INFLUENZA INFLUENCES ALL INFLUENTIAL CREATURES GREAT AND SMALL

AN INAUGURAL LECTURE,
2009/2010

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UNIVERSITY OF IBADAN

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An inaugural lecture delivered at the University of Ibadan

on Thursday, 10 June, 2010

By

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Ibadan University Press
Publishing House
University of Ibadan
Ibadan, Nigeria.

First Published 2010

ISBN: 978 – 978 – 8414 – 17 – 9

Printed by: Ibadan University Printery

The Vice-Chancellor, Deputy Vice-Chancellor (Administration), Deputy Vice-Chancellor (Academic), Registrar, Librarian, Provost of the College of Medicine, Dean of Faculty of Veterinary Medicine, Dean of the Postgraduate School, Deans of other Faculties and of students, Directors of Institutes, Distinguished ladies and Gentlemen.

Preamble

I feel highly honoured to be here to deliver this lecture, the tenth in the 2009/2010 series of inaugural lectures, on behalf of the Faculty of Veterinary Medicine, the 28th to be delivered by professors in my Faculty since 1976 when our founding Dean, Prof. Desmond H. Hill delivered the first. This lecture is the tenth from the Department of Veterinary Medicine, the first being that of Prof. Hill in 1976 followed by the second in 1981 by Prof. O. O. Oduye. Inaugural lectures from my Department have varied in topics in the broad field of Veterinary Medicine from perspectives on infectious and non-infectious diseases and their causal agents, through immunological responses and the art and science of diagnosis, to management of diseases. Today, however, we will be talking at the molecular level.

Although inaugural lectures are expected to give an account of one's scholarship and contributions to knowledge in a simplified form to varying categories of audience within one hour, it is a difficult task to simplify my contributions in the challenging field of molecular virology within this one hour. However, in this lecture, I will highlight my scholarship and contributions in the field of influenza virus research particularly the molecular aspects in so far as influenza viruses have influenced me and other creatures great and small. I also intend to showcase what I did with the influence.

Influenza or flu is the paradigm of a viral disease in which continued evolution of the virus is of paramount importance for epidemics and occasional pandemics of disease in humans as well as animals and birds (Webster *et al.* 1993). It is an infectious viral disease characterized mainly by respiratory signs in man, animals and birds which also show gastro-

intestinal manifestations, depression, muscle pain, and reduced food and water intake. It leads to decreased productivity in man and working animals, and decrease in egg production and quality in birds, in which, especially land-based poultry, it could be very deadly with high rates of spread and death and serious economic losses. It is caused by influenza viruses (fig.1). It also has the potential for rapid global spread and in pandemic settings, it causes very high hospitalization rate in man and high death rate in poultry (fig. 2).

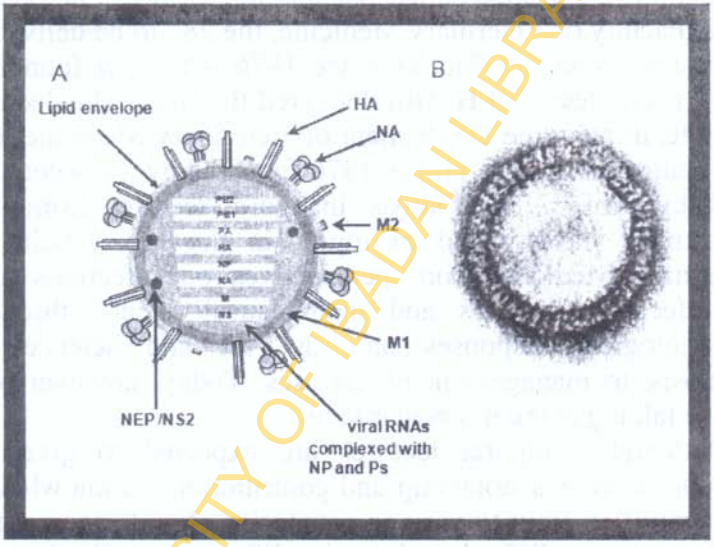


Fig. 1. Influenza virus particle

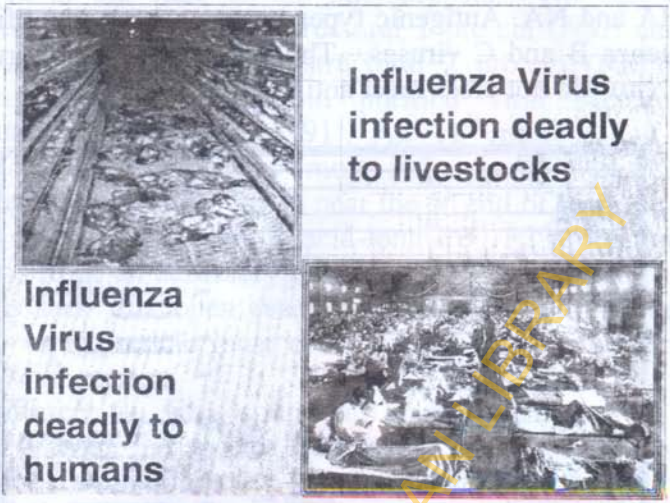


Fig. 2. High Mortality caused by HPAI H5N1 virus and high hospitalisation in pandemic H1N1 virus of 1918 - 1919

The term "influenza" was coined in 1357 AD from the Italian word meaning "influence" because it was popularly believed at the time that the development of flu was influenced by the stars and the gods.

The Virus and its Replication

Influenza viruses belong to family ***Orthomyxoviridae*** which comprises viruses that have a negative-sense, single stranded and segmented RNA genome (fig. 3). The negative-sense genome is complementary to the mRNA which is positive sense (Baltimore 1971). This family comprises five genera: Influenza A virus, Influenza B virus, Influenza C virus, Thogoto virus, which includes Thogoto virus and Dhori virus, and Isavirus, which includes infectious salmon anaemia virus (ISAV) (Kawaoka *et al.* 2005). Influenza A viruses are further classified into subtypes based on the antigenicity of their surface protein molecules, the haemagglutinin (HA) and the neuraminidase (NA). Currently, 16 HA (H1-H16) and 9

NA (N1-N9) subtypes are known (Fouchier *et al.* 2005) which could give rise to 1,976,832,000 possible combinations of HA and NA. Antigenic types have not been identified for influenza B and C viruses. Thogoto virus, Dhori virus and ISA virus do not cross react antigenically.

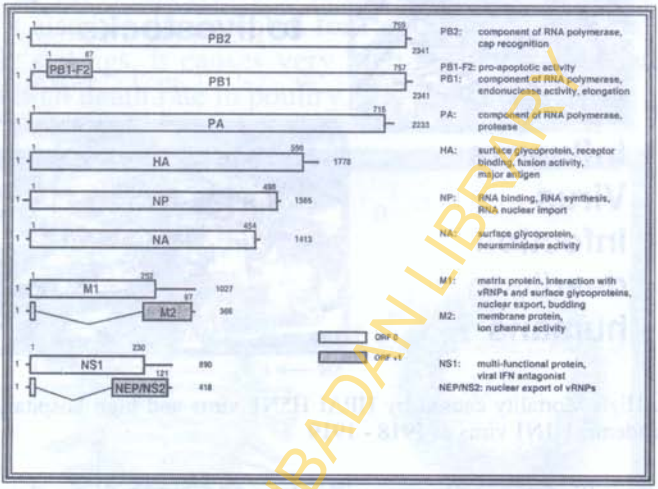


Fig. 3. Genome organization of influenza A virus

All A- and B- type flu viruses possess eight RNA segments whereas influenza C viruses have only seven RNAs (Palese 1980) while Thogoto and Dhori viruses isolated from ticks have six RNA segments and ISA viruses also have eight RNA segments (Hagmaier *et al.* 2003) (fig. 3). On initial isolation, influenza A viruses are small (80-120 nm in diameter), pleomorphic particles that later become generally spherical. These particles consist of a host-derived lipid bilayer envelope in which the virus-encoded glycoproteins, HA and NA and the matrix 2 protein (M2) are embedded, and an inner shell of matrix 1 protein (M); and, at the center, the nucleocapsids of the viral genome which are of negative sense. The RNA is loosely surrounded by multiple nucleoprotein (NP) molecules. Complexes containing the three viral polymerase proteins (basic polymerase 2, (PB2), basic

polymerase 1, (PB1), and acidic polymerase, (PA)) are situated at the ends of the nucleocapsids. The last RNA segment encodes the non-structural 1 protein (NSI) and the nuclear export protein/non-structural 2 protein (NEP/NS2) which are also present in purified viral preparations (Richardson and Akkina1991). An 11th protein has been found to be encoded by most influenza A viruses. An alternate open reading frame near the 5' end of the PB1 gene gives rise to the 87–amino acid-long PB1-F2 protein which appears to be an accessory protein because some influenza A viruses lack this open reading frame. To be infectious, a single virus particle must contain each of the eight unique RNA segments.

Different flu virus strains are named according to their genus or type, the species from which the virus is isolated (omitted if human), isolate location, isolate number, the year of isolation, and in the case of influenza A viruses, the HA and NA subtypes. Thus, the 220th isolate of an H5N1 subtype virus isolated from chickens in Hong Kong in 1997 is designated A/chicken/Hong Kong/220/97 (H5N1) while an 8th H1N1 isolate from human in Puerto Rico in 1934 is designated A/PR/8/34 (H1N1).

How do Influenza Viruses multiply in their Hosts?

Transcription, Translation and Replication

Flu viruses replicate themselves by first binding to sialic acid receptors on cell surfaces with their attachment protein, the HA, to initiate infection and replication (fig. 4). They then enter into the cells through 5 different mechanisms of endocytosis (fig. 5).

