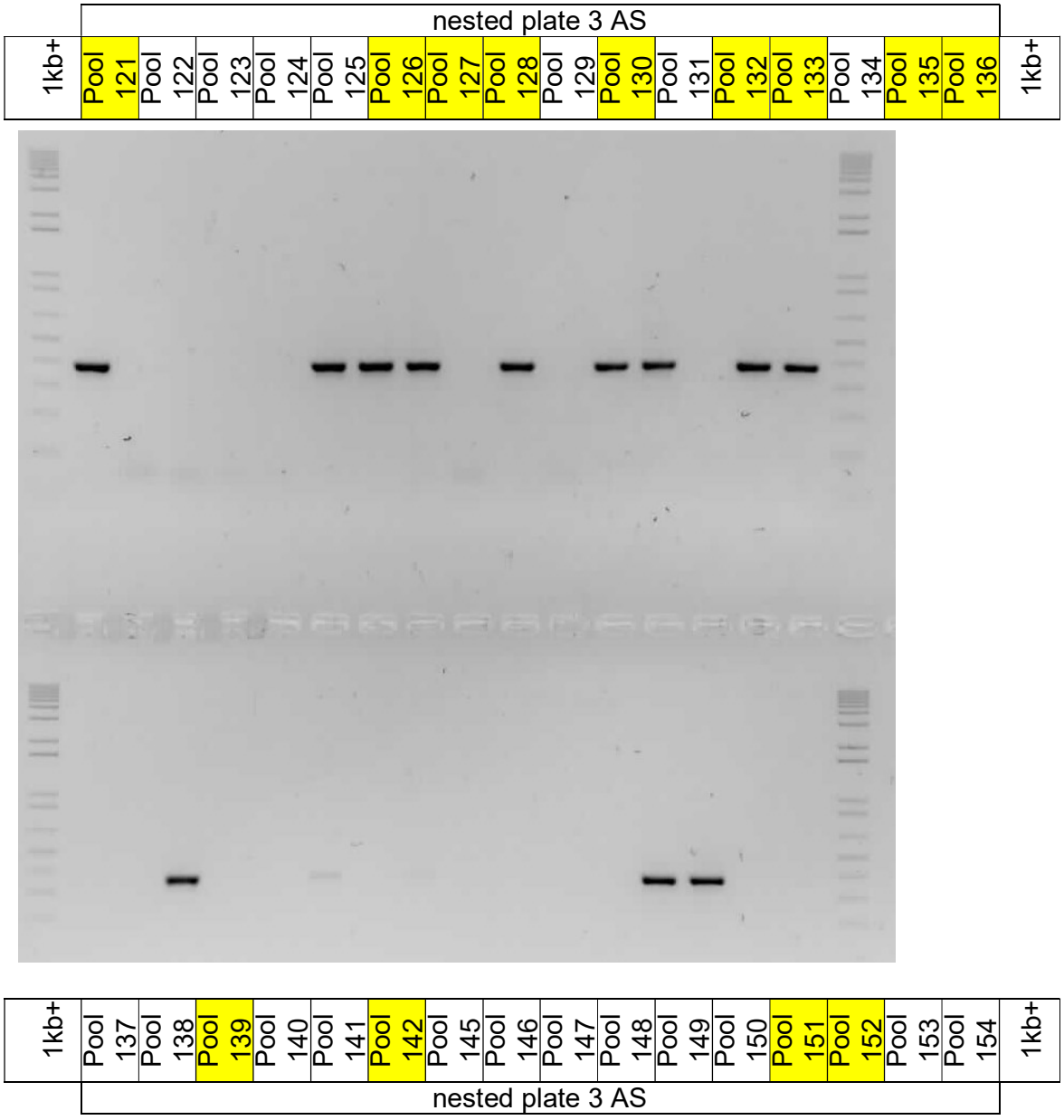
20130617_IBV_DET_NESTED_PLATE2_REDO_CHICKENPOOLS_NIE13																			
1Kb+	Pool101	Pool102	Pool103	Pool104	Pool105	Pool106	Pool107	Pool108	Pool109	Pool110	Pool111	Pool112	Pool113	Pool114	Pool115	Pool116	Pool117	pool118	1Kb+

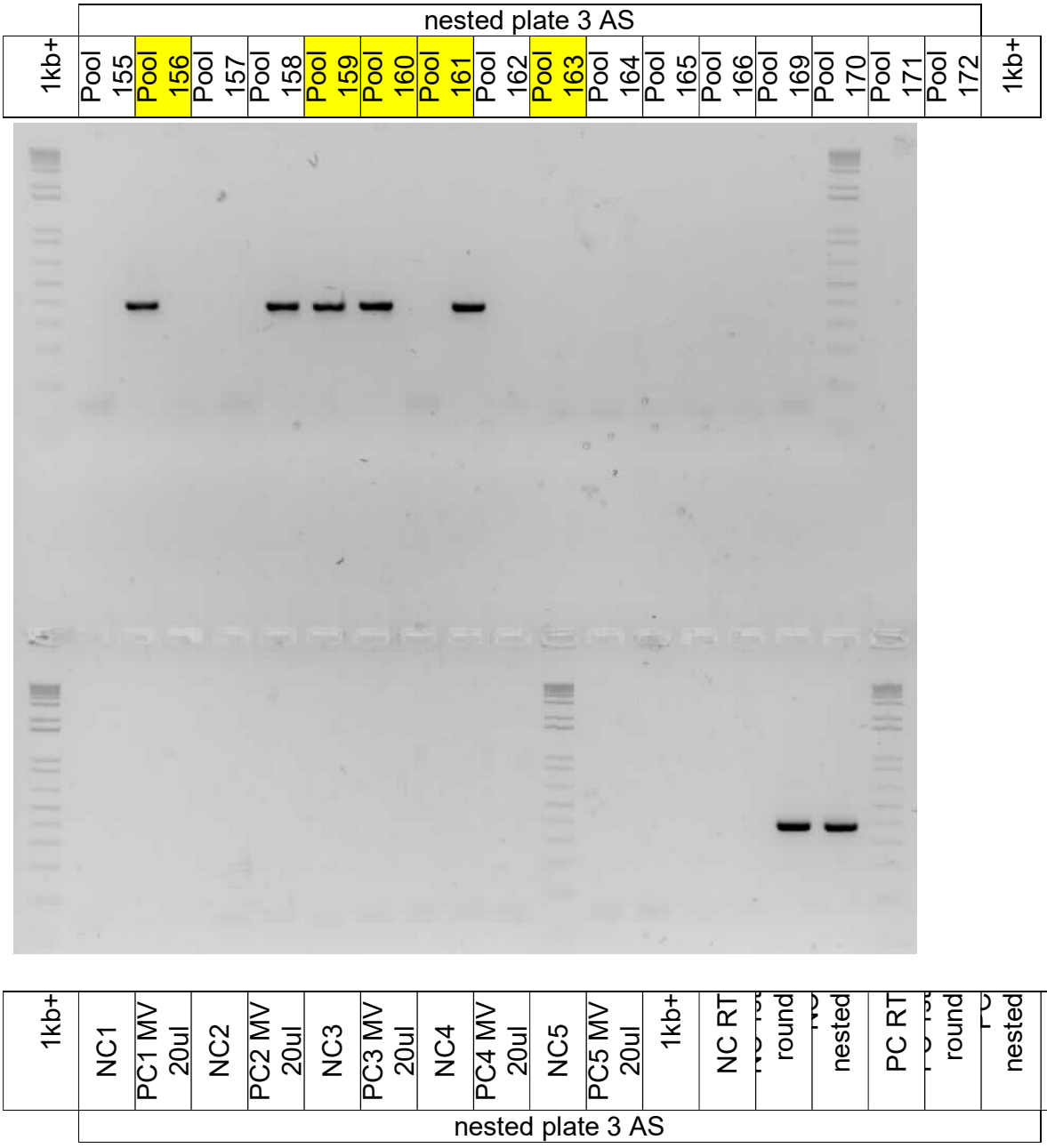


1Kb+	NC PCR	PC PCR	NESTED	NESTED	1Kb+


1st round plate 3 AS
1kb+
Pool 121
Pool 122
Pool 123
Pool 124
Pool 125
Pool 126
Pool 127
Pool 128
Pool 129
Pool 130
Pool 131
Pool 132
Pool 133
Pool 134
Pool 135
Pool 136
1kb+
1kb+
Pool 137
Pool 138
Pool 139
Pool 140
Pool 141
Pool 142
Pool 145
Pool 146
Pool 147
Pool 148
Pool 149
Pool 150
Pool 151
Pool 152
Pool 153
Pool 154
1kb+
1st round plate 3 AS

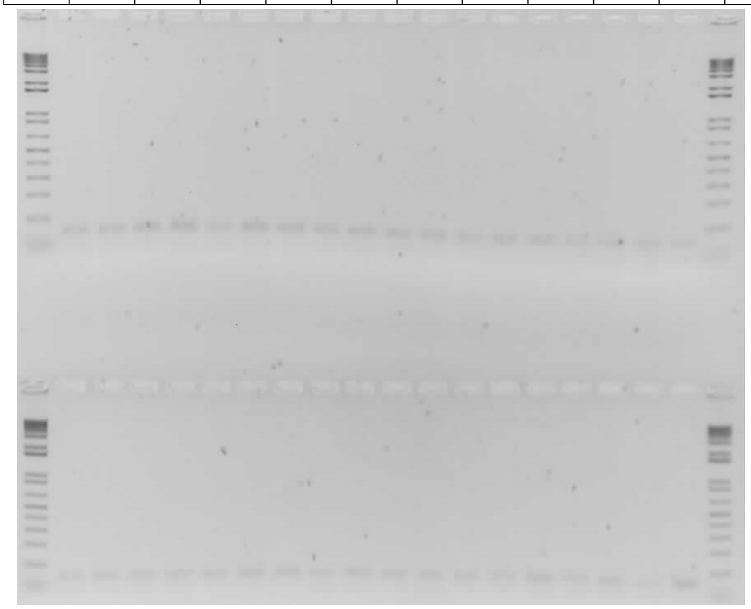

1st round plate 3 AS
1kb+
Pool 155
Pool 156
Pool 157
Pool 158
Pool 159
Pool 160
Pool 161
Pool 162
Pool 163
Pool 164
Pool 165
Pool 166
Pool 169
Pool 170
Pool 171
Pool 172
1kb+
1kb+
NC1
PC1 MV 20ul
NC2
PC2 MV 20ul
NC3
PC3 MV 20ul
NC4
PC4 MV 20ul
NC5
PC5 MV 20ul
1kb+
NC RT
NC PCR
PC RT
PC PCR
1kb+
NC4
PC3 MV 20ul
1kb+
1st round plate 3 AS


nested plate 3 AS
1kb+
Pool 121
Pool 122
Pool 123
Pool 124
Pool 125
Pool 126
Pool 127
Pool 128
Pool 129
Pool 130
Pool 131
Pool 132
Pool 133
Pool 134
Pool 135
Pool 136
1kb+
1kb+
Pool 137
Pool 138
Pool 139
Pool 140
Pool 141
Pool 142
Pool 145
Pool 146
Pool 147
Pool 148
Pool 149
Pool 150
Pool 151
Pool 152
Pool 153
Pool 154
1kb+
nested plate 3 AS


nested plate 3 AS
1kb+
Pool 155
Pool 156
Pool 157
Pool 158
Pool 159
Pool 160
Pool 161
Pool 162
Pool 163
Pool 164
Pool 165
Pool 166
Pool 169
Pool 170
Pool 171
Pool 172
1kb+
1kb+
NC1
PC1 MV 20ul
NC2
PC2 MV 20ul
NC3
PC3 MV 20ul
NC4
PC4 MV 20ul
NC5
PC5 MV 20ul
1kb+
NC RT
nested
PC RT
nested
1kb+
nested plate 3 AS

20130612_IBV_DET_NESTED_REDO_PLATE4_CHICKEN_NIE13

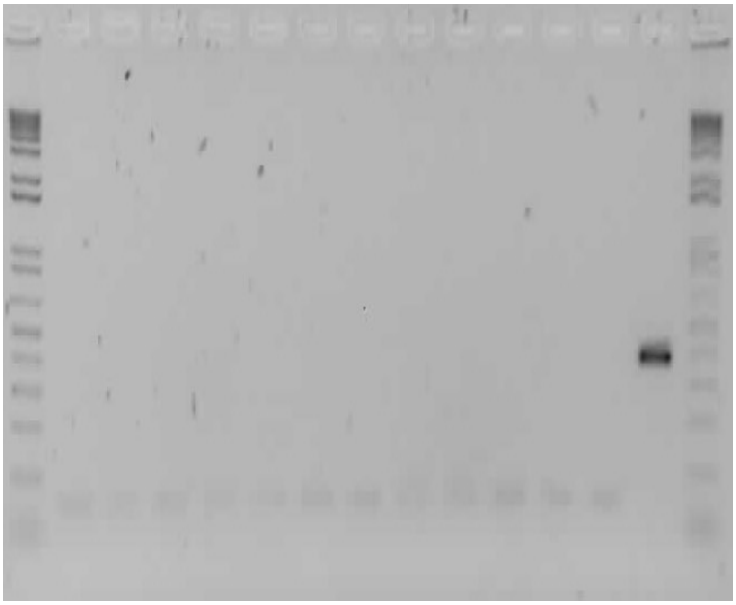
1Kb+	Pool173	Pool174	Pool175	Pool176	Pool177	Pool178	Pool179	Pool180	Pool181	Pool182	Pool183	Pool184	Pool185	Pool186	Pool187	Pool188	Pool189	Pool190	1Kb+

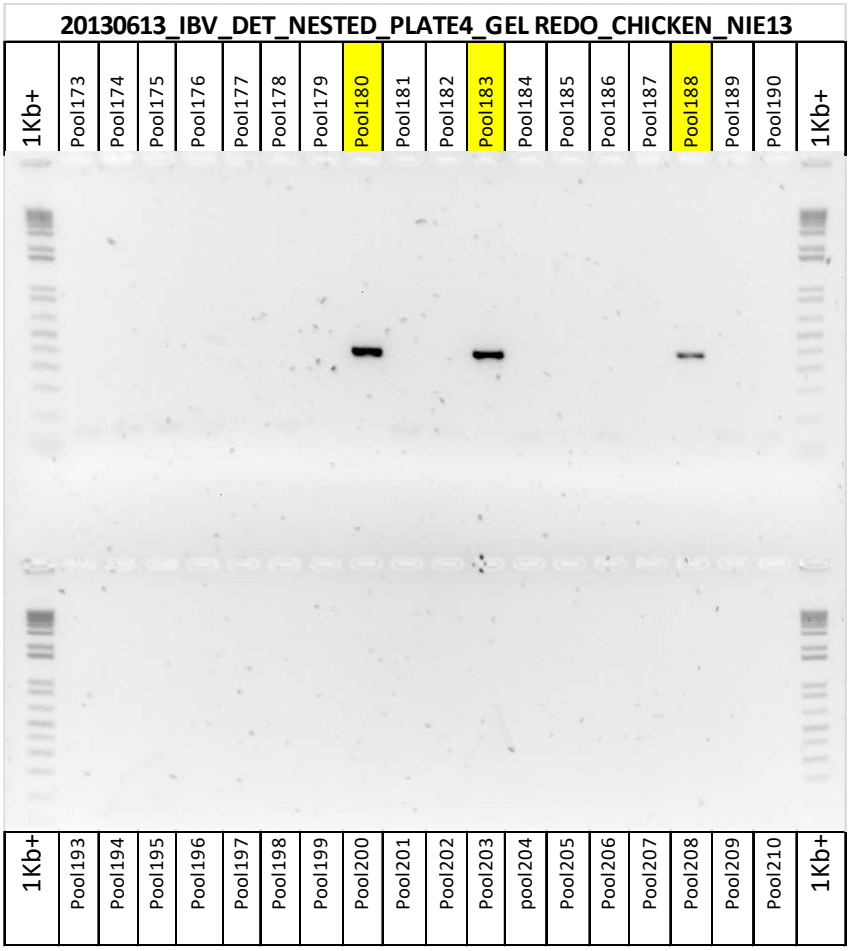


1Kb+	Pool193	Pool194	Pool195	Pool196	Pool197	Pool198	Pool199	Pool200	Pool201	Pool202	Pool203	Pool204	Pool205	Pool206	Pool207	Pool208	Pool209	Pool210	1Kb+