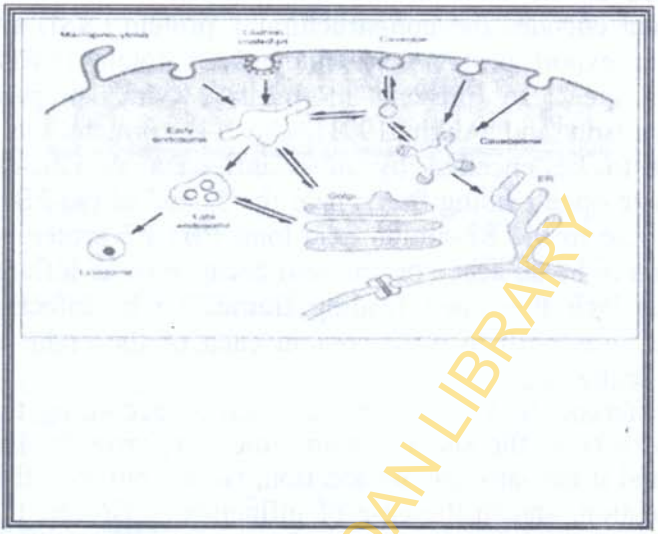


Fig. 4. Endocytic pathways used by the viruses to enter an infected cell

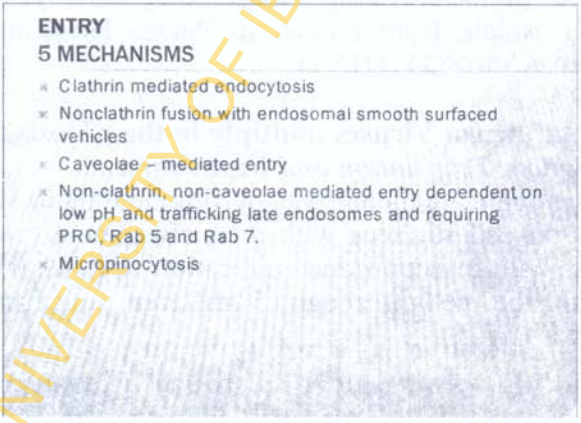


Fig. 5. Entry mechanisms used by flu viruses

On entering an infected cell, the virus uncoats by fusion of its envelope membrane with that of the endosome at a low pH which allows the surface protein HA to undergo a

conformational change by being cleaved into two, HA1 and HA2. A pore is then opened by the concerted effort of several HA molecules and the viral nucleic acid, the RNP, is released into the cell cytoplasm and transported to the nucleus where multiplication takes place.

The life cycle of the flu virus is dependent on nuclear functions. All viral RNA synthesis occurs in the nucleus, and the trafficking of the viral genome into and out of the nucleus is a tightly regulated process (Cros and Palese 2003).

In the nucleus, the incoming negative-sense viral RNA (vRNA) is transcribed into messenger RNA (mRNA) by a primer-dependent mechanism. Replication occurs in two steps: a full length positive copy of viral RNA is first synthesized to serve as a template for the synthesis of the daughter viral RNA which is negative sense (fig. 6).

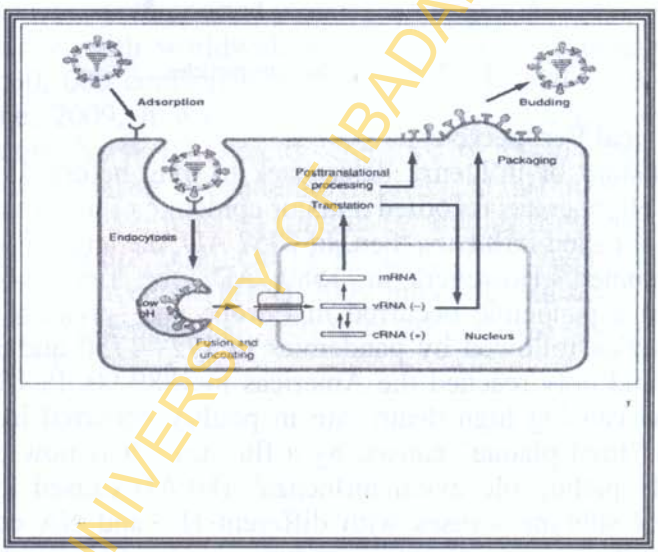


Fig. 6. Flu virus replication cycle

Flu viruses assemble and bud from the apical plasma membrane of polarized cells (e.g. lung epithelial cells of the infected host) (Buolan and Sabatini 1978). The individual viral envelope proteins are seen to accumulate at the same

polar surface where virus budding occurs, suggesting that they determine the maturation site (Boulan and Pendergast 1980) (fig. 7).

Fig. 7. Budding flu virus particles

Historical Perspective

The history of influenza dates back to long before 412 BC when Hippocrates recorded a major epidemic of a disease that was not called influenza then. In 1357 AD, the term influenza was coined. However, in 1580 AD, the first recorded influenza pandemic occurred in Europe and spread to Asia and Africa followed by pandemics in 1729-1730 and 1830-1884 and only reached the Americas in 1889-90. In 1878, a disease causing high death rate in poultry occurred in Italy called "fowl plaque" caused by a flu virus. It is now called "highly pathogenic avian influenza" (HPAI) caused by H5 and H7 subtype viruses with different HA and NA combinations.

Major known pandemics in the 20th century are those of 1918-1919, the "Spanish Flu" that circled the globe caused by an H1N1 flu virus, causing the worst known pandemic which killed an estimated 50-100m people worldwide, followed by the Asian flu in 1957-58 caused by H2N2 virus and the Hong

Kong flu in 1968-69 caused by an H3N2 virus all due to genetic shift.

The swine virus was first isolated in 1930 followed by human, avian and equine viruses from 1933 through 1963. In 1984 the first human flu virus was isolated in Nigeria by Prof. Tam David-West and others. From 1983 through 2008, deadly bird flu outbreaks occurred worldwide caused by H5 and H7 bird viruses. In 1991, equine H3N8 viruses were isolated for the first time in Ibadan, Nigeria in tropical Africa (Adeyefa and McCauley 1994). In January 2006, the first reported outbreak of deadly bird flu occurred in Nigeria followed by outbreaks across the country till mid-2008 with one reported human death in Lagos in 2007.

In March/April, 2009 the new swine origin (S-OIV) H1N1 influenza outbreaks in man and pigs in the USA and Mexico were reported, followed by human-to-human transmission with worldwide spread to over 111 countries with 1,630, 000 confirmed cases and over 17,500 deaths. On 11 June, 2009, it was declared the first pandemic in the 21st century by WHO Director–General, by which time, the virus became known as pandemic influenza H1N1 2009. The genes of this virus originated from three sources (Smith *et al.* 2009, Garten *et al.* 2009). On 18 January, 2010 Nigeria announced the first pandemic H1N1 2009 infection and death. As at 20 April, 11 people had been infected and two deaths recorded (fig. 8).

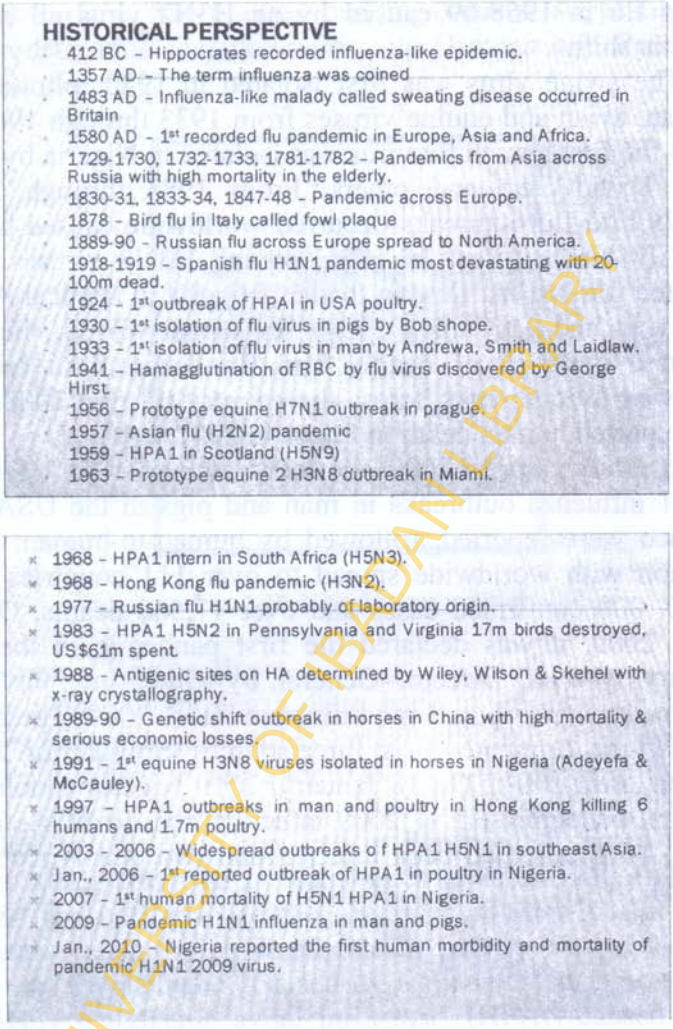


Fig. 8. History of flu viruses

Origin, Evolution, Genetic Relatedness, Ecology and Epidemiolgy of Influenza Viruses: From Where Did They Come?

Available body of evidence from extensive studies strongly suggests that all the genes of flu viruses currently circulating among mammals and birds originated from avian flu viruses

whose reservoirs are wild aquatic birds, although separate host-specific gene pools exist (fig. 9). The general structural features, genome organization and sequence analyses of A, B and C flu viruses indicate that they originated from a common ancestor distinct from other negative strand viruses (Webster *et al.* 1992)). The gene pool of influenza A viruses in aquatic birds provides all genetic diversity required for the emergence of pandemic influenza viruses for humans, lower animals and birds. Continuing evolution is most prominent in the surface glycoproteins of flu viruses but also occurs in each of the eight gene segments of both type A and type B viruses.

Fig. 9. Origin and ecology of flu viruses

The variability results from accumulation of molecular changes in the eight RNA segments that can occur by a number of different mechanisms including point mutations (antigenic drift), gene re-assortment (genetic shift), defective interfering particles and RNA recombination. With the realization that avian and swine flu viruses can be directly transmitted to humans, flu viruses are now considered a major global health threat.

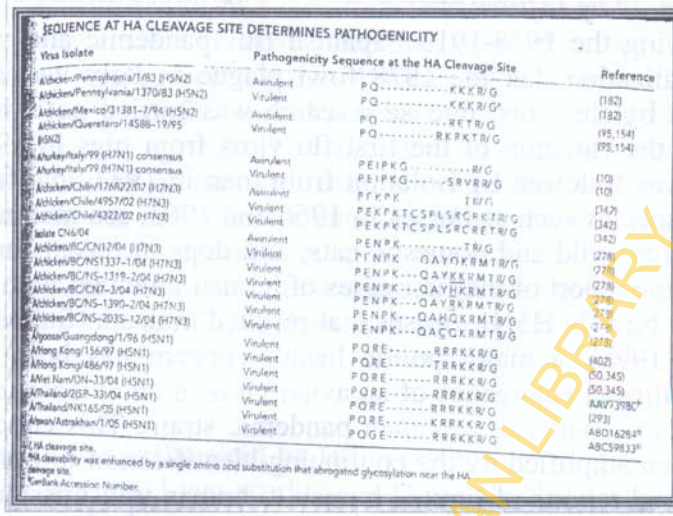
Surveillance is very important for the viruses in view of the threat of pandemic influenza. Weekly summaries of influenza activity are published in the morbidity and mortality weekly report from the US Center for Disease Control and Prevention (CDC) during the influenza season and in the weekly epidemiologic record published by the World Health Organization (WHO) and the FAO. Additional components of surveillance include estimate of the burden and intensity of epidemics using weekly tracking of the proportion of deaths due to influenza and pneumonia (Doushoff *et al.* 2006).

Pathogenesis and Pathogenicity: How do Flu Viruses Cause Disease in their Hosts?

Flu virus proteins HA, M2, NS1, BP1-F2, PB2 and NA play significant roles in viral pathogenesis and pathogenicity.

Molecular Basis of Pathogenicity

The mechanisms involved in virulence and pathogenicity include multibasic amino acid sequence at the HA cleavage site, carbohydrate side chain, M2 activity, host cell proteases, mutations in the BP2 gene segment, insertions in the HA gene segment, deletions in the NA and NS gene segments and the roles of NS1 and BP1-F2 proteins (figs. 10 and 11).



SEQUENCE AT HA CLEAVAGE SITE DETERMINES PATHOGENICITY

Virus Isolate	Pathogenicity	Sequence at the HA Cleavage Site	Reference
A/chicken/Pennsylvania/1/83 (H5N2)	Avirulent	PQ----------KKKR/G	(182)
A/chicken/Pennsylvania/1370/83 (H5N2)	Virulent	PQ----------KKKR/G*	(182)
A/chicken/Mexico/31381–7/94 (H5N2)	Avirulent	PQ----------RETR/G	(95,154)
A/chicken/Queretaro/14588–19/95 (H5N2)	Virulent	PQ----------RKRKTR/G	(95,154)
A/turkey/Italy/99 (H7N1) consensus	Avirulent	PEIPKG----------R/G	(10)
A/turkey/Italy/99 (H7N1) consensus	Virulent	PEIPKG----------SRVRR/G	(10)
A/chicken/Chile/176R22/02 (H7N3)	Avirulent	PEKPK---------IRR/G	(342)
A/chicken/Chile/4957/02 (H7N3)	Virulent	PEKPKTCSPLSRCRKTR/G	(342)
A/chicken/Chile/4322/02 (H7N3)	Virulent	PEKPKTCSPLSRCRETR/G	(342)
Isolate CN6/04	Avirulent	PENPK----------TR/G	(278)
A/chicken/BC/CN12/04 (H7N3)	Virulent	PENPK---QAYQKRMTR/G	(278)
A/chicken/BC/NS1337–1/04 (H7N3)	Virulent	PENPK---QAYKKRMTR/G	(278)
A/chicken/BC/NS–1319–2/04 (H7N3)	Virulent	PENPK---QAYHKRMTR/G	(278)
A/chicken/BC/CN7–3/04 (H7N3)	Virulent	PENPK---QAYRKRMTR/G	(278)
A/chicken/BC/NS–1390–2/04 (H7N3)	Virulent	PENPK---QAHQKRMTR/G	(278)
A/chicken/BC/NS–2035–12/04 (H7N3)	Virulent	PENPK---QACQKRMTR/G	(278)
A/goose/Guangdong/1/96 (H5N1)	Virulent	PQRE-------RRRKKR/G	(402)
A/Hong Kong/156/97 (H5N1)	Virulent	PQRE-------TRKKR/G	(50,345)
A/Hong Kong/486/97 (H5N1)	Virulent	PQRE-------RRKKR/G	(50,345)
A/Viet Nam/ON–33/04 (H5N1)	Virulent	PQRE-------RRRKKR/G	AAV73980*
A/Thailand/2(SP–33)/04 (H5N1)	Virulent	PQRE-------RRRKKR/G	(293)
A/Thailand/NK165/05 (H5N1)	Virulent	PQRE-------KRRKKR/G	ABD16284*
A/Iran/Astrakhan/1/05 (H5N1)	Virulent	PQGE-------RRRKKR/G	ABC59833*

* HA cleavage site.
† HA cleavability was enhanced by a single amino acid substitution that abrogated glycosylation near the HA cleavage site.
‡ GenBank Accession Number.

Fig. 10. Sequence requirement for high cleavability of the HA

- 1 Sequence requirement for high cleavability of the HA and virulence
- 2 Carbohydrate side chain.
- 3 M2 activity.
- 4 Host cell proteases.
- 5 Mutations in the PB2 RNA gene segment.
- 6 Insertions in the HA RNA gene segment.
- 7 Deletions in the NA and NS gene segment.
- 8 Role of NS1 and PB1-F2 protein genes in pandemic potential.
- NS1 inhibits the production of type 1 interferon and pro-inflammatory cytokines which are parts of host defence mechanism. Inhibition of the function of dentritic cells in producing pro-inflammatory cytokines and chemokines results in multi-organ failure while PB1-F2 localizes to the mitochondria where it interferes with two cellular components, ANT3 and VDAC1, responsible for apoptosis control, another host defence mechanism.

Fig. 11. Mechanisms involved in virulence and pathogenicity

How Other Creatures Great and Small have been influenced by Influenza

Following the 1918-1919 "Spanish flu" pandemic and with the realization that the 1878 fowl plague in Italy was also caused by flu virus, intense research was carried out which led to the isolation of the first flu virus from pigs in 1930. This was followed by isolation from man in 1933, and from other species such as horses in 1956 and 1963, sea mammals and birds, wild and domestic cats, and dogs in recent times. Since the report of the first cases of human infection with the deadly bird flu H5N1 viruses that resulted in death (Subbarao *et al.* 1998), a major public health concern has been the possibility of emergence of an avian virus or an avian-human re-assortant virus as a human pandemic strain. This concern has been amplified by the continuing identification of human cases and reports of limited human to human transmission of H5N1 viruses within a family cluster in southeast Asia (Normile 2005), and more recently in 2009 the emergence of the new swine-origin flu virus (S-OIV) H1N1 which spread globally with human-to-human transmission that resulted in it's declaration in June 2009 as the first pandemic of the 21st century (Garten *et al.* 2009, Smith *et al.* 2009).

These concerns have resulted in extensive WHO flu expert committee meetings and stockpiling of vaccines and antiviral drugs, particularly in industrialized nations. Extensive research for vaccine candidate viruses and effective antiviral drugs has been intensified across the globe. Serious economic losses have resulted from severe outbreaks of deadly bird, swine and equine flu. For instance, in Pennsylvania in 1983, a sum of US$61m was spent in destroying 17m birds due to an outbreak of deadly bird flu caused by H5N2 virus. Mexico spent millions of US dollars on vaccination following outbreaks of deadly bird flu across the country. In outbreak settings, thermal detectors are installed in airports and there is contact tracing of people showing influenza-like illnesses.

During the Nigerian outbreaks in 2006, a crises center was established in Aso rock directed by the President himself while a disease control steering committee was formed headed alternatively by the Ministers of Health and Agriculture. The avian flu control project in Nigeria comprises three components: agriculture (veterinary), health and information. At the onset of the outbreaks, the international community came to our aid; international organizations, donor agencies and a number of countries gave us protective materials, lab equipment, dressings and medications, and the World Bank gave us a grant. When Niger had her outbreaks, the Nigerian Government sent personnel to eradicate the disease in that country.

How I was Influenced by Influenza

Prior to 1989, I was working in the fields of parasitology and virology looking at insect vectors with the aim of focusing on African horse sickness virus whose vector is the common midge ("kotonkan" in Yoruba). However, research grants were difficult to come by in this area at the time and University of Ibadan Senate research grant then was a paltry N2,750.00 for an individual grant and about N7,000.00 for a multidisciplinary grant from 1987 to 1991 which could not grant you anything because you could access only half of it. Literarily, the research grants were actually "kotonkan." We had to spend part of our meager salary which could not take you home on procuring reagents and chemicals, biologicals and even some equipment.

Fortunately, Prof. Oyewale Tomori who was my co-supervisor then, being a virologist, sometime in 1989 represented Nigeria at a WHO meeting in Geneva, and on his return, he informed me that WHO Fellowship was available for influenza research. So I switched over to flu virus research and promptly went with him to the WHO office in the Federal Ministry of Health, Federal Secretariat in Lagos to obtain, fill and submit a Fellowship form. I also got a placement in Prof. Robert G. Webster's laboratory in the Department of Virology and Molecular Biology, St. Jude

Children Research Hospital, University of Memphis in USA, which is a WHO influenza collaborating center and a center of excellence for influenza research. Unfortunately, to my disappointment the Fellowship did not come through. I then quickly sort placement in another world-class laboratory in the flu group under Dr. John McCauley in the Pirbright laboratory of the AFRC Institute for Animal Health, U.K. in anticipation of a Royal Society Fellowship in 1990. Unfortunately, this also did not materialize.