20130612_IBV_DET_NESTED_REDO_PLATE4_CHICKEN_NIE13

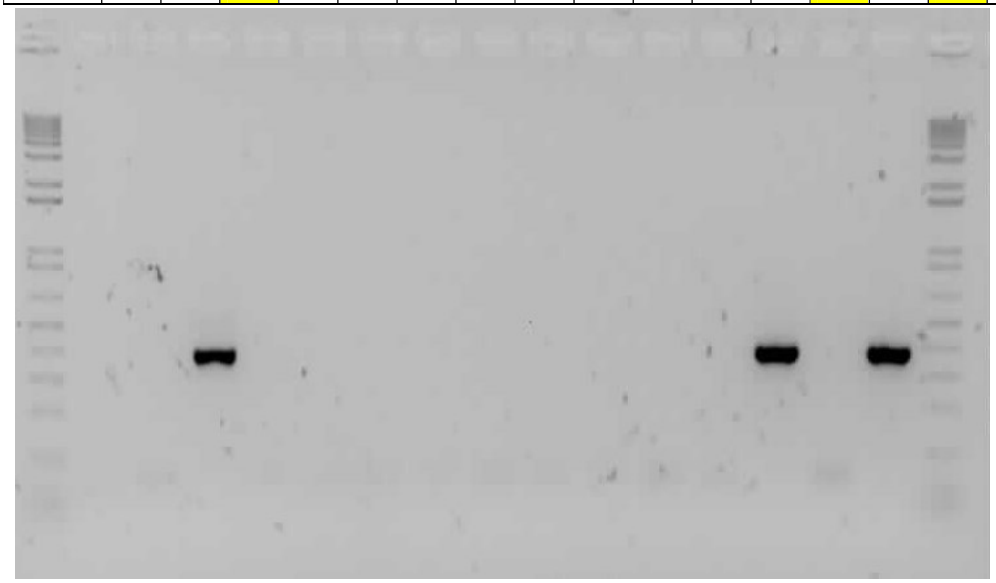
1Kb+	Pool211	Pool212	Pool213	Pool214	Pool217	Pool218	Pool219	Pool220	NC6	PC6	NC7	NC PCR	PC PCR	1Kb+




20130613_IBV_DET_NESTED_PLATE4_GEL REDO_CHICKEN_NIE13
1Kb+
Pool173
Pool174
Pool175
Pool176
Pool177
Pool178
Pool179
Pool180
Pool181
Pool182
Pool183
Pool184
Pool185
Pool186
Pool187
Pool188
Pool189
Pool190
1Kb+
1Kb+
Pool193
Pool194
Pool195
Pool196
Pool197
Pool198
Pool199
Pool200
Pool201
Pool202
Pool203
pool204
Pool205
Pool206
Pool207
Pool208
Pool209
Pool210
1Kb+

20130613_IBV_DET_NESTED_PLATE4_GEL REDO_CHICKEN_NIE13

1Kb+	Pool211	Pool212	Pool213	Pool214	pool217	Pool218	Pool219	Pool220	NC6	PC6	NC7	NC NESTED	PC NESTED	NC 1STROUND	PC 1STROUND	1Kb+



Appendix X1: Polymerase Chain Reaction of cloaca and trachea in commercial and local chickens in Lagos, Ogun and Oyo states

SAMPLE ID	LOCATION	TYPE OF SWAB	SAMPLE 1	SAMPLE 2	SAMPLE 3	POOL (1+2+3)
LAC1	LAGOS	CLOACA	NIE13 - A -635	NIE13 - A- 636	NIE13 - A - 637	P ool 18
LAC2	LAGOS	CLOACA	NIE13 - A -640			pool19
LAC3	LAGOS	CLOACA	NIE13 -A- 646	NIE13 - A- 647		Pool 20
LAC4	LAGOS	CLOACA	NIE13 -A -650			pool 21
LAC5	LAGOS	CLOACA	NIE13 - A- 660	NEI13 - A -661	NIE13 - A - 662	pool 22
LAC6	LAGOS	CLOACA	NIE13 - A-665	NIE13 A -666	NIE13-A- 667	Pool 25
LAC7	LAGOS	CLOACA	NIE13-A-766	NIE13 -A- 767	NIE13-A- 768	Pool 37
LAC8	LAGOS	CLOACA	NIE13- A- 796	NIE13 -A- 797	NIE13-A- 798	Pool 38
LAC9	LAGOS	CLOACA	NIE13 - A- 806	NIE13 -A -807	NIE13 -A - 808	Pool 39
LAC10	LAGOS	CLOACA	NIE13 - A - 871	NIE13 -A -872	NEI13 -A - 873	Pool 46
LAC11	LAGOS	CLOACA	NIE13- A- 881	NIE13 - A- 882	NIE13 -A - 883	Pool 49
LAC12	LAGOS	CLOACA	NIE13 - A - 891	NIE13 - A - 892	NIE13 - A 893	Pool 50
LAC13	LAGOS	CLOACA	NIE13 - A - 986	NIE13 -A - 987	NIE13 - A- 988	Pool 61
LAC14	LAGOS	TRACHEA	N1E13-A-638	NIE13 - A- 639		Pool 81
LAC15	LAGOS	TRACHEA	NIE13 - A - 648	NIE13 - A-648		Pool 82
LAC16	LAGOS	TRACHEA	NIE13 - A - 663	NIE13 - A - 664		Pool 83
LAC17	LAGOS	TRACHEA	NIE13 - A -668	NIE13 - A -669		Pool 84
LAC18	LAGOS	TRACHEA	NIE13 - A -769	NIE13- A- 770		Pool 98
LAC19	LAGOS	TRACHEA	NIE13 - A 799	NIE13 - A -800		Pool 99
LAC20	LAGOS	TRACHEA	NIE13 - A - 809	NIE13 - 810		Pool 100
LAC21	LAGOS	TRACHEA	NIE13 -A - 874	NIE13-A- 875		Pool 107
LAC22	LAGOS	TRACHEA	NIE13 -A- 884	NIE13 - A-885		Pool 108
LAC23	LAGOS	TRACHEA	NIE13 - A - 894	NIE13 - A -895		Pool 109
LAC24	LAGOS	TRACHEA	NIE13-A- 899	NIE13- A- 900		Pool 110
LAC25	LAGOS	CLOACA	NIE13 - A - 641	NIE13-A- 642		Pool 129
LAC26	LAGOS	CLOACA	NIE13-A- 645			Pool 130
LAC27	LAGOS	CLOACA	NIE13-A- 651	NIE13 - A - 652		Pool 131
LAC28	LAGOS	CLOACA	NIE13 - A - 655	NIE13 - A -656	NIE13 - A - 657	Pool 132
LAC29	LAGOS	CLOACA	NIE13 - A -781	NIE13 - A -	NIE13 - A -	Pool 145

				782	783	
LAC30	LAGOS	CLOACA	NIE13 -A -786	NIE13-A-787	NIE13 - A - 788	Pool 146
LAC31	LAGOS	CLOACA	NIE13 - A - 791	NEI13 - A - 792	NIE13- A- 793	Pool 147
LAC32	LAGOS	CLOACA	NIE13 - A - 801	NIE13 - A - 802	NIE13- A- 803	Pool 148
LAC33	LAGOS	CLOACA	NIE13 - A - 991	NIE13 -A- 992	NIE13 -A - 993	Pool 163
LAC34	LAGOS	TRACHEA	NIE13 - A -643	NIE13 -A- 644		Pool 177
LAC35	LAGOS	TRACHEA	NIE13 - A -653	NEI13 - A -654		Pool 178
LAC36	LAGOS	TRACHEA	NIE13- A - 658	NIE13- A- 659		Pool 179
LAC37	LAGOS	TRACHEA	NIE13 - A - 784	NIE13 - A - 785		Pool 190
LAC38	LAGOS	TRACHEA	NIE13 - A -789	NIE13 - A -790		Pool 193
LAC39	LAGOS	TRACHEA	NIE13 - A - 794	NIE13 - A -795		Pool 194
LAC40	LAGOS	TRACHEA	NIE13 - A -804	NIE13 - A - 805		Pool 195
LAC41	LAGOS	TRACHEA	NIE13 - A - 994			Pool 210
LAC42	LAGOS	TRACHEA	NIE13 - A - 989	NEI13 - A 990		Pool 212
LAC43	LAGOS	CLOACA	NIE13 - A-886	NIE13-A-887	NIE13 - A - 888	Pool 217
LAC44	LAGOS	CLOACA	NIE13 -A - 966	NIE13 - A -967	NIE13 - A - 968	Pool 218
LAC45	LAGOS	TRACHEA	NEI13 - A -969	NIE13 - A -970		Pool 220
LAC46	LAGOS	CLOACA	NIE13-A-1002	NIE13-A-1003	NIE13-A- 1004	pool 23
LAC47	LAGOS	CLOACA	NIE13-A- 1007	NIE13-A- 1008	NIE13-A- 1009	pool 24
LAC48	LAGOS	CLOACA	NIE13-A- 1012	NIE13 -A- 1013	NIE13 -A- 1014	pool 40
LAC49	LAGOS	CLOACA	NIE13-A- 1017	NIE13-A- 1018		pool 47
LAC50	LAGOS	CLOACA	NIE13-A- 1021	NIE13-A-1022	NIE13-A- 1023	pool 48
LAC51	LAGOS	CLOACA	NIE13-A-1026	NIE13-A-1027		pool71
LAC52	LAGOS	TRACHEA	NIE13-A-1030	NIE13-A-1031		pool 72
LAC53	LAGOS	TRACHEA	N1E13-A- 1005	NIE13-A- 1006		pool 95
LAC54	LAGOS	TRACHEA	NIE13-A-1010	NIE13-A- 1011		pool 96
LAC55	LAGOS	TRACHEA	NIE13-A-1015	NIE13-A- 1016		pool 119
LAC56	LAGOS	TRACHEA	NIE13-A-1019	NIE13-A- 1020		pool 120
LAC57	LAGOS	TRACHEA	NIE13-A-1024	NIE13-A- 1025		pool 143
LAC58	LAGOS	TRACHEA	NIE13-A-1023	NIE13-A-1024		pool 144
LAC59	LAGOS	TRACHEA	NIE13-A-1025	NIE13-A-1026		pool 153
LAC60	LAGOS	TRACHEA	NIE13-A-1028	NIE13-A-1029		pool 156
OGC1	OGUN	CLOACA	NIE13 - A -676	NIE13 - A - 677	NIE13 - A - 678	Pool 26
OGC2	OGUN	CLOACA	NIE13 - A -681	NIE13 - A - 682	NIE13 - A - 683	Pool 27

OGC3	OGUN	CLOACA	NIE13 - A -736	NIE13 - A- 737	NIE13 - A - 738	Pool 33
OGC4	OGUN	CLOACA	NIE13 - A -741	NIE13 - A - 742	NIE13 - A - 743	Pool 34
OGC5	OGUN	CLOACA	NIE13 - A - 751	NIE13 - A -752	NIE13- A - 753	Pool 35
OGC6	OGUN	CLOACA	NIE13 - A -761	NIE13 - A -762	NIE13 - A - 763	Pool 36
OGC7	OGUN	CLOACA	NIE13-A-896	NIE13-A-897	NIE13-A-898	Pool 51
OGC8	OGUN	CLOACA	NIE13-A-906	NIE13-A907	NIE13-A-908	Pool 52
OGC9	OGUN	CLOACA	NIE13- A -956	NIE13-A-957	NIE13-A-958	Pool 58
OGC10	OGUN	CLOACA	NIE13- A- 961	NIE13-A-962	NIE13-A-963	Pool 59
OGC11	OGUN	CLOACA	NIE13- A -976	NIE13-A- 977	NIE13-A-978	Pool 60
OGC12	OGUN	CLOACA	NIE13-A-996	NIE13-A- 997		Pool 62
OGC13	OGUN	CLOACA	NIE13-A-998			Pool 63
OGC14	OGUN	TRACHEA	NIE13-A-679	NIE13-A-680		Pool 85
OGC15	OGUN	TRACHEA	NIE13-A-684	NIE13-A- 685		Pool 86
OGC16	OGUN	TRACHEA	NIE13-A- 739	NIE13-A-740		Pool 92
OGC17	OGUN	TRACHEA	NIE13-A- 744	NIE13-A- 745		Pool 93
OGC18	OGUN	TRACHEA	NIE13-A- 754	NIE13-A-755		Pool94
OGC19	OGUN	TRACHEA	NIE13-A-764	NIE13-A-765		Pool 97
OGC20	OGUN	TRACHEA	NIE13-A-819	NIE13-A-820		Pool101
OGC21	OGUN	TRACHEA	NIE13-A-909	NIE13-A- 910		Pool 111
OGC22	OGUN	TRACHEA	NIE13 -A-959	NIE13 -A- 960		Pool 117
OGC23	OGUN	TRACHEA	NIE13-A- 964	NIE13-A-965		Pool118
OGC24	OGUN	CLOACA	NIE13-A-671	NIE13-A-672	NIE13-A-673	Pool 133
OGC25	OGUN	CLOACA	NIE13 -A-686	NIE13-A-687	NIE13-A-688	Pool 134
OGC26	OGUN	CLOACA	NIE13-A-691	NIE13-A-692	NIE13-A-693	Pool 135
OGC27	OGUN	CLOACA	NIE13-A-721	NIE13-A-722	NIE13-A-723	Pool 136
OGC28	OGUN	CLOACA	NIE13-A-726	NIE13-A- 727	NIE13-A-728	Pool 137
OGC29	OGUN	CLOACA	NIE13-A- 731	NIE13-A- 732	NIE13-A-733	Pool 138
OGC30	OGUN	CLOACA	NIE13-A-746	NIE13-A-747	NIE13-A-748	Pool 139
OGC31	OGUN	CLOACA	NIE13-A-756	NIE13-A- 757	NIE13-A-758	Pool 140
OGC32	OGUN	CLOACA	NIE13-A- 771	NIE13-A-772	NIE13 -A-773	Pool 141

OGC33	OGUN	CLOACA	NIE13 -A- 776	NIE13-A- 777	NIE13-A-778	Pool 142
OGC34	OGUN	CLOACA	NIE13-A-836	NIE13-A- 837	NIE13-A-838	Pool 150
OGC35	OGUN	CLOACA	NIE13-A- 841	NIE13-A- 842	NIE13-A-843	Pool 151
OGC36	OGUN	CLOACA	NIE13-A- 851	NIE13 -A-852	NIE13-A-853	Pool 152
OGC37	OGUN	CLOACA	NIE13-A-941	NIE13-A-942	NIE13-A-943	Pool 159
OGC38	OGUN	CLOACA	NIE13-A-946	NIE13-A-947	NE13-A-948	Pool 160
OGC39	OGUN	CLOACA	NIE13 -A- 971	NIE13 -A-972	NIE13-A-973	Pool 161
OGC40	OGUN	TRACHEA	NIE13-A-674	NIE13-A-675		Pool 180
OG41	OGUN	TRACHEA	NIE13-A-689	NIE13-A-690		Pool 181
OGC42	OGUN	TRACHEA	NIE13-A- 724	NIE13-A- 725		Pool 183
OGC43	OGUN	TRACHEA	NIE13-A-729	NIE13-A- 730		Pool 184
OGC44	OGUN	TRACHEA	NIE13 -A- 734	NIE13 -A - 735		Pool 185
OGC45	OGUN	TRACHEA	NIE13-A- 749	NIE13-A- 750		Pool 186
OGC46	OGUN	TRACHEA	NIE13-A- 759	NIE13-A-760		Pool 187
OGC47	OGUN	TRACHEA	NIE13-A- 774	NIE13-A-775		Pool 188
OGC48	OGUN	TRACHEA	NIE13 -A- 779	NIE13 -A- 780		Pool 189
OGC49	OGUN	TRACHEA	NIE13-A-839	NIE13-A- 840		Pool 197
OGC50	OGUN	TRACHEA	NIE13 -A- 844	NIE13-A-845		Pool 198
OGC51	OGUN	TRACHEA	NIE13-A-854	NIE13-A-855		Pool 199
OGC52	OGUN	TRACHEA	NEI13-A-864	NIE13-A-865		Pool 200
OGC53	OGUN	TRACHEA	NIE13-A-904	NIE13=A-905		Pool 203
OGC54	OGUN	TRACHEA	NIE13-A- 944	NIE13-A-945		Pool 206
OGC55	OGUN	TRACHEA	NIE13-A- 949	NIE13-A-950		Pool 207
OGC56	OGUN	TRACHEA	NIE13-A- 974	NIE13-A- 975		Pool 208
OGC57	OGUN	TRACHEA	NIE13-A- 979	NIE13 -A-980		Pool 211
OGC58	OGUN	TRACHEA	NIE13 -A-995			Pool 213
OGC59	OGUN	TRACHEA	NIE13 -A-999	NIE13 -A - 1000		Pool 214
OGC60	OGUN	TRACHEA	NIE13-A- 889	NIE13-A-890		Pool 219
OYC1	OYO	CLOACA	NIE13-A-501	NIE13-A-502	NIE13-A-503	Pool 1
OYC2	OYO	CLOACA	NIE13-A- 506	NIE13-A- 507	NIE13-A-508	Pool 2
OYC3	OYO	CLOACA	NIE13 -A- 511	NIE13-A- 512	NE13-A-513	Pool 3
OYC4	OYO	CLOACA	NIE13-A- 516	NIE13-A-517	NIE13 -A-518	Pool 4
OYC5	OYO	CLOACA	NIE13-A- 531	NIE13-A-532	NIE13-A-533	Pool 5
OYC6	OYO	CLOACA	NIE13-A-696	NIE13-A-697	NIE13 - A - 698	Pool 28