All the while, I was travelling all over the country following the trails of annual Polo tournaments to collect and store the necessary samples to work on abroad should a scholarship, fellowship or grant become available. The samples comprised sera, nasal, ocular and pharyngeal swabs, tracheal washes as well as tissues from dead animals. I was also carrying out simple laboratory tests on my samples at least to determine the status of equine influenza in Nigeria.

By a stroke of luck, I was nominated by my Dean then, Prof. Tom Aire for the 1991 Commonwealth Academic Staff Fellowship for my sabbatical leave tenable at the Pirbright laboratory. This would also have failed as the Commonwealth office in London was not willing to pay the £9000.00 bench fees required in Pirbright. But through divine intervention, the Commonwealth Secretary then, Mr. Herterrington, agreed to pay the bench fees after several telephone conversations with him. You can imagine what it was like in NITEL phone booths in Ibadan then.

In the last week of December I received my letter of award by courier and left for the UK on 31 December, 1991. I met my host, Dr. John McCauley, on 4 January, 1992 after several telephone conversations with him, here in Ibadan before I left Nigeria, and on arrival in the UK, to discuss what I would be doing for the 10-month duration of the fellowship in the flu lab. I was quite surprised but exited when he outlined what I had to do. The paper listed such high sounding procedures like molecular techniques, SDS-PAGE, gene cloning and genetic engineering, in-situ hybridization and

oligonucleotide fingerprinting, among others. I must confess, I only heard about one or two of these techniques in passing but had not read them to understand what they meant not to talk of doing them.

I was a young old man aged 43 years and about 3 years older than my host who was the oldest in the Flu group, a brilliant and hardworking world-class research scientist. And here I was, an outsider in the field of molecular biology/virology, being confronted with all these "high tech" laboratory techniques just to study flu viruses. After John left for the Compton Laboratory where he was based, I just didn't know what to do. I copied the programme and sent it to Profs. Tomori and T. U. Obi who were my supervisors back home. I started thinking about how I could cope with learning such procedures. John and I were to meet in a few days for him to take me round the laboratory. I was a Senior Lecturer of six years standing. I thought I could just play along for the 10 months, collect the £250 monthly stipend, eat, drink, generally enjoy my life in the UK and come back home. But then I realized my limitations back home with no Ph.D yet which you must have before you could be promoted to the Professorial grade. I knew I had to do something fast. I also remembered my burning ambition to become a world-class scientist and a Professor in a hostile environment back home with no electricity, water, laboratory consumables, reagents and chemicals; there were unending strikes, the take home pay could not take you home and was sometimes paid on the "40th day" of the month. Worst still, you had to contend with repressors, to say the least.

However, after a few days of serious thinking and putting things in proper perspective, I decided to give it whatever it would take to learn all I could learn to be able to work on my samples. Afterall, all the great names in Arts and Science had to learn at one time or the other in their career before they got a breakthrough to become world famous. I knew I had to read, so I pitched my tent in the Institute's library which was open 24 hours a day, 365 days a year, with my 'magnetic key', which also opened all the doors of the Institute for me.

So I read extensively the dictionary of molecular biology, journals, articles, on the molecular aspects of viruses and anything that would give me an insight into molecular biology.

Well, one needed a sound knowledge of biochemistry and cell biology at the molecular level to understand the complex ways viruses multiply but our knowledge of biochemistry in the pre-clinical medical and veterinary schools back home was limited. You can now imagine what I had to stuff in my head to cope with the demands of my studies on flu viruses at the time. And I did not want to expose my ignorance or limited knowledge in the presence of the scientists, technicians/technologists and graduate students in the lab. It was almost a hell on earth for me but thank God, I learnt and I learnt fast too. The intrinsic pride and work ethic of the good Nigerian in me ensured that I prevailed.

What did I do with the Influence?

In the Beginning: A Research Scientist in the Making

The foundation for my scientific research was laid in Cambridge Veterinary School in 1982. However, the Pirbright laboratory then was and is still a world-class, level 3 bio-security/bio-containment laboratory whose doors are under negative air pressure and where you have to shower in with caustic potash soap, change into laboratory clothes, shower out on completion of your tour of duties and then change into personal clothing. You also had to put your samples in three sets of sealed containers to prevent escape of viruses should there be an accident. Materials from the lab had to pass through a carbide filter to preclude virus escape. There were several virus groups working on different virus families, who gave their seminars on particular days of the week, but there was a compulsory overall seminar for every scientist and graduate student on Tuesdays between 10am and 12 noon and an outside seminar once in a while for other scientists from outside the laboratory. I was introduced to all the various laboratories, virus groups and personnel including the sectional HODs by my host on my first day in the

laboratory, and for the rest of the week, I was introduced to how the various state-of-the-art equipment, facilities, reagents and biologicals including radio-isotopes function. Most of these were centralized and you sometimes had to book down in the log books, for their use.

I started with the basic routine laboratory work such as virus isolation in fertile chicken eggs and tissue culture, preparation of chicken embryo fibroblasts and other monolayer cells. I then learnt virus purification in sucrose gradients using cold Beckman centrifuges, phenol/chloroform extraction of virus RNA (commercial extraction kits were not popular then as they are now), and analysis of virus polypeptides in pulse-chase SDS-PAGE. Then, I moved on to more sophisticated molecular techniques over many weeks, such as reverse transcription using radioisotopes to detect virus RNA segments in 4% polyacrylamide gels (fig. 12).

Fig. 12. Reverse transcripts of flu virus gene segments

I then learnt RT-PCR followed by partial gene sequence analysis. But my first hands-on sequence analysis was a disaster with a complicated Boehringer Mannahien protocol

because I did not couple the radio-labelled primer with the sequence reaction. This was because my understanding then of the biochemistry of natural viral replication was very much rudimentary. John, then realizing my mistake, quietly but in no unmistakable terms explained to me "sequence reactions" including the di-deoxy chain termination reaction which generated the sequence bands visualized on X-ray films, and at this point, I had to go back to the library after lab work and read up a lot on molecular processes in micro-organisms including bacteria and viruses. After another trial I got it right and was pleased with myself.

Now that I had become familiar with and influenced by influenza A virus transcription, replication and sequence reactions, we changed to Promega sequencing kit. My partial sequencing of a deadly flu virus segment 5 gene (fig. 13) was so successful that John was proud to bring Dr. Chris Bostock, the Head of Molecular Biology Division, who later became the Director of the Institute from Compton lab to see it and others that I carried out. This virus actually killed 7,129 turkey poults out of about 7,800 in Norfolk, England within five days in 1991 before I arrived in the UK. I was very happy, grinning from ear to ear and was quite proud of myself. I was thus launched into the world of molecular biology/virology and from then on, I became a confident scientist moving on to gene cloning in plasmid vectors and genetic engineering having learnt the use of restriction enzymes, design and synthesis of primers on Applied Biosystems in the "oligo suite" and their deprotection in the lab. I had finally arrived and got hooked to flu viruses.

Fig. 13. Partial nucleotide sequence of flu virus segments

What I did with the Influence

I moved on to develop a rapid method for the analysis of flu virus genes called "multiplex RT-PCR". This method relies on the use of the 12 and 13 terminal nucleotides at the 3' and 5' end respectively of each flu virus gene as primers. This we applied to elucidating the re-assortment of equine flu virus genes using the PCR product (fig. 14) generated with the terminal forward and reverse primers, to determine partial nucleotide sequences which can be used to search nucleotide sequence database and rapidly map the genetic origin of each RNA gene segment. We analysed over ten equine-1 flu viruses isolated between 1956 and 1977 to examine the origin of the genes of the ribonucleoprotein complex (RNP) and the other four genes.

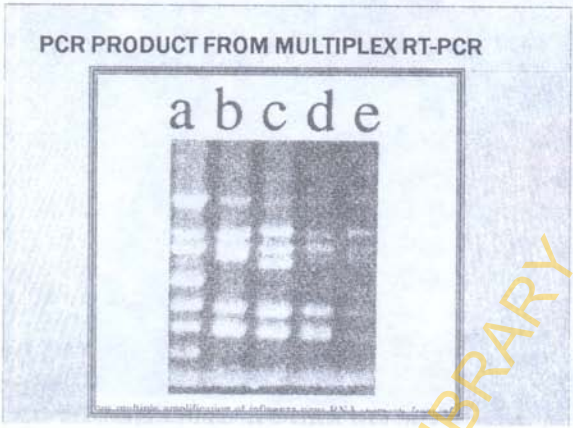


Fig. 14. PCR product used for nucleotide sequence analysis

We were thus able to definitely establish that the genes of the RNP complex and the NS polypeptides of equine-1 flu viruses isolated after 1966 have been usurped by equine-2 flu viruses particularly the London 1416/73 virus but the RNA gene segments encoding the other proteins were not (Adeyefa *et al.* 1994), (fig. 15).

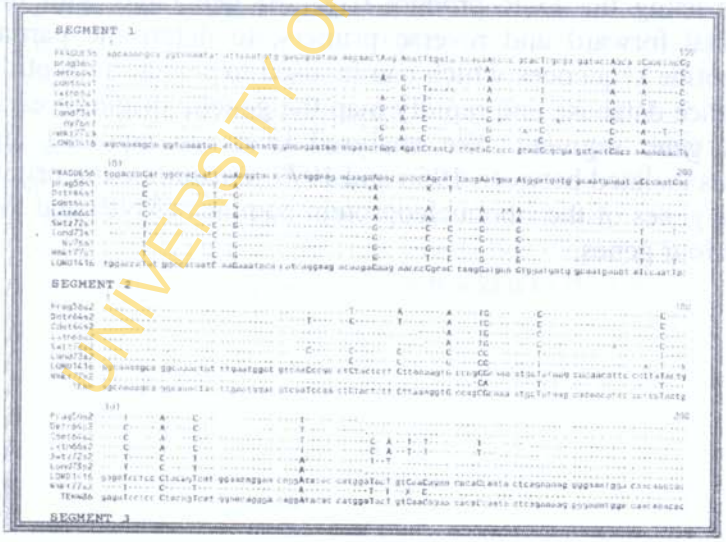


Fig. 15. Partial nucleotide sequence of equine-1 segments 1 and 2

This work actually launched me into the field of molecular virology and recognition as it was taken to at least three international conferences before it was published. The results extended our understanding of the details of genetic variation within equine flu viruses as a result of which existing equine vaccines are being regularly upgraded (Adeyefa *et al.* 1994).

The laboratory offered me a lot of opportunities for more work unhindered. Reagents were abundant and when out of stock, you only needed to call the suppliers who would bring them in immediately. Facilities were also adequate and well maintained and the laboratory environment which had well controlled temperature and humidity all year round was very conducive to research and learning.

So I ploughed on, undertaking more projects and spending considerable time in the lab. I also developed a method of direct sequencing of influenza A virus RNAs from clinical samples in flexible Dynatech ELISA plates without cDNA amplification and molecular cloning. We used the method to simultaneously determine and analyse the partial nucleotide sequences of the eight RNA gene segments of flu viruses from disease outbreaks (Adeyefa and McCauley 1997) (figs. 16 and 17). The method is capable of precluding the risk of cross contamination associated with PCR and obviating the differences observed in antigenic and genetic properties of viruses isolated in eggs or tissue culture compared to viruses predominantly circulating in the various host species. The method is also very stringent, rapid, sensitive, specific and cost effective. It's potential lies in its application for rapid diagnosis, molecular epidemiology, virus surveillance, vaccine strain selection and adaptability especially in areas where modern high tech, state-of-the-art equipment and facilities are not available or easily obtainable like in Nigeria where foreign exchange, and Custom's inspection and clearance are barriers to procuring materials for meaningful research.

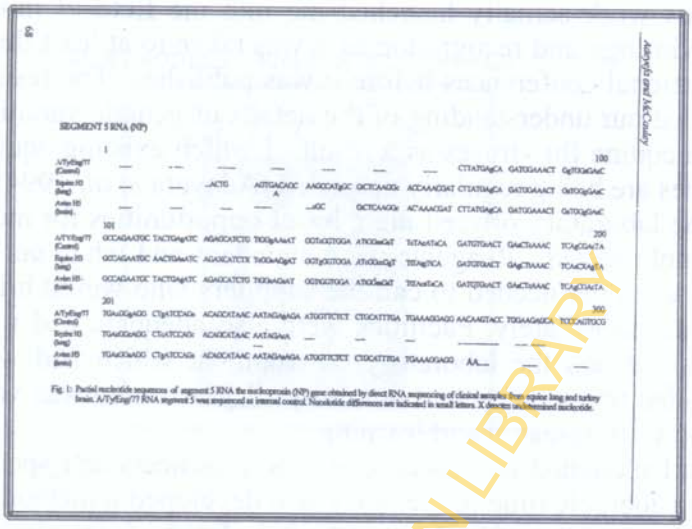


Fig. 1: Partial nucleotide sequences of segment 5 RNA the nucleoprotein (NP) gene obtained by direct RNA sequencing of clinical samples from equine lung and turkey brain. A/Tyr/Eng/77 RNA segment 5 was sequenced as internal control. Nucleotide differences are indicated in small letters. X denotes undetermined nucleotide.

Fig. 16. Partial nucleotide sequence of flu virus segments from clinical samples

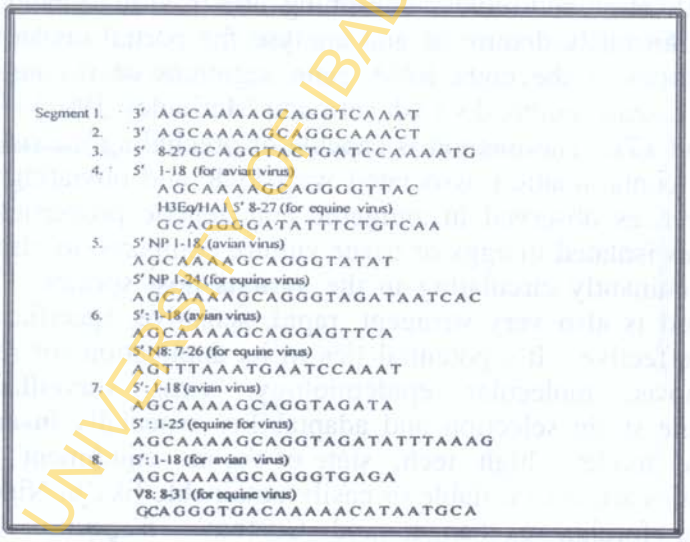


Fig. 17. Nucleotide sequence of segment-specific primers

I stated earlier that I collected and shipped samples to the UK for analysis. I could not work on these samples however

until I had completed my host's projects designed for my benefit. So I had a four-week break to attend the American Society for Virology conference in Ithaca, New York State, US from 7-10 July, 1992, followed by a holiday from 11 July to 5 August, to visit friends and classmates. These included Dr. Femi Obasaju (DVM, Ibadan), a world renowned expert in in-vitro fertilization in Long Island, New York, Prof. B. O. Oke on sabbatical leave in Georgetown University in Washington DC, Dr. Laitan Balogun (B.Sc. Agric Engineering, UI) in far away California, and of course Prof. Webster in Memphis. This was to solicit for assistance in getting primers, monoclonal antibody and polyclonal antisera panels to analyse my samples, and to arrange for a Rockefella Fellowship, after which I went back to the UK. From my equine sera collected from 1990-1991 and later on arrival at home from 1993-1994, we were able to detect equine-2 flu virus antibodies by heamagglutination inhibition (HI) and ELISA tests compared with sera from Western Europe, in collaboration with Dr. Chris Hambling in Pirbright and Dr. Anne Cullinane in Dublin, Ireland (Adeyefa *et al.* 1996a).

Also, from the swab and tracheal wash samples, particularly those from the January 1991 equine influenza outbreak among polo horses during the Ibadan tournament, I was able to isolate 3 independent equine-2 (H3N8) viruses named A/eq/Ib/4/91 (Ib4), A/eq/Ib/6/91 (Ib6) and A/eq/Ib/9/91 (Ib9) which were reported for the first time in Nigeria, in tropical Africa (Adeyefa and McCauley 1994). The two previous outbreaks on the African continent were from Algeria in 1972, caused by equine-1 (H7N7) virus and South Africa in 1989, caused by equine-2 (H3N8) virus respectively. We carried out extensive antigenic and genetic analyses on the three isolates at the molecular level. The detailed antigenic and complete nucleotide and amino acid sequences of the HA genes of these Ibadan viruses, the phylogenetic as well as the partial nucleotide sequence analyses of the other seven gene segments using primers, monoclonal antibody and polyclonal antisera panels kindly

provided by Prof. Webster and Dr. Alan Douglas of the National Institute for Medical Research, Mill Hill, London, established that despite the geographic location from which the viruses were isolated, two were similar to viruses that concurrently caused disease in Europe in 1989 and 1991 and were related to viruses that had been predominating in horses since 1985. The third was more closely related to viruses from 1991 onward in Europe and other parts of the world (Adeyefa *et al.* 1996b), (figs. 18 and 19).

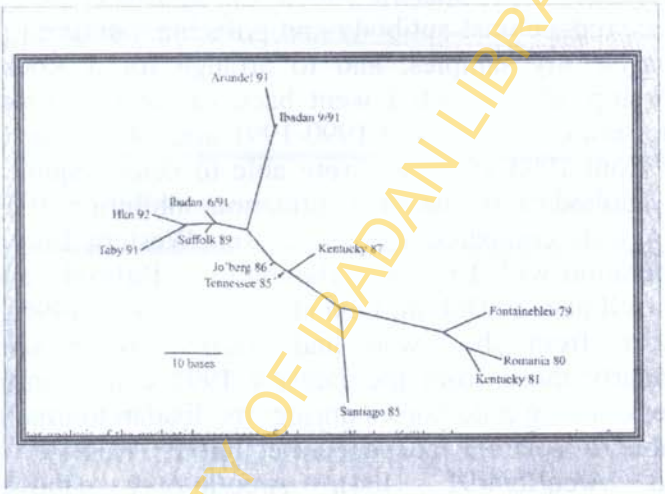


Fig. 18. Phylogenetic tree of equine-2 virus HAs

Table 3. *Nucleotide sequence variation between UK and Nigerian equine influenza viruses*

Base	Suffolk 89			Arundel 91	Amino acid sequence	
						position 6
83	C	C	T	C	Synonymous	—
245	G	G	A	A	Synonymous	—
308	T	T	C	C	Synonymous	—
361	A	A	G	G	N 96 S	HA1 antigenic site A
428	A	A	G	G	Synonymous	—
478	G	C	G	G	R 135 T	HA1 antigenic site A, potential N-linked glycosylation at N133
486	G	G	T	T	A 138 S	HA1 antigenic site A
540	A	A	G	G	K 156 E	HA1 antigenic site B
551	T	T	C	C	Synonymous	—
562	T	T	C	C	I 163 T	HA1 antigenic site B
599	T	T	C	C	Synonymous	—
658	T	T	C	C	Synonymous	—
693	G	G	A	A	E 207 K	HA1 antigenic site D
711	G	G	A	A	V 213 I	Buried
800	T	T	C	C	Synonymous	—
805	C	T	T	T	T244 M	T unique to Suffolk 89
824	C	C	T	C	Synonymous	—
856	G	G	A	A	R 261 K	HA1 antigenic site D/E
901	C	C	T	T	T 276 I	HA1 antigenic site C
943	A	A	A	G	N 290 S	HA1 antigenic site C
965	G	G	A	A	Synonymous	—
1028	C	T	T	T	Synonymous	—
1168	C	C	G	ND	A 36 G	HA2
1188	G	A	G	ND	A 43 T	HA2 unique to Ibadan 4/91 and 6/91
1271	C	C	T	ND	Synonymous	—
1298	T	T	C	ND	Synonymous	—
1322	C	C	T	ND	Synonymous	—
1430	G	G	A	ND	Synonymous	—
1571	A	A	G	ND	Synonymous	—

Fig. 19. Nucleotide sequence variation between UK and Nigerian equine-2 flu viruses

A comparison of the nucleotide sequences of two of the Ibadan viruses, Ib4 and Ib6, with a European prototype strain, A/eq/Suffolk/89 (H3N8) showed limited variation in the HA gene which resulted in amino acid substitutions in one of the five antigenic sites: this mutation resulted in the potential production of a new glycosylation site in antigenic site A of the HA (Adeyefa *et al.* 1997a). The other Ibadan virus, Ib9, showed a single amino acid change from another European strain, A/eq/Arundel/12369/91, while the Ib4 and Ib6 had several amino acid substitutions in antigenic sites B to E of the HA glycoprotein (Adeyefa *et al.* 1997a) (fig. 20). The nucleotide sequences have been deposited at the EMBL nucleotide sequence database at the European Bioinformatics Institute, Hingston, UK under accession numbers: X95637 and X95638.