OYC7	OYO	CLOACA	NIE13-A-701	NIE13- A- 702	NIE13-A-703	Pool 29
OYC8	OYO	CLOACA	NIE13-A- 706	NIE13-A- 707	NIE13-A-708	Pool 30
OYC9	OYO	CLOACA	NIE13-A- 711	NIE13-A- 712	NIE13-A-713	Pool 31
OYC10	OYO	CLOACA	NIE13-A- 716	NIE13- A- 717	NIE13-A-718	Pool 32
OYC11	OYO	CLOACA	NIE13-A-821	NIE13-A-822	NIE13-A-823	Pool 41
OYC12	OYO	CLOACA	NIE13-A-826	NIE13-A- 827	NIE13-A-828	Pool 42
OYC13	OYO	CLOACA	NIE13-A-831	NIE13-A-832	NIE13-A-833	Pool 43
OYC14	OYO	CLOACA	NIE13-A- 846	NIE13-A-847	NIE13-A-848	Pool 44
OYC15	OYO	CLOACA	NIE13-A- 856	NIE13-A-857	NIE13-A-858	Pool 45
OYC16	OYO	CLOACA	NIE13-A-921	NIE13-A-922	NIE13-A-923	Pool 53
OYC17	OYO	CLOACA	NIE13-A-926	NIE13-A-927	NIE13-A-928	Pool 54
OYC18	OYO	CLOACA	NIE13-A- 931	NIE13-A- 932	NIE13-A-933	Pool 55
OYC19	OYO	CLOACA	NIE13-A-936	NIE13-A-937	NIE13-A-938	Pool 56
OYC20	OYO	CLOACA	NIE13-A-951	NIE13-A-952	NIE13-A-953	Pool 57
OYC21	OYO	TRACHEA	NIE13-A- 504	NIE13-A-505		Pool 64
OYC22	OYO	TRACHEA	NIE13-A-509	NIE13-A-510		P00l 65
OYC23	OYO	TRACHEA	NIE13-A-514	NIE13-A-515		Pool 66
OYC24	OYO	TRACHEA	NIE13-A-519	NIE13-A-520		Pool 67
OYC25	OYO	TRACHEA	NIE13-A-534	NIE13-A-535		Pool 68
OYC26	OYO	TRACHEA	NIE13-A-699	NIE13-A=700		Pool 87
OYC27	OYO	TRACHEA	NIE13-A-704	NIE13-A-705		Pool 88
OYC28	OYO	TRACHEA	NIE13-A- 709	NIE13-A- 710		Pool 89
OYC29	OYO	TRACHEA	NIE13-A-714	NIE13-A-715		Pool 90
OYC30	OYO	TRACHEA	NIE13-A-719	NIE13-A-720		Pool 91
OYC31	OYO	TRACHEA	NIE13-A-824	NIE13-A-825		Pool 102
OYC32	OYO	TRACHEA	NIE13-A- 829	NIE13-A- 830		Pool 103
OYC33	OYO	TRACHEA	NIE13 - A- 834	NIE13-A-835		Pool 104
OYC34	OYO	TRACHEA	NIE13-A-849	NIE13-A-850		Pool 105
OYC35	OYO	TRACHEA	NIE13-A-859	NIE13-A-860		Pool 106
OYC36	OYO	TRACHEA	NIE13-A- 924	NIE13-A-925		Pool 112
OYC37	OYO	TRACHEA	NIE13-A- 929	NIE13-A-930		Pool 113
OYC38	OYO	TRACHEA	NIE13-A-934	NIE13-A-935		Pool 114
OYC39	OYO	TRACHEA	NIE13-A- 939	NIE13-A- 940		Pool 115
OYC40	OYO	TRACHEA	NIE13-A-954	NIE13-A-955		Pool 116

OYC41	OYO	CLOACA	NEI13-A- 521	NIE13-A-522	NIE13-A-523	Pool 121
OYC42	OYO	CLOACA	NIE13-A- 526	NIE13-A-527	NIE13-A-528	Pool 122
OYC43	OYO	CLOACA	NIE13-A- 536	NIE13-A-537	NIE13-A-538	Pool 123
OYC44	OYO	CLOACA	NIE13-A- 541	NIE13-A-542	NIE13-A-543	Pool 124
OYC45	OYO	CLOACA	NIE13-A- 811	NIE13-A-812	NIE13-A-813	Pool 149
OYC46	OYO	CLOACA	NIE13-A-866	NIE13-A-867	NIE13-A-868	Pool 154
OYC47	OYO	CLOACA	NIE13-A- 876	NIE13-A- 877	NIE13-A-878	Pool 155
OYC48	OYO	CLOACA	NIE13-A- 911	NIE13-A- 912	NIE13-A-913	Pool 157
OYC49	OYO	CLOACA	NIE13-A- 916	NIE13-A-917	NIE13-A-918	Pool 158
OYC50	OYO	CLOACA	NIE13-A-981	NIE13-A-982	NIE13-A-983	Pool 162
OYC51	OYO	TRACHEA	NIE13-A-524	NIE13-A-525		Pool 164
OYC52	OYO	TRACHEA	NIE13-A- 529	NIE13-A-530		Pool 165
OYC53	OYO	TRACHEA	NIE13-A- 539	NIE13-A- 540		Pool 166
OYC54	OYO	TRACHEA	NIE13-A- 544	NIE13-A-545		Pool 169
OYC55	OYO	TRACHEA	NIE13-A- 814	NIE13-A-815		Pool 196
OYC56	OYO	TRACHEA	NIE13-A-869	NIE13-A-870		Pool 201
OYC57	OYO	TRACHEA	NIE13-A- 879	NIE13-A-880		Pool 202
OYC58	OYO	TRACHEA	NIE13-A-914	NIE13-A- 915		Pool 204
OYC59	OYO	TRACHEA	NIE13-A-919	NIE13-A-920		Pool 205
OYC60	OYO	TRACHEA	NIE13-A- 984	NIE13-A-985		Pool 209
OYL1	OYO(L)	CLOACA	NIE13-A- 561	NIE13-A- 562	NIE13-A-563	Pool 6
OYL2	OYO(L)	CLOACA	NIE13-A- 564	NIE13-A- 565		Pool 7
OYL3	OYO(L)	CLOACA	NIE13-A- 576	NIE13-A- 577	NIE13-A-578	Pool 8
OYL4	OYO(L)	CLOACA	NIE13-A- 579	NIE13-A- 580		Pool 9
OYL5	OYO(L)	CLOACA	NIE13-A- 581	NIE13-A- 582	NIE-A- 583	Pool 10
OYL6	OYO(L)	CLOACA	NIE13 -A- 584	NIE13 -A- 585		Pool 11
OYL7	OYO(L)	CLOACA	NIE13-A- 608	NIE13 -A - 609	NIE13-A-610	Pool 12
OYL8	OYO(L)	CLOACA	NIE13-A- 611	NIE13 -A- 612	NIE13 -A - 613	Pool 13
OYL9	OYO(L)	CLOACA	NIE13 -A- 6I4	NIE13-A- 615		Pool 14
OYL10	OYO(L)	CLOACA	NIE13 - A-616	NIE13-A- 617		Pool 15
OYL11	OYO(L)	CLOACA	NIE13 - A- 618	NIE13 - A-619		Pool 16
OYL12	OYO(L)	CLOACA	NIE13 - A- 631	NIE13 -A- 632		Pool 17
OYL13	OYO(L)	TRACHEA	NIE13-A- 586	NIE13 -A- 587		Pool 69
OYL14	OYO(L)	TRACHEA	NIE13-A- 588	NIE13-A-589	NIE13-A-	Pool 70

					590	
OYL15	OYO(L)	TRACHEA	NIE1`3-A- 596	NIE13-A- 597	NIE13-A-598	Pool 73
OYL16	OYO(L)	TRACHEA	NIE13-A- 599	NIE13 - A- 600	NIE13 -A-601	Pool 74
OYL17	OYO(L)	TRACHEA	NIE13 - A- 602	NIE13 -A- 603	NIE13 - A-604	Pool 75
OYL18	OYO(L)	TRACHEA	NIE13 -A- 605	NIE13-A- 606	NIE13-A-607	Pool 76
OYL19	OYO(L)	TRACHEA	NIE13-A- 620	NIE13 -A- 621		Pool 77
OYL20	OYO(L)	TRACHEA	NIE13-A- 622	NIE13 - A- 623		Pool 78
OYL21	OYO(L)	TRACHEA	NIE13-A- 624	NIE13-A- 625		Pool 79
OYL22	OYO(L)	TRACHEA	NIE13-A- 633	NIE13 -A- 634		Pool 80
OYL23	OYO(L)	CLOACA	NIE13-A- 566	NIE13-A- 567	NIE13-A-568	Pool 125
OYL24	OYO(L)	CLOACA	NIE13 - A- 569	NIE13 -A - 570	NIE13-A-571	Pool 126
OYL25	OYO(L)	CLOACA	NIE13-A- 572	NIE13 - A- 573		Pool 127
OYL26	OYO(L)	CLOACA	NIE13 -A - 574	NIE13 -A- 575		Pool 128
OYL27	OYO(L)	TRACHEA	NIE13-A- 546	NIE13-A- 547		Pool 170
OYL28	OYO(L)	TRACHEA	NIE13 -A- 548	NIE13-A- 549		Pool 171
OYL29	OYO(L)	TRACHEA	NIE13 -A- 550	NIE13 -A-551		Pool 172
OYL30	OYO(L)	TRACHEA	NIE13 -A- 552	NIE13 -A- 553		Pool 173
OYL31	OYO(L)	TRACHEA	NIE13-A- 554	NIE13 -A- 555		Pool174
OYL32	OYO(L)	TRACHEA	NIE13-A- 591	NIE13-A- 592	NIE13-A-593	Pool 175
OYL33	OYO(L)	TRACHEA	NIE13-A- 594	NIE13 -A-595		Pool176
OYL34	OYO(L)	CLOACA	NIE13-A-1032	NIE13-A-1033	NIE13-A-1034	pool 167
OYL35	OYO(L)	CLOACA	NIE13-A- 1035	NIE13-A-1036	NIE13-A-1037	pool 168
OYL36	OYO(L)	CLOACA	NIE13-A-1038	NIE13-A-1039		pool 182
OYL37	OYO(L)	CLOACA	NIE13-A- 1040	NIE13-A- 1041	NIE13-A-1042	pool 191
OYL38	OYO(L)	TRACHEA	NIE13-A-1043	NIE13-A-1044		pool 192
OYL39	OYO(L)	TRACHEA	NIE13-A- 1045	NIE13-A- 1046		pool 215
OYL40	OYO(L)	TRACHEA	NIE13-A- 1047	NIE13-A-1048		pool 216
OGL1	OGUN(L)	CLOACA	NIE13-A-1049	NIE13-A-1050	NIE13-A-1051	pool 221
OGL2	OGUN(L)	CLOACA	NIE13-A-1052	NIE13-A-1053	NIE13-A-1054	pool 222
OGL3	OGUN(L)	CLOACA	NIE13-A-1055	NIE13-A-1056		pool 223
OGL4	OGUN(L)	CLOACA	NIE13-A- 1057	NIE13-A-1058	NIE13-A-1059	pool 224
OGL5	OGUN(L)	CLOACA	NIE13-A- 1060	NIE13-A- 1061	NIE13-A-1062	pool 225
OGL6	OGUN(L)	CLOACA	NIE13-A-1063	NIE13-A-1064	NIE13-A-1065	pool 226

OGL7	OGUN(L)	CLOACA	NIE13-A-1066	NIE13-A-1067	NIE13-A-1068	pool 227
OGL8	OGUN(L)	CLOACA	NIE13-A-1069	NIE13-A-1070		pool 228
OGL9	OGUN(L)	CLOACA	NIE13-A- 1071	NIE13-A- 1072		pool 229
OGL10	OGUN(L)	CLOACA	NIE13-A- 1073	NIE13-A- 1074	NIE13-A-1075	pool 230
OGL11	OGUN(L)	TRACHEA	NIE13-A- 1076	NIE13-A-1077		pool 231
OGL12	OGUN(L)	TRACHEA	NIE13 --A- 1078	NIE13-A- 1079		pool 232
OGL13	OGUN(L)	TRACHEA	NIE13-A- 1080	NIE13-A-1081		pool 233
OGL14	OGUN(L)	TRACHEA	NIE13-A-1082	NIE13-A- 1083		pool 234
OGL15	OGUN(L)	TRACHEA	NIE13-A-1084	NIE13-A-1085		pool 235
OGL16	OGUN(L)	TRACHEA	NIE13-A-1086	NIE13-A-1087		pool 236
OGL17	OGUN(L)	TRACHEA	NIE13-A-1088	NIE13-A-1089		pool 237
OGL18	OGUN(L)	TRACHEA	NIE13-A-1090	NIE13-A-1091		pool 238
OGL19	OGUN(L)	TRACHEA	NIE13-A-1092	NIE13-A-1093		pool 239
OGL20	OGUN(L)	TRACHEA	NIE13-A- 1094	NIE13-A-1095		pool 240
OGL21	OGUN(L)	CLOACA	NIE13-A-1096	NIE13-A-1097		pool 241
OGL22	OGUN(L)	CLOACA	NIE13-A-1098	NIE13-A-1099		pool 242
OGL23	OGUN(L)	CLOACA	NIE13-A-1100	NIE13-A-1101	NIE13-A-1102	pool 243
OGL24	OGUN(L)	CLOACA	NIE13-A-1103	NIE13-A-1104	NIE13-A-1105	pool 244
OGL25	OGUN(L)	CLOACA	NIE13-A-1106	NIE13-A-1107	NIE13-A-1108	pool 245
OGL26	OGUN(L)	CLOACA	NIE13-A-1109	NIE13-A-1110	NIE13-A-1111	pool 246
OGL27	OGUN(L)	CLOACA	NIE13-A-1112	NIE13-A-1113	NIE13-A-1114	pool 247
OGL28	OGUN(L)	CLOACA	NIE13-A-1115	NIE13-A-1116		pool 248
OGL29	OGUN(L)	CLOACA	NIE13-A-1117	NIE13-A-1118	NIE13-A-1119	pool 249
OGL30	OGUN(L)	CLOACA	NIE13-A-1120	NIE13-A-1121	NIE13-A-1122	pool 250
OGL31	OGUN(L)	TRACHEA	NIE13-A-1123	NIE13-A-1124		pool 251
OGL32	OGUN(L)	TRACHEA	NIE13-A-1125	NIE13-A-1126		pool 252
OGL33	OGUN(L)	TRACHEA	NIE13-A-1127	NIE13-A-1128		pool 253
OGL34	OGUN(L)	TRACHEA	NIE13-A-1129	NIE13-A-1130		pool 254
OGL35	OGUN(L)	TRACHEA	NIE13-A-1131	NIE13-A-1132		pool 255
OGL36	OGUN(L)	TRACHEA	NIE13-A-1133	NIE13-A-1134		pool 256
OGL37	OGUN(L)	TRACHEA	NIE13-A-1135	NIE13-A-1136		pool 257
OGL38	OGUN(L)	TRACHEA	NIE13-A-1137	NIE13-A-1138		pool 258
OGL39	OGUN(L)	TRACHEA	NIE13-A-1139	NIE13-A-1140		pool 259
OGL40	OGUN(L)	TRACHEA	NIE13-A-1141	NIE13-A-1142		pool 260
LAGL1	LAGOS(L)	CLOACA	NIE13-A-1143	NIE13-A-1144	NIE13-A-1145	pool 261
LAGL2	LAGOS(L)	CLOACA	NIE13-A-1146	NIE13-A-1147	NIE13-A-1148	pool 262
LAGL3	LAGOS(L)	CLOACA	NIE13-A-1149	NIE13-A-1150		pool 263