Fig. 20. HA monomer of equine H3 viruses

The new glycosylation site enhanced the infectivity of these viruses as investigated by plaque assay, virus titration in fertile chicken eggs and tunicamycin treatment (Adeyefa *et al.* 1997a and b). The results of this study showed that though the three viruses were infectious and that strains Ib4 and Ib6 were more infectious than Ib9, the latter did not contain the additional asparagine-(N)-linked glycosylation site which actually influenced the infectivity, antigenicity and immunogenicity of the viruses (Adeyefa *et al.* 1997b). In both H1 and H3 HAs of influenza A viruses, the addition of a sugar moiety can block antibody binding at a site to which in the absence of the carbohydrate, the virus will bind (Wiley and Skehel 1967). The H1 and H3 viruses use this mechanism to avoid antigenic virus surveillance in humans. It is thus probable that the observed additional N-linked glycosylation site in two of the viruses resulting in the addition of a carbohydrate side chain with subsequent escape of antibody binding and virus neutralization, invariably led to the overt

clinical infection of the horses as reported in Veterinary Record (Adeyefa and McCauley 1994) despite the demonstration of circulating antibodies in some of the affected horses (Adeyefa *et al.* 1996a).

The important role of N-linked glycosylation in relation to attachment, fusion, virus infectivity and antigenicity can thus not be overemphasized as observed in our other studies which showed distinct electrophoretic mobility of the HA of the two Ibadan viruses with an additional glycosylation site compared with the third (Adeyefa *et al.* 2000), (fig. 21); in an epidemic strain of human flu virus, A/Eng/878/69 which acquired a new glycosylation site at residue 63 with the resultant increased infectivity (Skehel *et al.* 1984); as well as in a cousin virus, a measles virus which showed heterogeneity in electrophoretic mobility of the HA having N-linked glycans (Hu *et al.* 1994). All these emphasize the importance of the sugar moiety on the virus attachment protein, the HA.

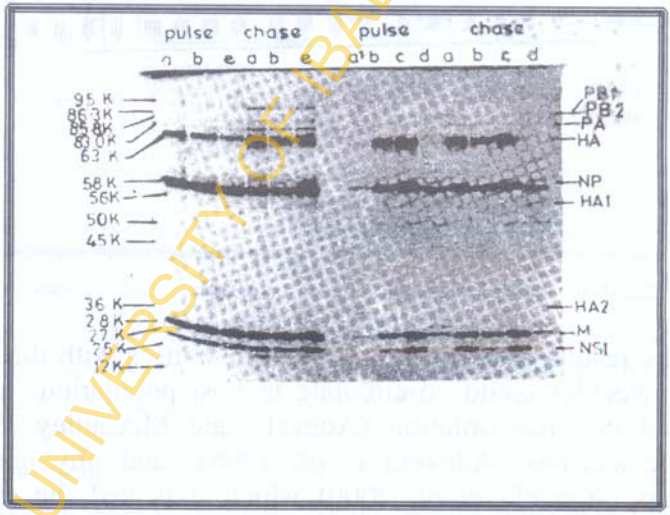


Fig. 21. Influenza A virus encoded polypeptides

We investigated the antigenic diversity of these viruses as well, apart from the genetic variation, in the light of antigenic drift known to occur with flu viruses. Antigenic analysis was

carried out with panels of monoclonal antibodies (mAbs) and polyclonal antisera (time will not allow me to dwell on monoclonal antibody production in which I also have expertise), and the results indicated that the three virus isolates were antigenically divergent, although they were all equine-2 subtype viruses related to 1963 –1987 viruses (Adeyefa *et al.* 1996b) (fig. 22). We also investigated viral protein synthesis by pulse-chase experiments which showed heterogeneity among the proteins of the RNP complex and HA glycoprotein (Adeyefa *et al.* 2000) (fig. 21) indicating that equine H3 HAs have evolved by a process of evolutionary divergence and mutational changes confirmed by genetic analysis above.

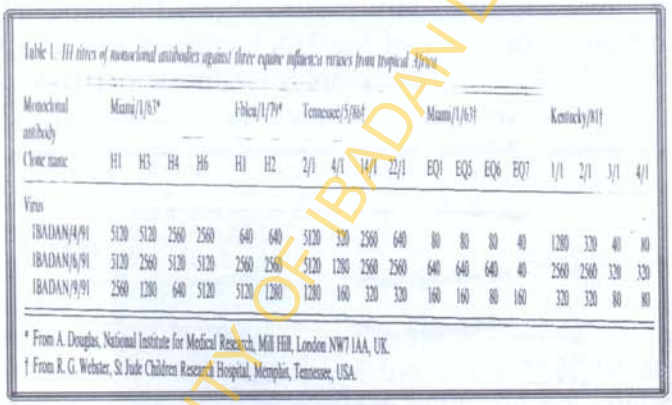


Table 1. *HI titres of monoclonal antibodies against three equine influenza viruses from tropical Africa*

Monoclonal antibody	Miami/1/63*				Fibia/1/79*		Tennessee/5/86†		Miami/1/63†				Kentucky/81†			
Clone name	H1	H3	H4	H6	H1	H2	2/1	4/1	14/1	22/1	EQ1	EQ5	EQ6	EQ7	1/1	2/1	3/1	4/1
Virus																		
IBADAN/4/91	5120	5120	2560	2560	640	640	5120	320	2560	640	80	80	80	40	1280	320	40	80
IBADAN/8/91	5120	2560	5120	5120	2560	2560	5120	1280	2560	2560	640	640	640	40	2560	2560	320	320
IBADAN/9/91	2560	1280	640	5120	5120	1280	1280	160	320	320	160	160	80	160	320	320	80	80

* From A. Douglas, National Institute for Medical Research, Mill Hill, London NW7 1AA, UK.
† From R. G. Webster, St Jude Children Research Hospital, Memphis, Tennessee, USA.

Fig. 22. HI titres of H3 equine flu viruses using monoclonal antibody panel

The results also confirmed that H3 viruses with different HA molecules could co-circulate in host populations as we showed in virus isolation (Adeyefa and McCauley 1994), genetic analysis (Adeyefa *et al.* 1996a) and phylogenetic analysis (Adeyefa *et al.* 2000) which indicated the evolutionary relationship of contemporary equine-2 H3 subtype flu viruses isolated over a period of 30 years, 1963-1993 (Adeyefa *et al.* 1997c). This study was carried out by nucleotide sequence analysis of the HAs of these viruses and constructing phylogenetic trees using distance, neighbour

joining and DNA PARS in the PHYLIP software package (Adeyefa *et al.* 1997b) (fig. 23). The results indicated that there are two pathways of the evolutionary tree, one of which comprises four evolution lineages while the other comprises three lineages to which viruses isolated between 1967 and 1993 belong, including the three Ibadan viruses and contemporary European strains. The first pathway contains viruses from 1963 to 1987, including the equine-2 prototype as well as North and South American viruses.

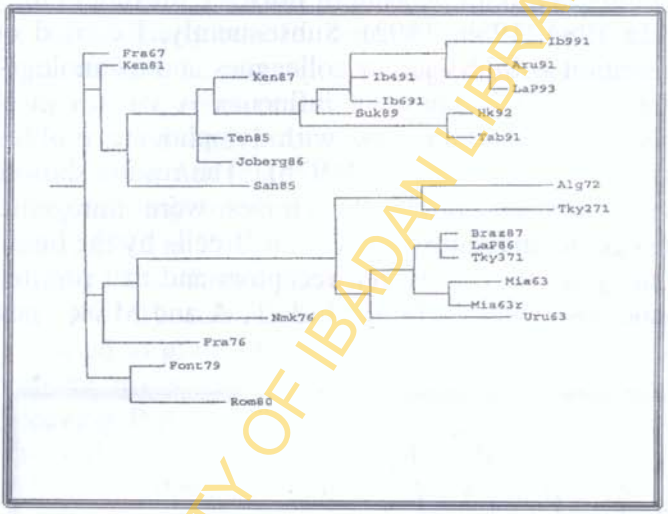


Fig. 23. Phylogenetic tree of equine H3 HA genes

These results emphasize the process of antigenic drift in these viruses over a period of 30 years along two evolutionary pathways rather than one arising from a single geographic gene pool proposed by Binns *et al.* (1993), but increased international movement of host species has inadvertently led to the emergence of drift strains despite repeated vaccinations.

Other studies indicate that nucleotide and amino acid changes in the HA of the Ibadan viruses map to functional domains of the HA molecule such as the signal peptide and

antigenic sites (McCauley 1987, McCauley and Mahy 1983, Adeyefa 1996, Adeyefa *et al.* 2010a- in press) (fig. 20).