LAGL4	LAGOS(L)	CLOACA	NIE13-A-1151	NIE13-A-1152		pool 264
LAGL5	LAGOS(L)	CLOACA	NIE13-A-1153	NIE13-A-1154	NIE13-A-1155	pool 265
LAGL6	LAGOS(L)	CLOACA	NIE13-A-1156	NIE13-A-1157	NIE13-A-1158	pool 266
LAGL7	LAGOS(L)	CLOACA	NIE13-A-1159	NIE13-A-1160	NIE13-A-1161	pool 267
LAGL8	LAGOS(L)	CLOACA	NIE13-A-1162	NIE13-A-1163		pool 268
LAGL9	LAGOS(L)	TRACHEA	NIE13-A-1164	NIE13-A-1165		pool 269
LAGL10	LAGOS(L)	TRACHEA	NIE13-A-1166	NIE13-A-1167		pool 270
LAGL11	LAGOS(L)	TRACHEA	NIE13-A-1168	NIE13-A-1169		pool 271
LAGL12	LAGOS(L)	TRACHEA	NIE13-A-1170	NIE13-A-1171		pool 272
LAGL13	LAGOS(L)	TRACHEA	NIE13-A-1172	NIE13-A-1173		pool 273
LAGL14	LAGOS(L)	TRACHEA	NIE13-A-1174	NIE13-A-1175		pool 274
LAGL15	LAGOS(L)	TRACHEA	NIE13-A-1176	NIE13-A-1177		pool 275
LAGL16	LAGOS(L)	TRACHEA	NIE13-A-1178	NIE13-A-1179		pool 276
LAGL17	LAGOS(L)	TRACHEA	NIE13-A-1180	NIE13-A-1181		pool 277
LAGL18	LAGOS(L)	TRACHEA	NIE13-A-1182	NIE13-A-1183		pool 278
LAGL19	LAGOS(L)	TRACHEA	NIE13-A-1184	NIE13-A-1185		pool 279
LAGL20	LAGOS(L)	TRACHEA	NIE13-A-1186	NIE13-A-1187		pool 280
LAGL21	LAGOS(L)	CLOACA	NIE13-A-1188	NIE13-A-1189	NIE13-A-1190	pool 281
LAGL22	LAGOS(L)	CLOACA	NIE13-A-1191	NIE13-A-1192		pool 282
LAGL23	LAGOS(L)	CLOACA	NIE13-A-1193	NIE13-A-1194		pool 283
LAGL24	LAGOS(L)	CLOACA	NIE13-A-1195	NIE13-A-1196	NIE13-A-1197	pool 284
LAGL25	LAGOS(L)	CLOACA	NIE13-A-1198	NIE13-A-1199	NIE13-A-1200	pool 285
LAGL26	LAGOS(L)	CLOACA	NIE13-A-1201	NIE13-A-1202	NIE13-A-1203	pool 286
LAGL27	LAGOS(L)	CLOACA	NIE13-A-1204	NIE13-A-1205	NIE13-A-1206	pool 287
LAGL28	LAGOS(L)	CLOACA	NIE13-A-1207	NIE13-A-1208	NIE13-A-1209	pool 288
LAGL29	LAGOS(L)	CLOACA	NIE13-A-1210	NIE13-A-1211		pool 289
LAGL30	LAGOS(L)	CLOACA	NIE13-A-1212	NIE13-A-1213		pool 290
LAGL31	LAGOS(L)	TRACHEA	NIE13-A-1214	NIE13-A-1215		pool 291
LAGL32	LAGOS(L)	TRACHEA	NIE13-A-1216	NIE13-A-1217		pool 292
LAGL33	LAGOS(L)	TRACHEA	NIE13-A-1218	NIE13-A-1219		pool 293
LAGL34	LAGOS(L)	TRACHEA	NIE13-A-1220	NIE13-A-1221		pool 294
LAGL35	LAGOS(L)	TRACHEA	NIE13-A-1222	NIE13-A-1223		pool 295
LAGL36	LAGOS(L)	TRACHEA	NIE13-A-1224	NIE13-A-1225		pool 296
LAGL37	LAGOS(L)	TRACHEA	NIE13-A-1226	NIE13-A-1227		pool 297
LAGL38	LAGOS(L)	TRACHEA	NIE13-A-1228	NIE13-A-1229		pool 298
LAGL39	LAGOS(L)	TRACHEA	NIE13-A-1230	NIE13-A-1231		pool 299
LAGL40	LAGOS(L)	TRACHEA	NIE13-A-1232	NIE13-A-1233		pool 300

Appendix X11: Sequences of 1b gene of cloaca and trachea in commercial and local chickens in Lagos, Ogun and Oyo states

>NIE13_pool121. 27475 nucleotides.

CAGAGCAATGCCAAATTTGCTACGTATAGCAGCATCTTTGGTACTTGCTCGTAAACACACTA
ATTGTTGTACTTGGTCTGAACGCATTTATAGGTTGTATAATGAATGCGCTCAGGTTTTATCT
GAAACTGTTTTAGCTACAGGTGGTATTTATGTAAAACCTGGTGGCACTAGCAGTGGTGATG
CTACTACTGCTTATGCAAACAGCGTTTTCAACATAATACAGGCTACATCTGCTAATGTTGCG
CGTCTTTTGAGTGTTATAACGCGTGATATTGTTTATGATGACATTAAGAGCCTGcAGTATGA
GTTGTACCAGCAGGTTTATAGGCGAGCTAATTTTGACCCTGCCTTTGTAGAAAAGTTTTATT
CTTACTTATGTAAGAATTTTTCTTTGATGATCTTGTCTGATGATGGTGTTGTTTGTTACAACA
ACACATTAGCCAAACAGGGTCTTGTAGCAGACATTTCTGGCTTTAGAGAAATTCTCTACTAC
CAAAATAA

>NIE13_pool127. 27475 nucleotides.

ACACTAATTGTTGTACTTGGTCTGAACGCATTTATAGGTTGTATAATGAATGCGCTCAAGTT
TTGTCTGAAACTGCAAGCCACATCAGCCAATGTTGCGCGTCTTCTGAGTGTTATAACGCGTG
ATATTGTTTRTGATGACATTAAGAGCCTGCAGTATGAGTTGTACCAGCAGGTTTATAGGCG
AGTTAATTTCGACCCAGCCTTTGTAGAAAAGTTTTATTCTTACTTATGTAAGAACTTTTCTTT
GATGATCTTGTCTGATGATGGTGTTGTTTGTTACAACAACACATTAGCCAAACAGGGTCTTG
TAGCAGACATTTCTGGCTTTAGAGAAATTCTCTACTACCAAAATAA

>NIE13_pool128. 27475 nucleotides.

TAGAGCAATGCCAAATTTGCTACGTATAGCAGCTTCTTTAGTACTTGCTCGTAAACACACTA
ATTGTTGTACTTGGTCTGAACGCATTTATAGGTTGTATAATGAATGCGCTCAAGTTTTGTCT
GAAACTGTCTTAGCTACAGGTGGTATCTATGTGAAACCTGGTGGCACTAGCAGTGGTGATG
CAACTACTGCTTATGCAAACAGTGTTTTTAACATAATACAAGCCACATCAGCCAATGTTGCG
CGTCTTCTGAGTGTTATAACGCGTGATATTGTTTATGATGACATTAAGAGCCTGCAGTATGA
GTTGTACCAGCAGGTTTATAGGCGAGTTAATTTCGACCCAGCCTTTGTAGAAAAGTTTTATT
CTTACTTATGTAAGAACTTTTCTTTGATGATCTTGTCTGATGAYGGTGTTGTTTGTTACAACA
ACACATTAGCCAAACAGGGTCTTGTAGCAGACATTTCTGGCTTTAGAGAAATTCTCTACTAC
CAAAATA

>NIE13_pool133. 27475 nucleotides.

CAGAGCAATGCCAAATTTGCTACGTATAGCAGCATCTTTGGTACTTGCTCGTAAACACACTA
ATTGTTGTACTTGGTCTGAACGCATTTATAGGTTGTATAATGAATGCGCTCAGGTATTATCT
GAAACTGTTTTAGCTACAGGTGGTATTTATGTAAAACCTGGTGGCACTAGCAGTGGTGATG
CTACTACTGCTTATGCAAACAGCGTTTTCAACATAATACAAGCTACATCTGCTAATGTTGCG
CGTCTTTTGAGTGTTATAACGCGTGATATTGTTTATGATGACATTAAGAGCCTGCAGTATGA
GTTGTACCAGCAGGTTTATAGGCGAGTTAATTTTGACCCAGCCTTTGTAGAAAAGTTTTATT
CTTACTTATGTAAGAACTTTTCTTTGATGATCTTGTCTGATGATGGTGTTGTTTGTTACAACA

ACACATTAGCCAAACAGGGTCTTGTAGCAGACATTTCTGGCTTTAGAGAAATCCTCTACTAC
CAAAATAATGTCTATATGGCTGACTCTAAGTGtTGGGTTGAACCAGACTTAGAAAAAG

>NIE13_pool135. 27475 nucleotides.

TAGAGCAATGCCAAATTTGCTACGTATAGCAGCATCGTTGGTACTTGCTCGTAAACACACTA
ATTGTTGTACTTGGTCTGAACGCATTTATAGGTTGTATAATGAATGCGCTCAAGTTTTGTCT
GAAACTGTCTTAGCCACAGGTGGTATCTATGTGAAACCTGGTGGCACTAGCAGTGGTGATG
CCACTACTGCTTATGCAAACAGCGTCTTCAACATAATACAGGCTACATCTGCTAATGTTGCG
CGTCTTTTGAGTGTTATAACGCGTGATATTGTTTATGATGACATTAAGAGCCTGCAGTACGA
GCTGTACCAGCAGGTTTATAGGCGAGTTAATTTTGATCCTGCCTTTGTAGAAAAGTTTTATT
CTTACTTATGTAAGAACTTTTCATTGATGATCTTGTCTGATGATGGTGTTGTTTGTTACAACA
ACACATTAGCCAAACAGGGTCTTGTAGCAGACATTTCTGGCTTTAGAGAAATTCTCTACTAC
CAAAATAA

>NIE13_pool159. 27475 nucleotides.

TAGAGCAATGCCAAATTTGCTGCGTATAGCAGCATCTTTGGTACTTGCTCGTAAACACACTA
ATTGTTGTACTTGGTCTGAACGCATTTATAGGTTGTATAATGAATGCGCTCAGGTTTTATCT
GAAACTGTTTTAGCTACAGGTGGTATTTATGTAAAACCTGGTGGCACTAGCAGTGGTGATG
CTACTACTGCTTATGCAAACAGTGTTTTCAACATAATACAGGCTACATCTGCTAATGTTGCG
CGTCTTTTGAGTGTTATAACGCGTGATATTGTTTATGATGACATTAAGAGCCTGCAGTATGA
GTTGTACCAGCAGGTTTACAGGCGAGTTAATTTTGACCCGGCCTTTGTAGAAAAGTTTTATT
CTTACTTATGTAAGAATTTTTCTTTGATGATCTTGTCTGATGATGGTGTTGTTTGTTACAACA
ACACATTAGCCAAACAGGGTCTTGTAGCAGATATTTCTGGCTTTAGAGAAATTCTCTACTAC
CAAAATAATGTCTATATGGCTGATTCTAAGTGTTGGGTTGAACCAGACTTAGAAAAAGGCC
CA

>NIE13_pool160. 27475 nucleotides.

CAGAGCAATGCCAAATTTGCTACGTATAGCAGCATCTTTGGTACTTGCTCGTAAACACACTA
ACTGTTGTACTTGGTCTGAACGCATTTATAGGTTGTATAATGAATGCGCTCAGGTTTTATCT
GAAACTGTCTTAGCTACAGGTGGTATTTATGTAAAACCTGGCGGCACTAGCAGTGGTGATG
CTACTACTGCTTATGCAAACAGCGTTTTCAACATAATACAGGCTACATCTGCTAATGTTGCG
CGTCTTTTGAGTGTTATAACGCGTGATATTGTTTATGATGACATTAAGAGCCTGCAGTATGA
GTTGTACCAGCAGGTTTATAGGCGAGTTAATTTTGAACCAGCCTTTGTAGAAAAGTTTTATT
CTTACTTATGTAAGAACTTTTCATTGATGATTTTGTCTGACGATGGTGTTGTTTGTTATAACA
ACACATTAGCTAAACAAGGTCTTGTAGCAGATATTTCTGGTTTTAGAGAGATTCTCTACTAC
CAAAATA

>NIE13_pool161. 27475 nucleotides.

CATCTTTGGTACTTGCTCGCAAACACACTAATTGTTGTACTTGGTCTGAACGGATTTATAGG
TTGTATAATGAATGCGCTCAGGTTTTATCTGAAACTGTTTTAGCTACAGGTGGTATTTATGT

AAAACCTGGTGGCACTAGCAGTGGTGATGCTACTACTGCTTATGCAAACAGCGTTTTCAAC
ATAATACAAGCTACATCTGCTAATGTTGCGCGTCTTTTGAGTGTTATAACGCGTGATATTGT
TTATGATGACATTAAGAGCCTGCAGTATGAGTTGTACCAGCAGGTTTATAGGCGAGTTAAT
TTTGACCCTGCCTTTGTAGAAAAGTTTTATTCTTACTTATGTAAGAACTTTTCTTTGATGATCT
TGTCTGATGATGGTGTTGTTTGTTATAACAACACATTAGCCAAACAGGGTCTTGTAGCAGA
CATTTCTGGCTTTAGAGAAATTCTCTACTACCAAAATAA

>NIE13_pool180. 27475 nucleotides.

CAGAGCAATGCCAAATTTGCTACGTATAGCAGCATCTTTGGTACTTGCTCGTAAACACACTA
ATTGTTGTACTTGGTCTGAACGCATTTATAGGTTGTATAATGAATGCGCTCAGGTATTATCT
GAAACTGTTTTAGCTACAGGTGGTATTTATGTAAAACCTGGTGGCACTAGCAGTGGTGATG
CTACTACTGCTTATGCAAACAGCGTTTTCAACATAATACAAGCTACATCTGCTAATGTTGCG
CGTCTTTTGAGTGTTATAACGCGTGATATTGTTTATGATGACATTAAGAGCCTGCAGTATGA
GTTGTACCAGCAGGTTTATAGGCGAGTTAATTTTGACCCAGCCTTTGTAGAAAAGTTTTATT
CTTACTTATGTAAGAACTTTTCTTTGATGATCTTGTCTGATGATGGTGTTGTTTGTTACAACA
ACACATTAGCCAAACAGGGTCTTGTAGCAGACATTTCTGGCTTTAGAGAAATCCTCTACTAC
CAAAATAA

>NIE13_pool20. 27475 nucleotides.

TAGAGCAATGCCAAATTTGCTACGTATAGCAGCATCTTTGGTACTTGCTCGTAAGCACACTA
ATTGTTGTACTTGGTCTGAACGCATTTATAGGTTGTATAATGAATGCGCTCAGGTTTTGTCT
GAAACTGTTTTAGCTACAGGTGGTATTTATGTAAAACCTGGTGGCACTAGCAGTGGTGATG
CCACTACTGCTTATGCCAACAGTGTCTTTAACATAATACAAGCCACATCTGCTAATGTTGCG
CGTCTTTTAAGTGTTATAACGCGTGATATTGTTTATGATGACATTAAGAGCTTGCAGTATGA
ATTGTACCAGCAGGTTTATAGGAGAGTTAATTTTGACCCAGCCTTTGTAGAAAAGTTCTATT
CTTACTTATGTAAGAACTTTTCATTGATGATTTTGTCTGACGATGGTGTTGTTTGTTATAACA
ACACATTAGCCAAACAGGGTCTTGTAGCAGACATTTCTGGCTTTAGAGAAATTCTCTACTAC
CAAAATAA

>NIE13_pool213. 27475 nucleotides.

TAGAGCAATGCCAAATTTGCTACGTATAGCAGCATCTTTGGTACTTGCTCGTAAACACACTA
ATTGTTGTACTTGGTCTGAACGCATTTATAGGTTGTATAACGAATGCGCTCAAGTTTTGTCT
GAAACTGTCTTAGCTACAGGTGGCATCTATGTGAAACCTGGTGGCACTAGCAGTGGTGATG
CCACTACTGCTTATGCAAATAGTGTTTTTAACATAATACAAGCCACATCAGCCAATGTTGCG
CGTCTTTTGAGTGTTATAACGCGTGATATTGTTTATGATGACATTAAGAGCCTGCAGTATGA
GTTGTACCAGCAGGTTTATAGGCGAGTTAATTTTGACCCTGCCTTTGTAGAAAAGTTTTATT
CTTACTTATGTAAGAATTTTTCTTTGATGATCTTGTCTGATGATGGTGTTGTTTGTTACAACA
ACACATTAGCCAAACAGGGTCTTGTAGCAGACATTTCTGGCTTTAGAGAAATTCTCTACTAC
CAAAATAATGTCTATATGGCTGATTCCAAGTGTTGGGTTGAGCCAGACTTGGAAAAAGGC

>NIE13_pool27. 27475 nucleotides.

CAGAGCAATGCCAAATTTGCTACGTATAGCAGCATCTTTGGTACTTGCTCGTAAACACACTA
ATTGTTGTACTTGGTCTGAACGCATTTATAGGTTGTATAATGAATGCGCTCAGGTTTTATCT
GAAACTGTTTTAGCTACAGGTGGTATTTATGTAAAACCTGGTGGCACTAGCAGTGGTGATG
CTACTACTGcTTATGCAAACAGCGTTTTCAACATAATACAGGCTACATCTGCTAATGTTGCG
CGTCTTTTGAGTGTTATAACGCGTGATATTGTTTATGATGACATTAAGAGCCTGCAGTATGA
GTTGTACCAGCAGGTTTATAGGCGAGCTAATTTTGACCCTGCCTTTGTAGAAAAGTTTTATT
CTTACTTATGTAAGAATTTTTCTTTGATGATCTTGTCTGATGATGGTGTTGTTTGTTACAACA
ACACATTAGCCAAACAGGGTCTTGTAGCAGACATTTCTGGCTTTAGAGAAATTCTCTACTAC
CAAAATAA

>NIE13_pool35. 27475 nucleotides.

TAGAGCAATGCCAAATTTGCTACGTATAGCAGCATCTTTGGTACTTGCTCGTAAACACACTA
ATTGTTGTACTTGGTCTGAACGCATTTATAGGTTGTATAATGAATGCGCTCAGGTTTTATCT
GAAACTGTTTTAGCTACAGGTGGTATTTATGTAAAACCTGGCGGCACTAGCAGTGGTGATG
CTACTACTGCTTATGCAAACAGCGTTTTCAACATAATACAGGCTACATCTGCTAATGTTGCG
CGTCTTTTGAGTGTTATAACGCGTGATATTGTTTATGATGACATTAAGAGCCTGCAGTATGA
GTTGTACCAGCAGGTTTATAGGCGAGTTAATTTTGACCCAGCCTTTGTAGAAAAGTTTTATT
CTTACTTATGTAAGAATTTTTCTTTGATGATCTTGTCTGATGATGGTGTTGTTTGTTACAATA
ACACATTAGCCAAACAGGGTCTTGTAGCAGATATTTCTGGCTTTAGAGAAATTCTCTACTAC
CAAAATAATGTCTAT

>NIE13_pool59. 27475 nucleotides.

TAGAGCAATGCCAAATTTGCTACGTATAGCAGCATCTTTGGTACTTGCTCGCAAACACACTA
ATTGTTGTACTTGGTCTGAACGCATTTATAGGTTGTATAATGAATGCGCTCAGGTTTTATCT
GAAACTGTTTTAGCTACAGGTGGTATTTATGTAAAACCTGGTGGCACTAGCAGTGGTGATG
CTACTACTGCTTATGCAAACAGCGTTTTCAACATAATACAAGCTACATCTGCTAATGTTGCG
CGTCTTTTGAGTGTTATAACGCGTGATATTGTTTATGATGACATTAAGAGCCTGCAGTATGA
GTTGTACCAGCAGGTTTATAGGCGAGTTAATTTTGACCCTGCCTTTGTAGAAAAGTTTTATT
CTTACTTATGTAAGAACTTTTCTTTGATGATCTTGTCTGATGATGGTGTTGTTTGTTATAACA
ACACATTAGCCAAACAGGGTCTTGTAGCAGACATTTCTGGCTTTAGAGAAATTCTCTACTAC
CAAAATAAT

>NIE13_pool65. 27475 nucleotides.

TAGAGCAATGCCAAATTTGCTACGTATAGCAGCATCTTTGGTACTTGCTCGTAAACACACTA
ATTGTTGTACTTGGTCTGAACGCATTTATAGGTTGTATAATGAATGCGCTCAGGTTTTATCT
GAAACTGTTTTAGCTACAGGTGGTATTTATGTAAAACCTGGTGGCACTAGCAGTGGTGATG
CTACTACTGCTTATGCAAACAGCGTTTTCAACATAATACAGGCTACATCTGCTAATGTTGCG
CGGCTTTTGAGTGTTATAACGCGTGATATTGTTTATGATGACATTAAGAGCCTGCAGTATGA
GTTGTACCAGCAGGTTTATAGGCGAGTTAATTTTGACCCTGCCTTTGTAGAAAAGTTTTATT
CTTACTTATGTAAGAATTTTTCTTTGATGATCTTGTCTGATGATGGTGTTGTTTGTTATAACA

ACACATTAGCCAAACAGGGTCTTGTAGCAGACATTTCTGGCTTTAGAGAAATTCTCTACTAC
CAAAATAATGTCTATATGG

>NIE13_pool70. 27475 nucleotides.

TAGAGCAATGCCAAATTTGCTACGTATAGCAGCATCTTTGGTACTTGCTCGTAAGCACACTA
ATTGTTGTACTTGGTCTGAACGCATTTATAGGTTGTATAATGAATGCGCTCAGGTTTTGTCT
GAAACTGTTTTAGCTACAGGTGGTATTTATGTAAAACCTGGTGGCACTAGCAGTGGTGATG
CCACTACTGCTTATGCCAACAGTGTCTTTAACATAATACAAGCCACATCTGCTAATGTTGCG
CGTCTTTTAAGTGTTATAACGCGTGATATTGTTTATGATGACATTAAGAGCTTGCAGTATGA
ATTGTACCAGCAGGTTTATAGGMGAGTTAATTTTGACCCAGCCTTTGTAGAAAAGTTCTAT
TCTTACTTATGTAAGAACTTTTCATTGATGATcTTGTCTGATGATGGcGTTGTTTGTTATAAC
AACACATTGGCCAAgCAaGGTCTTGTTGCAGACATTTCtGGtTTTAGAGAgATTCTCTACTAC
CAAAATAAT

Appendix X111: Sequences of S1 gene of cloaca and trachea in commercial and local chickens in Lagos, Ogun and Oyo states

>Pool126. 3558 nucleotides.

TCTTTAACAGGTATGATTCCAGAGAATCAGATTCGTATTTCTGCTATGAAAGGTAGAAGTTT
GTTTTATAACTTAACAGTTGATGTGACTAAATATCCTAAATTTAAGTCGCTTCAGTGTGTTAA
TAATTTTACATCTGTATACTTAAATGGTGATCTCGTTTTTACTTCTAATGCTACTAAAGATGT
TAGTGCAGCAGGTGTTCATTTTAAAAGTGGTGGACCTATAACTTATAAGTTTATGAAATAA
GTTGATGTCCTGG

>Pool127. 3558 nucleotides.

GTTACAAAGATGGTGCGCATGAATGTCCTTTAACAGGTATGATTCCACAGAATCAGATTCG
TATTTCTGCTATGAAAGGTAGCAGTTTGTTTTATAACTCAACAGTTGGTGTGACTAAATATC
CTAAATTTAAGTCGCTTCAGTGTGTTAATAATTTTACAGCTGTATACTTAAATGGTGATCTCG
TTTTTACTTCTAATGACACTAAAGATGTTAGTGCAGCAGGTGTTTATTTCAAAAGTGGTGGA
CCTATAACTTATAAGGTTATGAAACAAGTTGATGTCCT

>Pool132. 3558 nucleotides.

GTAGTGCCGCTTTGTTTKATAATAATGAAACCGTTTACTACTWCCAAAGTGCCTTCCGACCA
TCTGATGGTTGGCATATGCATGGGGGTGCTTATGCAGTAGTTAATGTTTCTATAGAATATA
ACAATGCACAGCAAGTATATGTACTGCAGGGGCTATCTATTGGAGTAAAAATTTTACTGCA
TCTTCTGTAGCCATGACAGCACCTGATACAGGTATGTCTTGGTCAGCCTCCCAATTCTGTAC
GGCCCACTGTAATTTTACAGATTTTACAGTGTTTGTTACACATTGTTACAAAAAAGGTGCTA
GTGAATGTCCTTTAACAGGTCTGATTCCAAAGAATCATATTCGTATTTCTGCTATGAAAGGT
AGCAGTTTGTTTTATAACTTAACAGTTGCTGTGACTAAATATCCTAAATTTAAGTCGCTTCAG
TGTGTTAATAATTTTACATCTGTATACTTAAATGGTGATCTCGTTTTTACTTCTAATGATACTA
AAGATGTTAGTGCAGCAGGTGTTCATTTTAAAAGTGGTGGACCTATAACTTATAAGGTTAT
GAGACAAGTTGATGTCCTAGCTTATTTTATTAATGGTACAGCACAAGATGTTATTTTGTGTG
A

>Pool135. 3558 nucleotides.

CCACCTGATGGTTGGCATATACATGGTGGTGCTTACGCAGTAGTTAAAACTTTTAATCAAAC
CAACAATGCTGGTGCACAGTCACAGTGCACAGCTGGTGTTATTAAAGGTGGTCATAGTTTT
AATGCCTCTTCTGTAGCTATTACTGCACCACCTTCAGGTATGACCTGGTCAGCATCCCAATTT
TGTACAGCGCATTGTAATTTTAGTGATATTACAGTGTTTGTAACACATTGTTTTATAGATGG
AGTTTAATCTTGTCTACTTACAGGCAAAATCCCACAGAACTTTCTTCGTATTTCTGCTCTTAA
AGGAGGCAGGCTGTTTTATAATTTAACAGTTAGTGTAGCTAAGTACCCTAATTTTAAATCTT
TTCAATGTGTTAATAATCAGACATCTGTATATTTAAATGGTGATCTTGTTTTTACTTCTAATG
AGACTATAAATGTTAAGGACGCTGGTGTTTACTTTAAAGCTGGCGGACCTGTACGCTATAA
AGTTATGAGAGAGGTCAAAGTTCTGGCCTACTTTGTTAATG

>Pool139. 3558 nucleotides.

TAATTTGATTGGTGATTCTTATGTTTACTACTACCAGAGTGAGTTCAGACCACCTGATGGTT
GGAATTTACATGGTGGTGCTTAYGCAGTAGTTAATACTTTTAATGAAACCAACAATGCTGGT
GCAARWYSGGAGTGCACAGTTGGTGTTATTAGCGGTGGTCATGGTTTTAATGCCTCTTCTG
TAGCTATTACTGCACCACCTTCAGGTATGGCCTGGTCAAAATCCCAATTTTGTACAGCGCAT
TGTAATTTTAGTGATATTACAGTGTTTGTAACACATTGTTTTAAAGATGGTGCTGGATCTYG
TCCAATTACTGGCAAAATCCCACAGAACTTTCTTCGCATTTCTGCTCTTAGAGGAGGCAGGC
TGTTTTATAATTTAACAGTTAGTGTAGCTAAGTACCCTAATTTTAAATCTTTTCAATGTGTTA
ATAATCAGACATCTGTATATTTAAATGGTGATCTTGTTTTTACTTCTAATGAGACTATAGATG
TTAAGGACGCTGGTGTTTACTTTAAAGCTGGCGGACCTGTATYCTATAAAGTTATGAGAGA
GGTCAAAGTTCTGGCCTACTTTGTTAATGGCACTGTACAAGATGTTATTTTATGTGATG

>Pool160. 3558 nucleotides.-
TATTCGTATTTCTGCTATGAAAAATAGCAGTTTGTTTTATAACTTAACAGTTTCTGTGACTAA
ATATCCTACATTTAGGTCGCTTCAGTGTGTTAATAATTTTACATCTGTATACCTAAATGGTGA
TCTCGTGTTTACTTCTAATGACACTAAAGATGTTAGTGCAGCAGGTGTTTATTTTAAAGGTG
GTGGACCTATAACTTATAAGGTTATGAGACAAGTTGCTGTCCTGGCTTATTTTGTTAATGGT
A

>Pool161.

3558 nucleotides.

TGCCGCTTTGTTTGATAATAATGAAACCGTTTACTACTACCAAAGTGCCTTCCGACCATTTAA
TGGTTGGCATATGCATGGGGGTGCTTATGCAGTAGTTAATGTTTCTGTAGAATATAACAAC
GCAGGCTCAAGTCAAACTTGTACTGCAGGGGCTATCCATTGGAGTAAGAATTTTTCTGCAT
CTTCTGTAGCCATGACAGCACCTGGTGCAGGTATGTCTTGGTCAGCCAGTGAGTTCTGTAC
GGCCCACTGTAACTTTACAGATTTTACAGTGTTTGTTACACATTGTTACAAAGCTGGTCAAT
GTCCTTTAACAGGTATGATTCCACAGAATCATATTCGTATTTCTGCTATGAGAAATGGCGGG
TTGTTTTATAACTTAACAGTTGCTGTGACTAAATATTCTAAATTTAAGTCGCTTCAGTGTGTT
AATAATTTAACAACTGTATACTTAAATGGTGATCTCGTTTTTAGTTCTAATGATACTAAAGAT
GTTAGTGCAGCAGGTGTTCATTTTAAAAGTGGTGGACCTATAACTTATAAGGTTATGAGGC
AAGTTGATGTCCTAGCTTATTTTGTTAATGGTACAGCACAAGATATTATTTTGTG

>Pool163. 3558 nucleotides.

GCTGTTTTGTATGACAGTAGTTCTTACGTGTACTACTACCAAAGTGCCTTCAGACCACCTGA
TGGTTGGCATTTACATGGGGGTGCGTATGCGGTTGTTAATATTTCTAGTGAATCTAATAATG
CAGGCTCTTCATCTGGGTGTACTGTTGGTATTATTCATGGTGGTCGTGTTGTTAATGCTTCTT
CTATAGCTATGACGGCACCGTCAYCAGGTATGGCTTGGTCTAGCAGKCAGTTTTGTACTGCA
TACTGTAACTTTTCAGATACTACAGTGTTTGTTACACATTGTTATAAACATGGTGGGTGTCCT
ATAACTGGCATGCTTCAACAGCATTCTATACGTGTTTCTGCTATGAAAAATGGCCAGCTTTT
TTATAATTTAACAGTTAGTGTAGCTAAGTACCCTACTTTTAAATCATTTCAGTGTGTTAATAA
TTTAACATCCGTATATTTAAATGGTGATCTTGTTTACACCTCTAATGAGACCACAGATGTTAC

ATCTGCAGGTGTTTATTTTAAAGCTGGTGGACCTATAACTTATAAAGTTATGAGAGAAGTTA
GAGCCCTGGCTTATTTTGTTAATGGTACTGCACAAGATGTTATTTTGTGT

>Pool20. 3558 nucleotides.