At this time, I also investigated the immunological response of the host to the flu virus, the foundation of which was laid while I was training in Prof. E. J. L. Soulsby's immunoparasitology lab in Cambridge Vet School in 1982. Then as a fulfillment of the requirement of University of Wales for my M.Sc degree, I studied the immune response of the horse to mitogenic fractions of *Strongylus vulgaris*, a notorious and devastating helminth parasite of horses (Adeyefa *et al.* 1985; Adeyefa 1984, 1989, 1992). Subsequently, I carried out, in collaboration with my junior colleagues and technologist, the cellular immune response of influenza A viruses in equine models *in-vivo* and *in-vitro* with lymphocyte proliferation experiments (Adeyefa *et al.* 1997b). The results showed that both subtypes of equine flu viruses were mitogenic and immunogenic, inducing both T- and B-cells by the binding of virus antigen, the HA, to cell receptors and that proliferating lymphocytes produced IgG1, 2, 3, T, A and M isotypes (fig. 24).

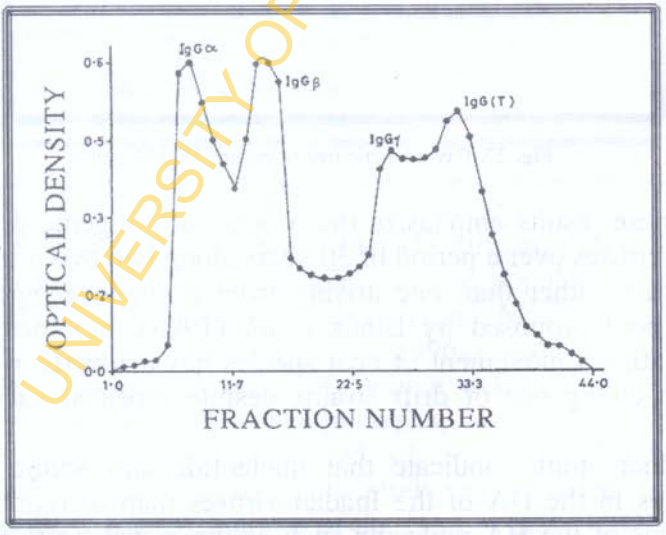


Fig. 24. Immunoglobulin isotypes

We also studied viral protein synthesis in three RNA virus families including Orthomyxo-, Paramyxo-, and Birna (Diai 2004). Part of these studies were carried out in the Biotechnology laboratory of the International Institute of Tropical Agriculture (IITA) Ibadan, part in Prof. Nwagwu's Cellular Parasitology laboratory in the Department of Zoology, University of Ibadan and part in the Virology Department, College of Medicine, University of Ibadan.

While in Pirbright, I learnt hybridoma technology under Dr. Robin Butcher. We then produced monoclonal antibodies against a non-deadly bird flu virus A/turkey/Eng/77 (H7N7). We characterized the monoclonal antibodies and I used them back home in Nigeria to carry out H7 flu virus surveillance studies in equine and avian host species (Adeyefa *et al.* 2010b; Olusa and Adeyefa 2010).

It is gratifying to note that I owed my last month in November, 1992 in Pirbright laboratory to two elderly ladies in particular, Ina Busby and Mrs. Skinner who practically provided for my subsistence because the Commonwealth 0ffice refused to grant me the one month extension I needed to complete my studies on my Ibadan viruses. However, while leaving Pirbright, John gave me an ample supply of reagents and biologicals. I also brought a lot of my mini-preps (DNAs) of the H3 isolates, my extracted vRNAs of both H7 and H3 equine flu viruses with which we carried out a number of studies. He also sent me over thirty oligo-nucleotide primers each worth fifty pounds sterling to facilitate my research, and over the years, anytime I asked him for anything on research, he sent them promptly. One couldn't have a better friend and collaborator in research. He even facilitated the admission and employment of some of my students in the UK.

On arrival from the UK in late November, 1992 and from subsequent travels, I mentored many undergraduate and post-graduate students as well as laboratory technologists in influenza virus research in areas of equine, swine and avian flu viruses which resulted in publications that have advanced

our knowledge in this field. My studies were also supported by University of Ibadan Senate Research grant numbers SRG/FVM/2000/8A (individual) for flu virus project and SRG/FVM/2000/6B multidisciplinary for "Potentials of Biotechnology and Molecular Biology Techniques in the Diagnosis, Epidemiology, Prevention and Control of Paramyxovirus Infections in Nigeria".

From 1994, long before the advent of the deadly bird flu outbreaks in Nigeria, the rest of Africa, Asia, Middle East and Europe, I had embarked on molecular epidemiological studies on flu viruses in Nigeria. For these, I had International Foundation for Science (IFS) grant of US$12,000.00 from which equipment, reagents and journals were procured and sent home to me by IFS agents. I also obtained the Third World Academy of Science grant of US$6,000 for my work on the mechanism of RNA editing in Paramyxovirus replication from which equipment and reagents were also procured and sent to me, all cleared by the passages office of our great University, thanks to the then acting Vice-Chancellor, Prof. Dosu Ojengbede and the then bursar, late Mr. Alao.

Those equipment and reagents which were insured by the University, thanks to the immediate past bursar, Alh. Bankole, assisted me tremendously in training these students and my junior colleagues and in publishing the results of our studies. We know that equine-1 thought to be extinct, and equine-2 flu viruses are still circulating among horses in the country (Olusa and Adeyefa; Olusa *et al*. 2010).

In the year 2000, I went to the National Medical Research Institute, Mill Hill, London for my sabbatical leave to work on swine flu viruses with Dr. Alan Hay, the then Director of the WHO Influenza Collaborating Center in the Institute. Little did we know then that the pandemic swine flu virus of 2009 would emerge. I have asked John to send me biologicals to study these swine viruses in Nigeria.

Prior to 2005, reports of outbreaks of the deadly bird flu were limited to Asia; however, by 2005, the virus had spread

to Russia and Western Europe (Normile 2005; Perdue and Swayne 2005). The first official report of outbreaks in Africa was in Nigeria, on 8 February, 2006. In December, 2005 and January, 2006, chickens in Kano and Kaduna States in northern Nigeria developed gastrointestinal and respiratory diseases with high rates of spread and death approaching 90%. Initially, Newcastle disease virus (NDV) or fowl typhoid was suspected as the main cause of disease; however, FAO reported in February 2006 that the deadly bird flu virus was responsible for the outbreaks and that the circulating virus was genetically similar to viruses isolated in southeast Asia and in European countries. However, phylogenetic analysis of the surface genes from several Nigerian deadly bird flu viruses suggested three separate introductions into the country and the timing of the initial introductions was not known (Ducatez *et al.* 2006). In addition, whether these introductions occurred via wild migratory birds or by human transport of infected animals remained uncertain (Ducatez *et al.* 2007) but I was determined to find these out.

Inspite of paucity of funds, facilities, reagents, basic infrastructure such as electricity and water here in Ibadan, we managed to struggle on in the field of flu virus research. Long before the reported January, 2006 outbreak of disease in Nigeria we had in place an ongoing flu virus surveillance in poultry at the poultry clinic in my Department where my then Ph.D student, Dr. Comfort Oluladun Aiki-Raji and other members of my research team (Drs. Omolade Oladele, Dimeji Oluwayelu, Ben Emikpe) and my postgraduate students were collecting nasopharyngeal and cloacal swabs as well as tissues from sick and dead birds found in poultry farms or brought by poultry farmers in the southwest of Nigeria notably, Oyo, Ogun and Lagos States. These were routinely injected by standard method into 10-11 days old fertile chicken eggs obtained from commercial hatcheries in Ibadan. Dr. Aiki-Raji won a McArthur grant in 2004 and took our analysed samples to Mt. Sinai School of Medicine to work with Dr. Chris Basler for three months in the laboratory of

Prof. Peter Palese, a world renowned flu molecular virologist and reverse geneticist (in fact, he was the first, along with other colleagues to conceive the idea of reverse genetics in 1991). The results were not conclusive then.

We however continued with our surveillance and routine tests, and fortunately during the first wave of the 2006 Nigerian outbreaks of deadly bird flu, in which investigations I was fully involved, carcasses were brought from affected poultry farms in the southwest to the poultry clinic. We independently isolated three deadly bird flu viruses and 12 Newcastle disease viruses from these samples on initial biological characterization. Dr. Aiki-Raji then took our isolates to the US in 2006 for further genetic, phylogenetic and pathogenicity analyses, in collaboration with members of Dr. Chris Basler's laboratory in New York, and Dr. David Swayne in the Southeast Poultry Research Laboratory in Athens, Georgia where the pathogenicity studies were carried out. Molecular analyses including gene cloning and transfection studies were carried out in Mt. Sinai. The three deadly viruses were designated A/ck/Nig/228-5/2006 (Nig228-5), A/ck/Nig/228-6/2006 (Nig228-6) and A/ck/Nig/228-10/2006 (Nig228-10) respectively (Aiki-Raji *et al.* 2008). Nucleotide and amino acid sequences of all their eight gene segments demonstrated that the isolates possessed multibasic amino acids at the HA cleavage site with the sequence PQGERRRKKR which was identical to those of deadly viruses from Europe, Russia, Asia, Nigeria and other African countries (fig. 25).