TTTGTTACACATTGTTTTAAAGACGGTGTTGGATCTTGTCCACTTACAGGCAAAATCCCACA
GAACTTTCTTCGTATTTCTGCTCTTAGAGGAGGCAGGCTGTTTTATAATTTAACAGTTAGTG
TAGCTAAGTACCCTACTTTTAAATCTTTTCAATGTGTTAATAATCAGACATCTGTATACTTAA
ATGGTGATCTTGTTTTTACTTCTAATGAGACTATAGATGTTAAGGACGCTGGTGTTCACTTT
AAAGCTGGCGGACCTGTACTCTATAAAGTTATGAGAGAGGTCAAAGTTCTTTTTCAGATGG
CTTCTATCCTTTTACTAATTCTAGTTTAGTTAAGGAAAAGTTCATTGTGTATCGAGAAAGTA
GTGTTAATACTACTTTGGAATTAATTACATTTAATTTTACTAATGTAACTAATGCCTCTCCTA
ATACTGGTGATGTTAGTACTTTCAAATTATATCAAACGAAAACAGCTCAGAGTGGTTATTAT
AAATTTGATTTTGGATTTCTGAGTAATTTTCGTTATGTTTCTTCAGATTTTATGTATGGATCTT
ATCATCCTAAGTGTAGTTTTAGACCTGAGACTATTAATAATGGTTTGTGGTTTAACTACTTGT
CTGTTTCACTAACTTATGGACCCCTTCAAGG--

>Pool213. 3558 nucleotides.

GTGCTGCTTTGTTTKATAATAMTGAAACCGTTTACTACTACCAAAGTGCCTTCCGACCATCT
AACGGTTGGCATATGCATGGGGGTGCTTATGCAGTAGTTAATGTTTCTTTAGAATATAACA
ACGCAGGCACAGCACCTACGTGTACTGCGGGGGCTATTTATTGGAGTAAAAATTTTAGTGC
ATCTTCTGTAGCCATGACAGCACCCGGTACAGGTATGTCTTGGTCAACCRGAGAGTTCTGT
ACGGCCCACTGTAACTTTACAGATTTTACAGTGTTTGTTACACATTGTTACAAAAATGGTCA
GGGTGAATGTCCTTTAACAGGTATGATTCCACAGAATCAGATTCGTATTTCTGCTATGAAAG
GTAGCATTTTGTTTTATAACTTAACAGTTGCTGTGACTAAATATCCTAAATTTAAGTCGCTTC
AGTGTGTTAATAATTTTACATCTGTATACTTAAATGGTGATCTCGTCTTTACTTCTAATGATA
CTAAAGATGTTAGTGCAGCAGGTGTTTATTTTAAAAGTGGTGGACCTATAACTTATAAGGT
TATGGAACAAGTTGATGTCCTAGCTTATTTTATTAATGGTACAGCACAAGAGGTTATTTTGT
GTGATAAT

>Pool35. 3558 nucleotides.

TTTTCAGATGGCTTCTATCCTTTTACTAATTCTAGTTTAGTTAAGGAAAAGTTCATTGTGTAT
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GCCCACCCTAATAGTGGTGGTGTTAACACTTTTCAATTGTATCAAACGCAAACAGCTCAGAG
TGGTTATTATAAATTTGATTTTGGATTTCTGAGTGGTTTTCGTTATGTTAGTTCAGATTTTAT
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TTAACTACTTGTCTGTTTCACTTACTTATGGACCCCTTCAAGG

Nucleoprotein gene sequences

Appendix X1V: Sequences of Nucleoprotein of cloaca and trachea in commercial in vaccinated chickens in Lagos, Ogun and Oyo states

>Cloc2

GGAATTAGGAGGGCGTGTTAAGCAATGCTTCAACCTTGTTCCCTAGCAGCCATGCTTGCCT
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ACTACTGTGGTGCCACGTGATGACCCGCAGTTTGATAATTATGTGAAAATTTGTGATCAGT
GTGTCGATGGTGTAGGGACGCGTCCAAAAGACGATGAACCGAGACCAAAGTCACGCCCAA
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AGGAACAATGA

>Cloc7

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CAATGA

>Cloc9

GATTAGGAGGGCGTGTTCAGCCTATGCTCAACCCTAGTTCCTAGGCAGGTCATGCCTTGTC
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>Lung18

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AATGA

>Lung21

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TGAA

Appendix XV

NCBI Resources How To Sign in to NCBI

Nucleotide Nucleotide Search Advanced Help

GenBank Send to:

Infectious bronchitis virus isolate NGA1 1b gene, partial cds

GenBank: MK886445.1

FASTA Graphics PopSet

Go to:

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            Orthocoronavirinae; Gammacoronavirus; Igacovirus.
REFERENCE   1  (bases 1 to 504)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O., Owoade,A.A. and
            Fagbohun,O.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-MAY-2019) Veterinary Microbiology, University of
            Ibadan, 1 Oyo Road, Ibadan, Oyo 200005, Nigeria
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      481 agaaattctc tactaccaaa ataa
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Analyze this sequence
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Infectious bronchitis virus isolate NGA1 1b gene, partial cds Nucleotide
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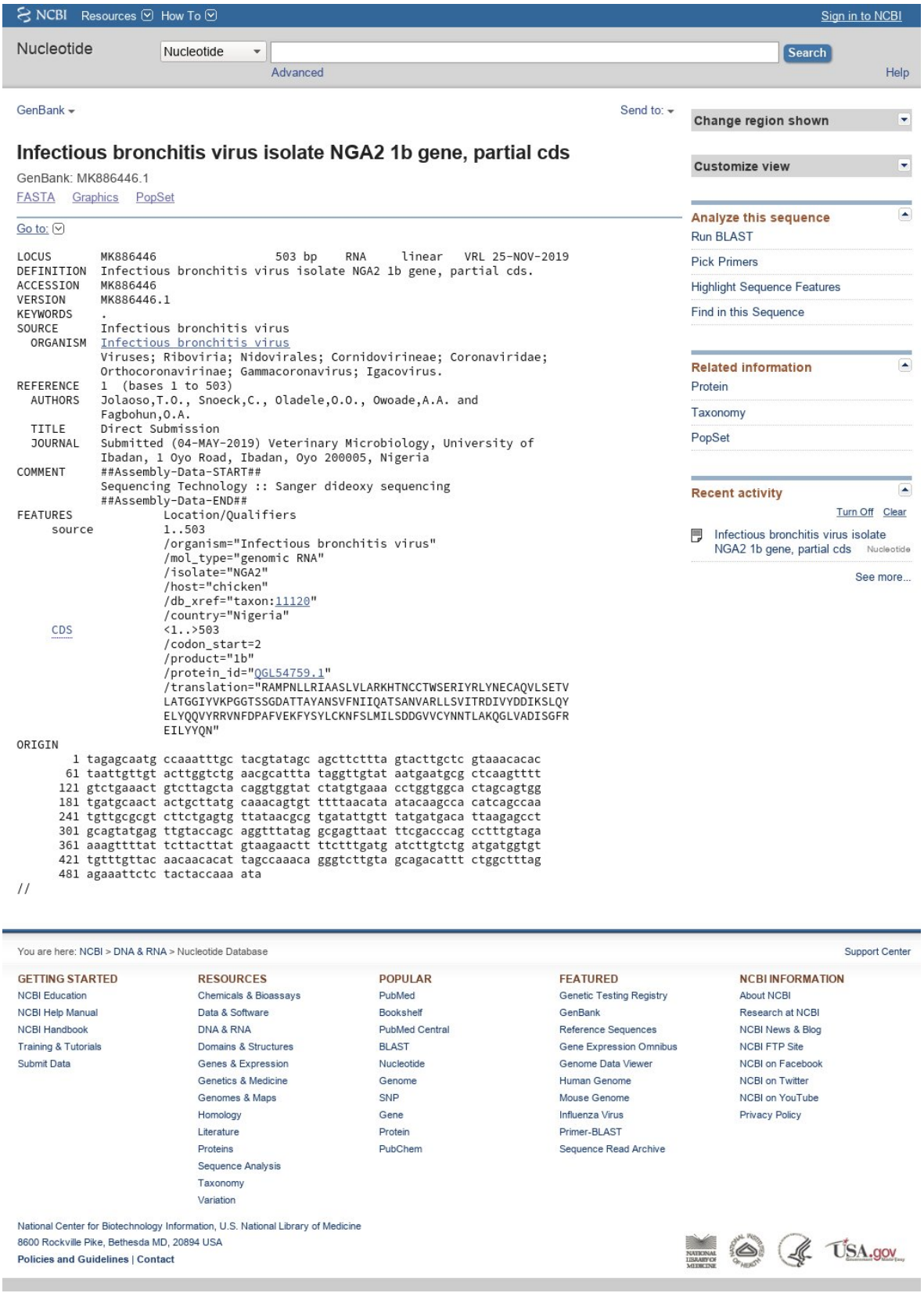
Appendix XV



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GenBank Send to:

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Infectious bronchitis virus isolate NGA2 1b gene, partial cds

GenBank: MK886446.1

FASTA Graphics PopSet

Go to:

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VERSION     MK886446.1
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  ORGANISM  Infectious bronchitis virus
            Viruses; Riboviria; Nidovirales; Cornidovirineae; Coronaviridae;
            Orthocoronavirinae; Gammacoronavirus; Igacovirus.
REFERENCE   1  (bases 1 to 503)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O., Owoade,A.A. and
            Fagbohun,O.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-MAY-2019) Veterinary Microbiology, University of
            Ibadan, 1 Oyo Road, Ibadan, Oyo 200005, Nigeria
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Taxonomy
PopSet

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Gene Expression Omnibus
Genome Data Viewer
Human Genome
Mouse Genome
Influenza Virus
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GenBank Send to:

Infectious bronchitis virus isolate NGA3 1b gene, partial cds

GenBank: MK886447.1

FASTA Graphics PopSet

Go to:

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VERSION     MK886447.1
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  ORGANISM  Infectious bronchitis virus
            Viruses; Riboviria; Nidovirales; Cornidovirineae; Coronaviridae;
            Orthocoronavirinae; Gammacoronavirus; Igacovirus.
REFERENCE   1  (bases 1 to 554)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O., Owoade,A.A. and
            Fagbohun,O.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-MAY-2019) Veterinary Microbiology, University of
            Ibadan, 1 Oyo Road, Ibadan, Oyo 200005, Nigeria
COMMENT     ##Assembly-Data-START##
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Change region shown

Customize view

Analyze this sequence

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Find in this Sequence

Related information

Protein
Taxonomy
PopSet

Recent activity

Turn Off Clear

Infectious bronchitis virus isolate NGA3 1b gene, partial cds Nucleotide

See more...

GenBank Send to:

Infectious bronchitis virus isolate NGA4 1b gene, partial cds

GenBank: MK886448.1

FASTA Graphics PopSet

Go to:

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VERSION     MK886448.1
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  ORGANISM  Infectious bronchitis virus
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            Orthocoronavirinae; Gammacoronavirus; Igacovirus.
REFERENCE   1  (bases 1 to 504)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O., Owoade,A.A. and
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  TITLE     Direct Submission
  JOURNAL   Submitted (04-MAY-2019) Veterinary Microbiology, University of
            Ibadan, 1 Oyo Road, Ibadan, Oyo 200005, Nigeria
COMMENT     ##Assembly-Data-START##
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Infectious bronchitis virus isolate NGA4 1b gene, partial cds Nucleotide

See more...

GenBank Send to:

Infectious bronchitis virus isolate NGA5 1b gene, partial cds

GenBank: MK886449.1

FASTA Graphics PopSet

Go to:

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REFERENCE   1  (bases 1 to 559)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O., Owoade,A.A. and
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  TITLE     Direct Submission
  JOURNAL   Submitted (04-MAY-2019) Veterinary Microbiology, University of
            Ibadan, 1 Oyo Road, Ibadan, Oyo 200005, Nigeria
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Infectious bronchitis virus isolate NGA5 1b gene, partial cds Nucleotide

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GenBank Send to:

Infectious bronchitis virus isolate NGA6 1b gene, partial cds

GenBank: MK886450.1
FASTA Graphics PopSet

Go to:

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  ORGANISM  Infectious bronchitis virus
            Viruses; Riboviria; Nidovirales; Cornidovirineae; Coronaviridae;
            Orthocoronavirinae; Gammacoronavirus; Igacovirus.
REFERENCE   1  (bases 1 to 503)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O., Owoade,A.A. and
            Fagbohun,O.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-MAY-2019) Veterinary Microbiology, University of
            Ibadan, 1 Oyo Road, Ibadan, Oyo 200005, Nigeria
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Change region shown

Customize view

Analyze this sequence
Run BLAST
Pick Primers
Highlight Sequence Features
Find in this Sequence

Related information
Protein
Taxonomy
PopSet

Recent activity
Turn Off Clear
Infectious bronchitis virus isolate NGA6 1b gene, partial cds Nucleotide
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	Taxonomy			
	Variation			

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GenBank Send to:

Infectious bronchitis virus isolate NGA8 1b gene, partial cds

GenBank: MK886452.1

FASTA Graphics PopSet

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  ORGANISM  Infectious bronchitis virus
            Viruses; Riboviria; Nidovirales; Cornidovirineae; Coronaviridae;
            Orthocoronavirinae; Gammacoronavirus; Igacovirus.
REFERENCE   1  (bases 1 to 504)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O., Owoade,A.A. and
            Fagbohun,O.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-MAY-2019) Veterinary Microbiology, University of
            Ibadan, 1 Oyo Road, Ibadan, Oyo 200005, Nigeria
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Change region shown

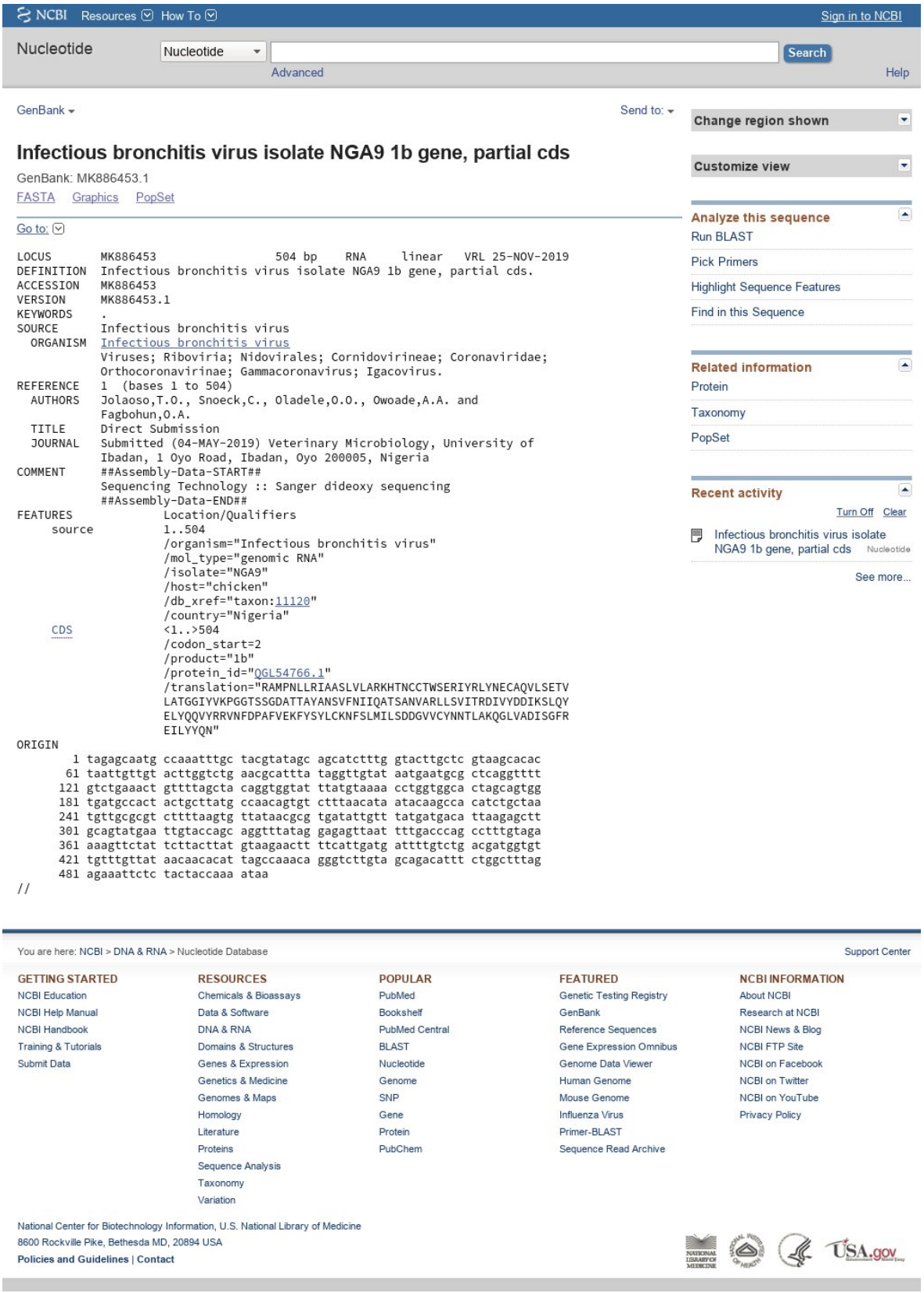
Customize view

Analyze this sequence
Run BLAST
Pick Primers
Highlight Sequence Features
Find in this Sequence

Related information
Protein
Taxonomy
PopSet

Recent activity
Turn Off Clear
Infectious bronchitis virus isolate NGA8 1b gene, partial cds Nucleotide
See more...

Appendix XV



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Infectious bronchitis virus isolate NGA9 1b gene, partial cds

GenBank: MK886453.1

FASTA Graphics PopSet

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REFERENCE   1  (bases 1 to 504)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O., Owoade,A.A. and
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  TITLE     Direct Submission
  JOURNAL   Submitted (04-MAY-2019) Veterinary Microbiology, University of
            Ibadan, 1 Oyo Road, Ibadan, Oyo 200005, Nigeria
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Analyze this sequence: Run BLAST; Pick Primers; Highlight Sequence Features; Find in this Sequence

Related information: Protein; Taxonomy; PopSet

Recent activity: Turn Off Clear

Infectious bronchitis virus isolate NGA9 1b gene, partial cds Nucleotide

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Submit Data	Genes & Expression	Nucleotide	Genome Data Viewer	NCBI on Facebook
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	Genomes & Maps	SNP	Mouse Genome	NCBI on YouTube
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	Proteins	PubChem	Sequence Read Archive	
	Sequence Analysis			
	Taxonomy			
	Variation			

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GenBank Send to:

Infectious bronchitis virus isolate NGA10 1b gene, partial cds

GenBank: MK886454.1

FASTA Graphics PopSet

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VERSION     MK886454.1
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            Orthocoronavirinae; Gammacoronavirus; Igacovirus.
REFERENCE   1  (bases 1 to 556)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O., Owoade,A.A. and
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  TITLE     Direct Submission
  JOURNAL   Submitted (04-MAY-2019) Veterinary Microbiology, University of
            Ibadan, 1 Oyo Road, Ibadan, Oyo 200005, Nigeria
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Change region shown

Customize view

Analyze this sequence

Run BLAST
Pick Primers
Highlight Sequence Features
Find in this Sequence

Related information

Protein
Taxonomy
PopSet

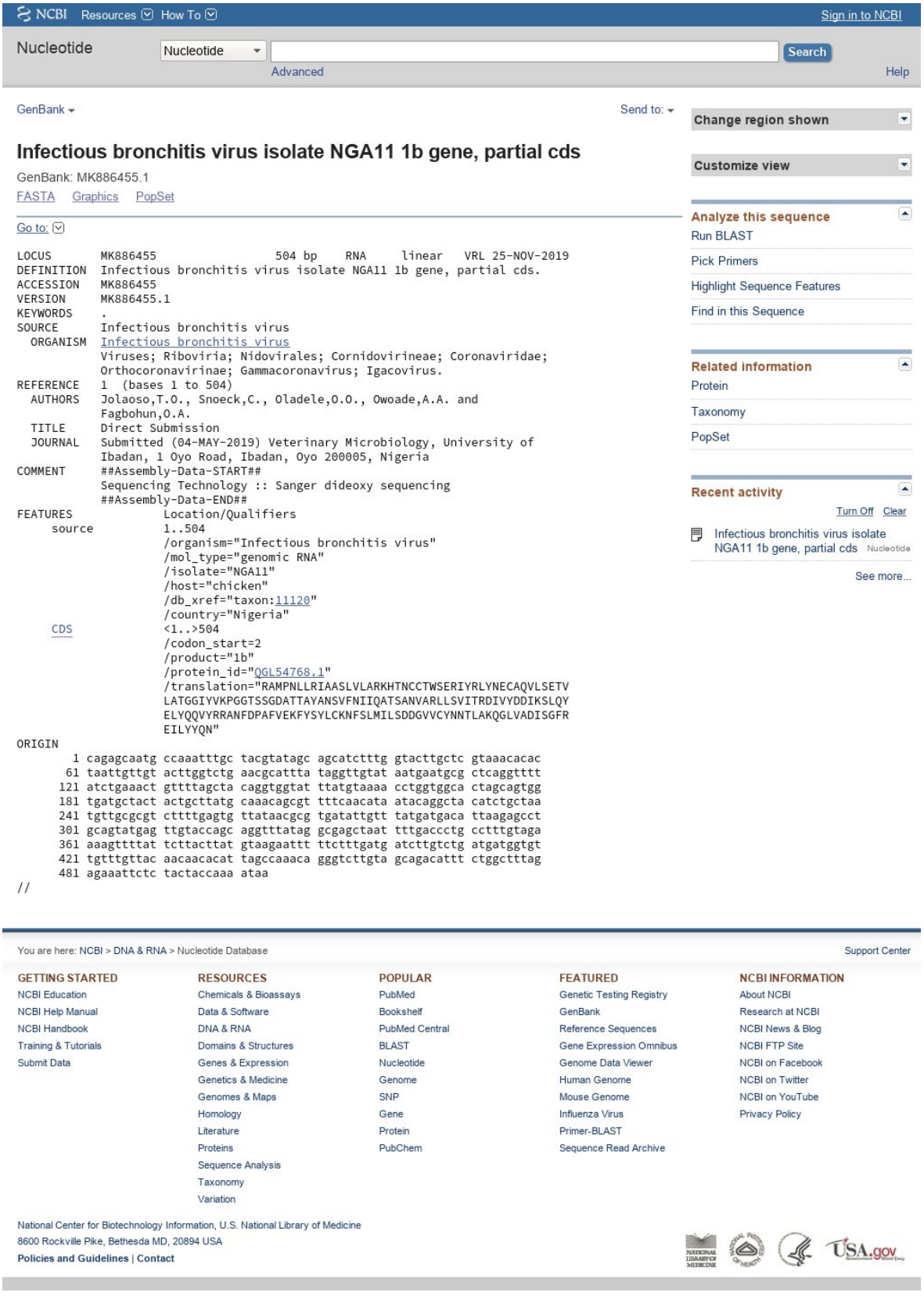
Recent activity

Turn Off Clear

Infectious bronchitis virus isolate NGA10 1b gene, partial cds Nucleotide

See more...

Appendix XV



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GenBank Send to:

Infectious bronchitis virus isolate NGA11 1b gene, partial cds

GenBank: MK886455.1

FASTA Graphics PopSet

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            Viruses; Riboviria; Nidovirales; Cornidovirineae; Coronaviridae;
            Orthocoronavirinae; Gammacoronavirus; Igacovirus.
REFERENCE   1  (bases 1 to 504)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O., Owoade,A.A. and
            Fagbohun,O.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-MAY-2019) Veterinary Microbiology, University of
            Ibadan, 1 Oyo Road, Ibadan, Oyo 200005, Nigeria
COMMENT     ##Assembly-Data-START##
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```

Analyze this sequence
- Run BLAST
- Pick Primers
- Highlight Sequence Features
- Find in this Sequence

Related information
- Protein
- Taxonomy
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Recent activity — Turn Off Clear

Infectious bronchitis virus isolate NGA11 1b gene, partial cds Nucleotide

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	Genomes & Maps	SNP	Mouse Genome	NCBI on YouTube
	Homology	Gene	Influenza Virus	Privacy Policy
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	Taxonomy			
	Variation			

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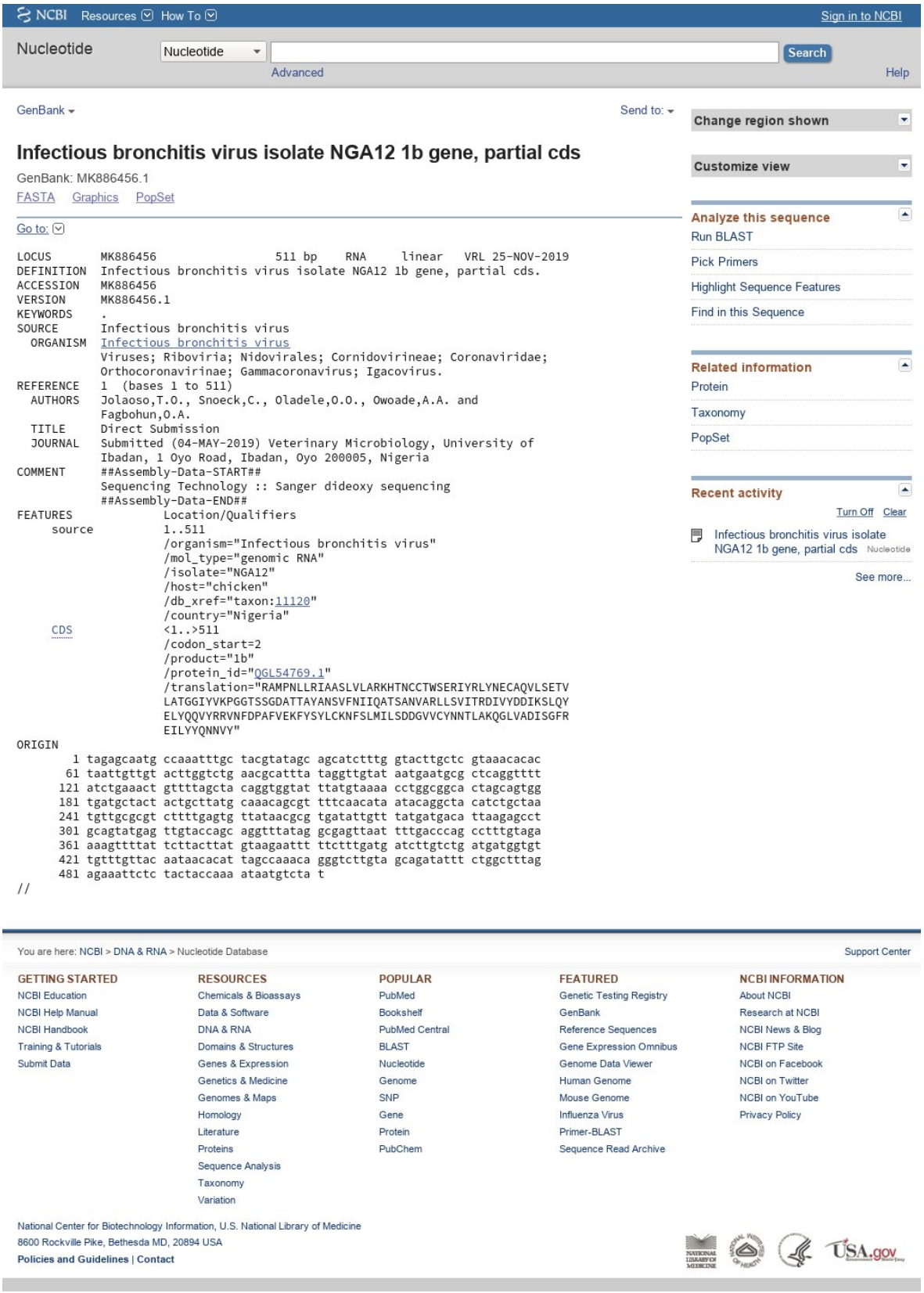

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GenBank Send to:

Change region shown

Customize view

Infectious bronchitis virus isolate NGA12 1b gene, partial cds

GenBank: MK886456.1

FASTA Graphics PopSet

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REFERENCE   1  (bases 1 to 511)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O., Owoade,A.A. and
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  TITLE     Direct Submission
  JOURNAL   Submitted (04-MAY-2019) Veterinary Microbiology, University of
            Ibadan, 1 Oyo Road, Ibadan, Oyo 200005, Nigeria
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Related information

Protein

Taxonomy

PopSet

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Infectious bronchitis virus isolate NGA12 1b gene, partial cds Nucleotide

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Genome
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Protein
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Genome Data Viewer
Human Genome
Mouse Genome
Influenza Virus
Primer-BLAST
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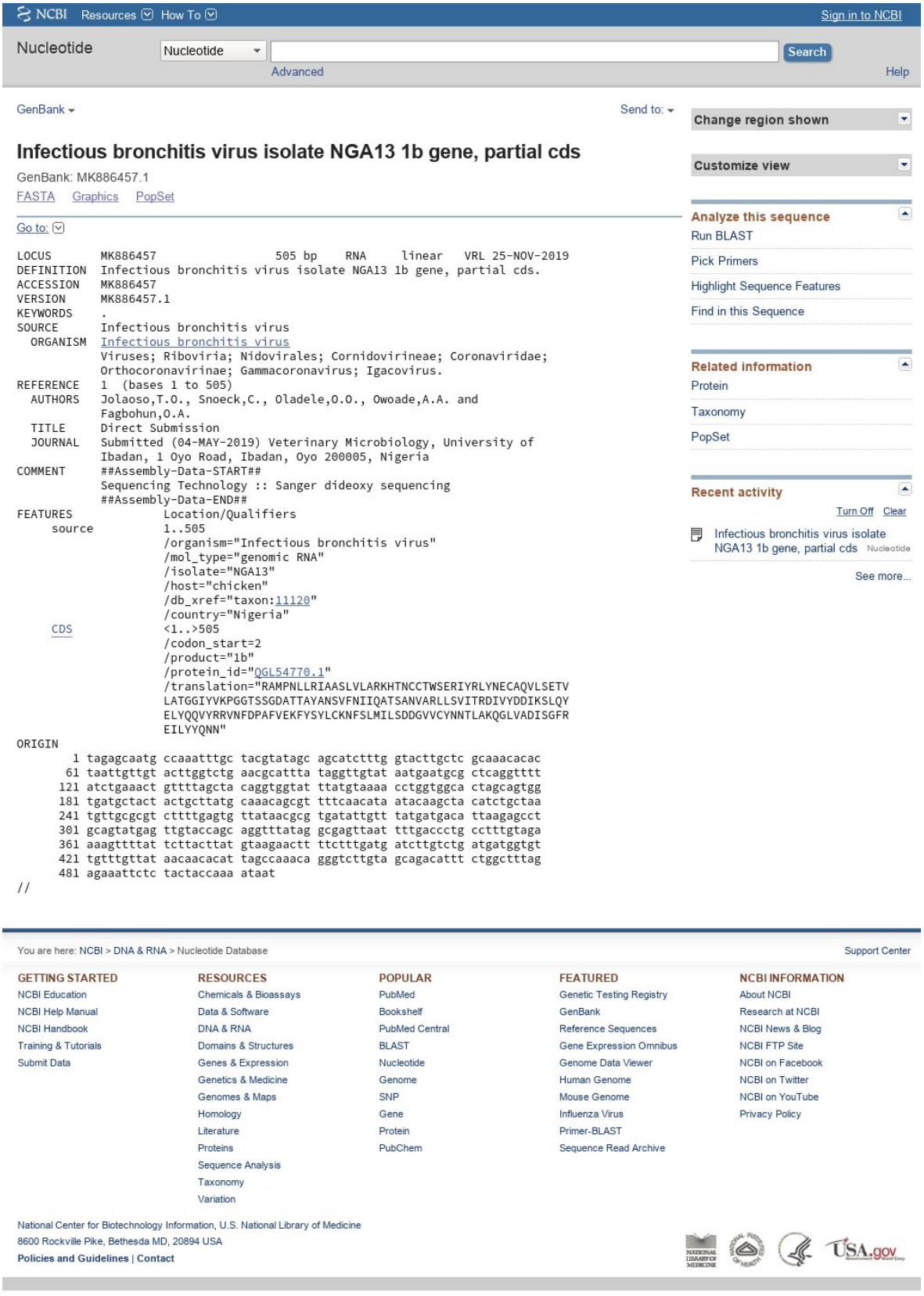

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Nucleotide Nucleotide Search

Advanced Help

GenBank Send to:

Infectious bronchitis virus isolate NGA13 1b gene, partial cds

GenBank: MK886457.1

FASTA Graphics PopSet

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            Orthocoronavirinae; Gammacoronavirus; Igacovirus.
REFERENCE   1  (bases 1 to 505)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O., Owoade,A.A. and
            Fagbohun,O.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-MAY-2019) Veterinary Microbiology, University of
            Ibadan, 1 Oyo Road, Ibadan, Oyo 200005, Nigeria
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Change region shown

Customize view

Analyze this sequence
Run BLAST
Pick Primers
Highlight Sequence Features
Find in this Sequence

Related information
Protein
Taxonomy
PopSet

Recent activity
Turn Off Clear
Infectious bronchitis virus isolate NGA13 1b gene, partial cds Nucleotide
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	Variation			

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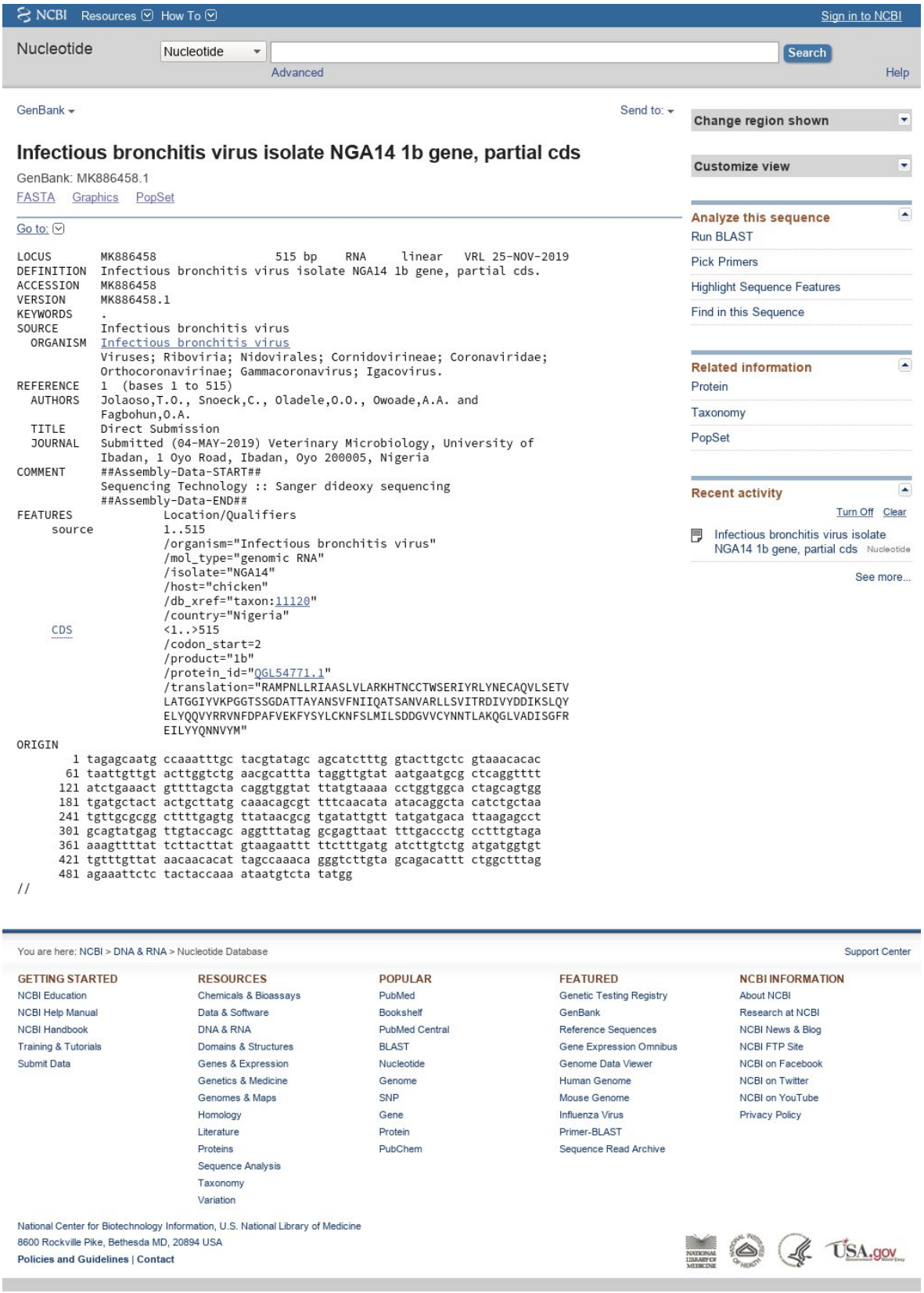

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GenBank Send to:

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Customize view

Infectious bronchitis virus isolate NGA14 1b gene, partial cds

GenBank: MK886458.1

FASTA Graphics PopSet

Go to:

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            Orthocoronavirinae; Gammacoronavirus; Igacovirus.
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  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O., Owoade,A.A. and
            Fagbohun,O.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-MAY-2019) Veterinary Microbiology, University of
            Ibadan, 1 Oyo Road, Ibadan, Oyo 200005, Nigeria
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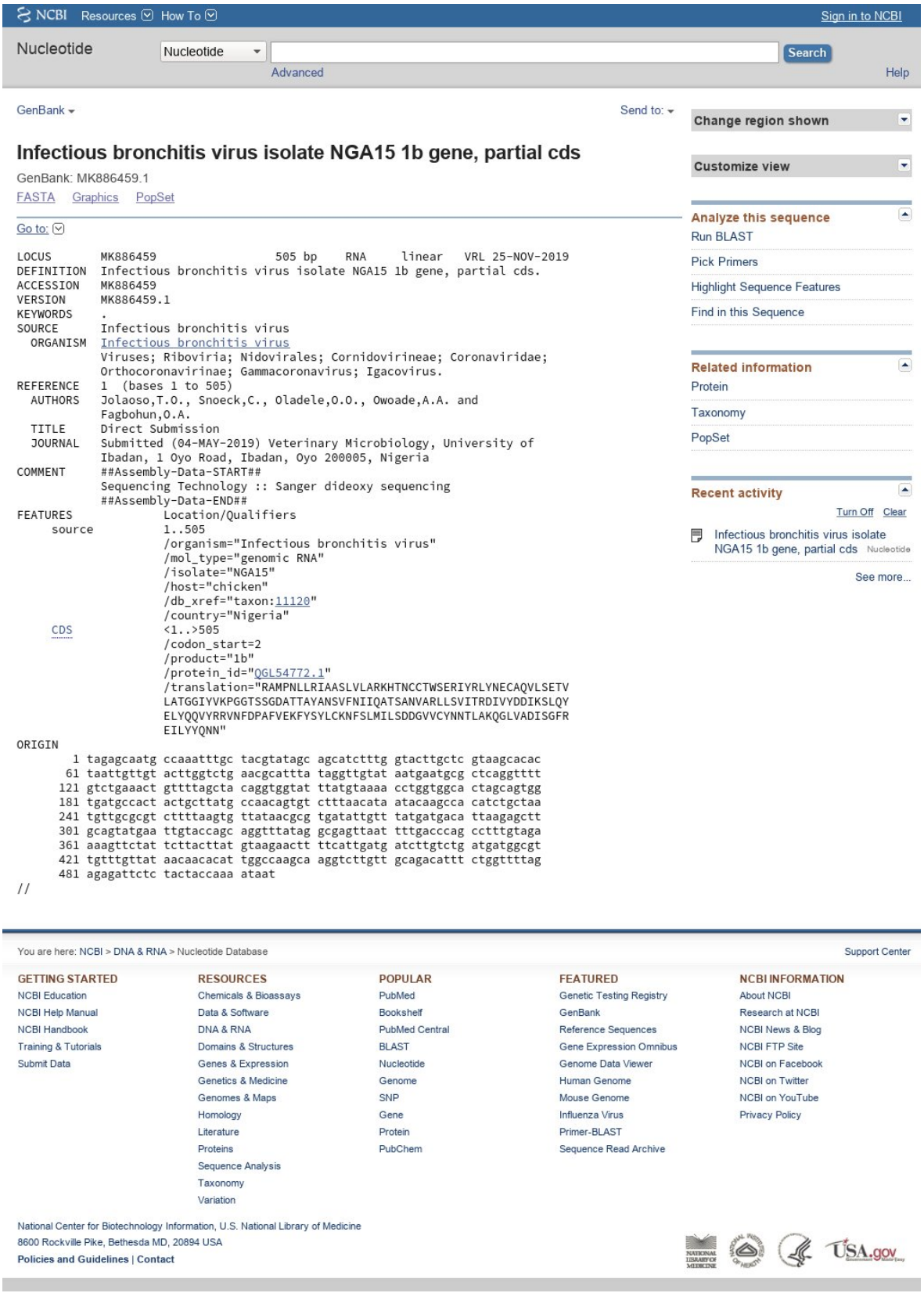

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Infectious bronchitis virus isolate NGA15 1b gene, partial cds

GenBank: MK886459.1

FASTA Graphics PopSet

Go to:

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VERSION     MK886459.1
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  ORGANISM  Infectious bronchitis virus
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            Orthocoronavirinae; Gammacoronavirus; Igacovirus.
REFERENCE   1  (bases 1 to 505)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O., Owoade,A.A. and
            Fagbohun,O.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-MAY-2019) Veterinary Microbiology, University of
            Ibadan, 1 Oyo Road, Ibadan, Oyo 200005, Nigeria
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            ##Assembly-Data-END##
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Related information: Protein, Taxonomy, PopSet

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GenBank Send to:

Infectious bronchitis virus isolate NGA1 spike glycoprotein 1 gene, partial cds

GenBank: MN082397.1

FASTA Graphics PopSet

Go to:

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ACCESSION   MN082397
VERSION     MN082397.1
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  ORGANISM  Infectious bronchitis virus
            Viruses; Riboviria; Nidovirales; Cornidovirineae; Coronaviridae;
            Orthocoronavirinae; Gammacoronavirus; Igacovirus.
REFERENCE   1  (bases 1 to 224)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O. and Fagbohun,O.A.
  TITLE     Molecular characterization of infectious bronchitis virus in
            chickens in Nigeria
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 224)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O. and Fagbohun,O.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-JUN-2019) Veterinary Microbiology, University of
            Ibadan, 1 Oyo Road, Ibadan, Oyo 200005, Nigeria
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Infectious bronchitis virus isolate NGA1 spike glycoprotein 1 g Nucleotide
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Infectious bronchitis virus isolate NGA4 spike glycoprotein 1 gene, partial cds

GenBank: MN082400.1
FASTA Graphics PopSet

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  ORGANISM  Infectious bronchitis virus
            Viruses; Riboviria; Nidovirales; Cornidovirineae; Coronaviridae;
            Orthocoronavirinae; Gammacoronavirus; Igacovirus.
REFERENCE   1  (bases 1 to 404)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O. and Fagbohun,O.A.
  TITLE     Molecular characterization of infectious bronchitis virus in
            chickens in Nigeria
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 404)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O. and Fagbohun,O.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-JUN-2019) Veterinary Microbiology, University of
            Ibadan, 1 Oyo Road, Ibadan, Oyo 200005, Nigeria
COMMENT     ##Assembly-Data-START##
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//
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Analyze this sequence
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Infectious bronchitis virus isolate NGA4 spike glycoprotein 1 g Nucleotide
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Infectious bronchitis virus isolate NGA5 spike glycoprotein 1 gene, partial cds

GenBank: MN082401.1

FASTA Graphics PopSet

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REFERENCE   1  (bases 1 to 235)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O. and Fagbohun,O.A.
  TITLE     Molecular characterization of infectious bronchitis virus in
            chickens in Nigeria
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 235)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O. and Fagbohun,O.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-JUN-2019) Veterinary Microbiology, University of
            Ibadan, 1 Oyo Road, Ibadan, Oyo 200005, Nigeria
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Infectious bronchitis virus isolate NGA6 spike glycoprotein 1 gene, partial cds

GenBank: MN082402.1

FASTA Graphics PopSet

Go to:

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  ORGANISM  Infectious bronchitis virus
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REFERENCE   1  (bases 1 to 416)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O. and Fagbohun,O.A.
  TITLE     Molecular characterization of infectious bronchitis virus in
            chickens in Nigeria
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 416)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O. and Fagbohun,O.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-JUN-2019) Veterinary Microbiology, University of
            Ibadan, 1 Oyo Road, Ibadan, Oyo 200005, Nigeria
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            ##Assembly-Data-END##
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Infectious bronchitis virus isolate NGA6 spike glycoprotein 1 gene, partial cds

GenBank: MN082402.1

FASTA Graphics PopSet

Go to:

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LOCUS       MN082402                416 bp    RNA     linear   VRL 12-FEB-2020
DEFINITION  Infectious bronchitis virus isolate NGA6 spike glycoprotein 1 gene,
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ACCESSION   MN082402
VERSION     MN082402.1
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  ORGANISM  Infectious bronchitis virus
            Viruses; Riboviria; Nidovirales; Cornidovirineae; Coronaviridae;
            Orthocoronavirinae; Gammacoronavirus; Igacovirus.
REFERENCE   1  (bases 1 to 416)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O. and Fagbohun,O.A.
  TITLE     Molecular characterization of infectious bronchitis virus in
            chickens in Nigeria
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 416)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O. and Fagbohun,O.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-JUN-2019) Veterinary Microbiology, University of
            Ibadan, 1 Oyo Road, Ibadan, Oyo 200005, Nigeria
COMMENT     ##Assembly-Data-START##
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Infectious bronchitis virus isolate NGA6 spike glycoprotein 1 gene, partial cds

GenBank: MN082402.1

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Go to:

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LOCUS       MN082402                416 bp    RNA     linear   VRL 12-FEB-2020
DEFINITION  Infectious bronchitis virus isolate NGA6 spike glycoprotein 1 gene,
            partial cds.
ACCESSION   MN082402
VERSION     MN082402.1
KEYWORDS    .
SOURCE      Infectious bronchitis virus
  ORGANISM  Infectious bronchitis virus
            Viruses; Riboviria; Nidovirales; Cornidovirineae; Coronaviridae;
            Orthocoronavirinae; Gammacoronavirus; Igacovirus.
REFERENCE   1  (bases 1 to 416)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O. and Fagbohun,O.A.
  TITLE     Molecular characterization of infectious bronchitis virus in
            chickens in Nigeria
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 416)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O. and Fagbohun,O.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-JUN-2019) Veterinary Microbiology, University of
            Ibadan, 1 Oyo Road, Ibadan, Oyo 200005, Nigeria
COMMENT     ##Assembly-Data-START##
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Infectious bronchitis virus isolate NGA6 spike glycoprotein 1 g Nucleotide

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GenBank Send to:

Infectious bronchitis virus isolate NGA7 spike glycoprotein 1 gene, partial cds

GenBank: MN082403.1

FASTA Graphics PopSet

Go to:

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DEFINITION  Infectious bronchitis virus isolate NGA7 spike glycoprotein 1 gene,
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ACCESSION   MN082403
VERSION     MN082403.1
KEYWORDS    .
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  ORGANISM  Infectious bronchitis virus
            Viruses; Riboviria; Nidovirales; Cornidovirineae; Coronaviridae;
            Orthocoronavirinae; Gammacoronavirus; Igacovirus.
REFERENCE   1  (bases 1 to 612)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O. and Fagbohun,O.A.
  TITLE     Molecular characterization of infectious bronchitis virus in
            chickens in Nigeria
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 612)
  AUTHORS   Jolaoso,T.O., Snoeck,C., Oladele,O.O. and Fagbohun,O.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-JUN-2019) Veterinary Microbiology, University of
            Ibadan, 1 Oyo Road, Ibadan, Oyo 200005, Nigeria
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