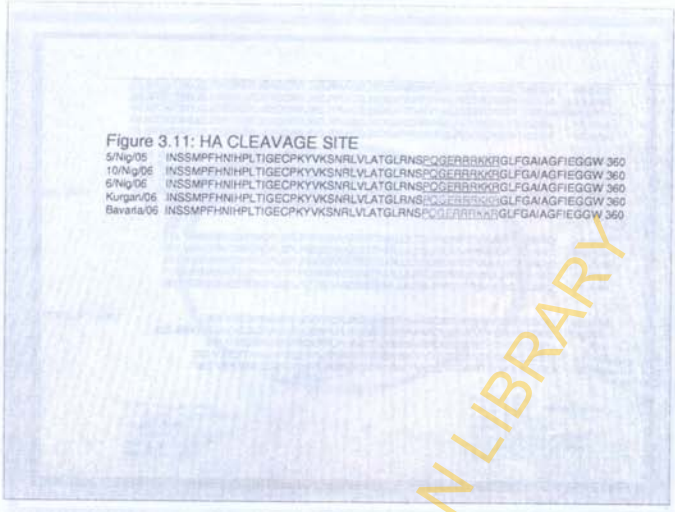


Fig. 25. Amino acid sequence of H5 HA cleavage site showing multi-basic amino acids

Other notable features of the sequences include the absence of the histidine to tyrosine substitution at residue 27 (H274Y genetic change) associated with high level resistance to oseltamivir in flu virus N1 subtype viruses and absence of known amantadine resistance-linked mutation in the M2 protein. These are medications for treating flu and these findings are of public health significance particularly in a pandemic influenza setting. The NS1 gene (fig. 24) of our viruses had a 5-amino acid deletion at positions 80-84, as observed since 2005 in viruses from southeast Asia. One of our isolates, Nig228-10, however also had a 7-amino acid extension at the C-terminus (fig. 26) which is predicted to affect the function of the PDZ ligand domain otherwise present at the C-terminus of the NS1 protein.

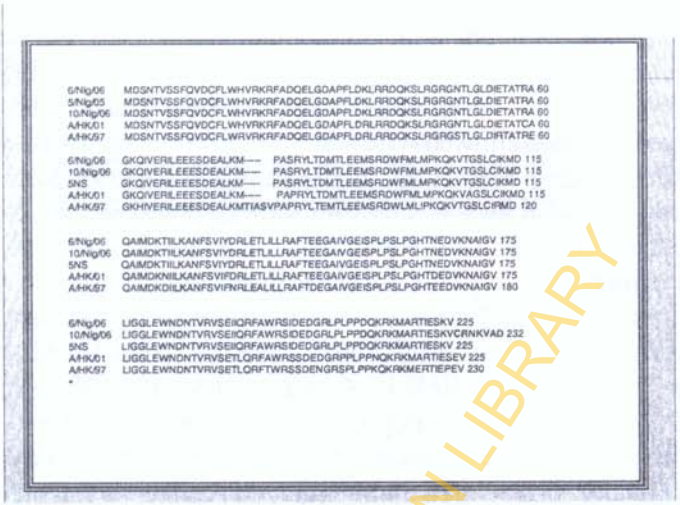


Fig. 26. Amino acid sequence of H5 NS1 protein showing the deletion and the extension

The PDZ motif in NS1 is a potential virulence determinant (Obenaeuer *et al.* 2006). Proteins that contain PDZ domains play significant roles in many cellular functions in cells infected by the deadly bird flu viruses. When introduced into humans, such viruses have the opportunity to bind and to presumably disrupt many PDZ domain protein-mediated pathways. Disruptions of these pathways at the cellular level contribute to higher death rates reported in recent outbreaks in both poultry and humans although multiple genes and gene products may be involved (Obenaeuer *et al.* 2006). But Mr. Vice-Chancellor Sir, the good news here is that this our finding reveals an entirely new means by which bird flu viruses may interact with host cell proteins which may prove valuable as targets for antiviral therapy. We only hope funds will be available to pursue this line of research in the near future. However, this 7-amino acid extension did not detectably affect the ability of NS1 of our viruses to block interferon induction when transiently expressed in 293T cells (fig. 27).

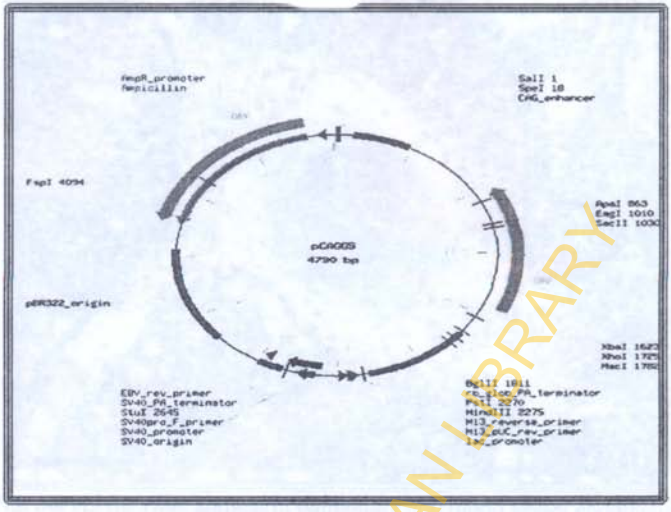


Fig. 27. Plasmid vector used in transfection studies

The (PB2) protein of the three viruses possesses a lysine residue at position 627, an amino acid previously implicated in mammalian adaptation of the deadly bird flu viruses (Aiki-Raji *et al.* 2008). However, the nature of this amino acid at position 627 does not affect the cell tropism of the virus but rather its replicative ability in birds and mammals including humans (Neuman and Kawaoka 2006). The lysine in position 627 in the present studies was probably responsible for the efficient replication of the viruses in most tissues of the affected birds as evident in our pathogenicity, virus infectivity and immmunohistochemical studies (figs. 28-30).

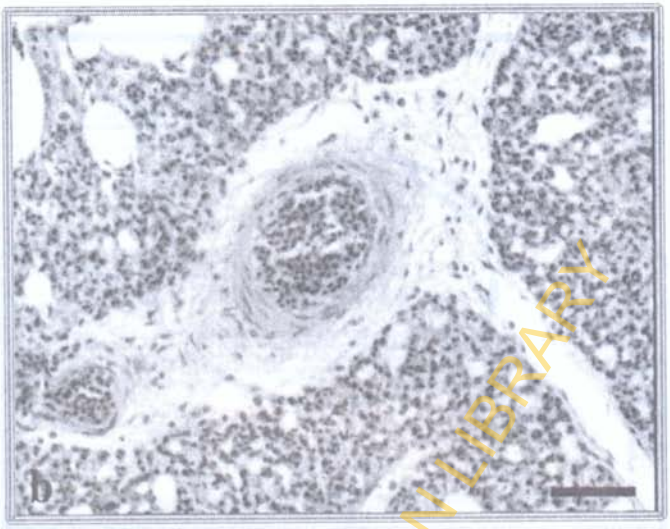


Fig. 28. Immunohistochemistry of lung epithelium showing pneumonia with edema

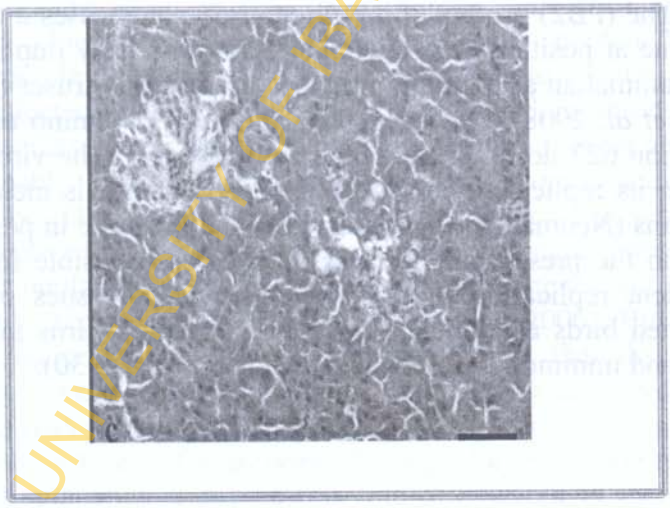


Fig. 29. Pancreatic acinar epithelium showing mild-to-moderate degeneration and necrosis

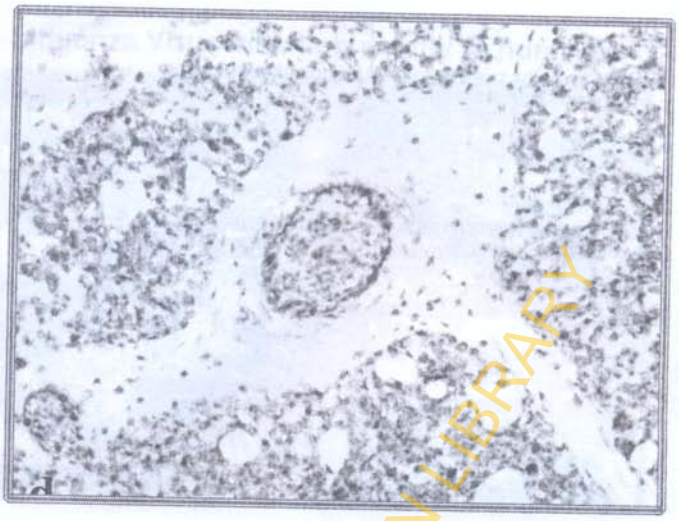


Fig. 30. H5 flu virus antigen in pulmonary and blood vessel endothelial cells

Several other findings underscore the importance of residue 627 of basic protein 2; some of the deadly viruses isolated from patients in Vietnam (Putanava *et al.* 2005) and Nigeria (Nasidi *et al.* 2007) possess lysine at position 627 which made them capable of efficient multiplication in humans and chickens.

Our three viruses also express the full length (87 amino acids) dangerous protein (PB1-F2) (fig. 31). This is a gene product known to disrupt the mitochondria permeability transition pore complex by interfering with two components of mitochondria inner and outer membranes, adenine nucleotide terminator-3 (ANT3) and voltage dependent anion channel-1 (VDAC-1) respectively, leading to increased virulence of the viruses in chickens and humans (figs. 32 and 33).

TRANSLATION OF THE PB1-F2 NUCLEOTIDE SEQUENCE FOR A/CHICKEN/NIGERIA/228-5/2006

MGQGQDTPWTQSTEHTNIQKRGSGQKTQRLEHPNSTRLMDHYLR
IMSPVVMHKQIVYWKQWLSLKNPTQGSLETRVLKRWKLFNKQEWIN

Fig. 31. Amino acid sequence of PB1- F2 protein

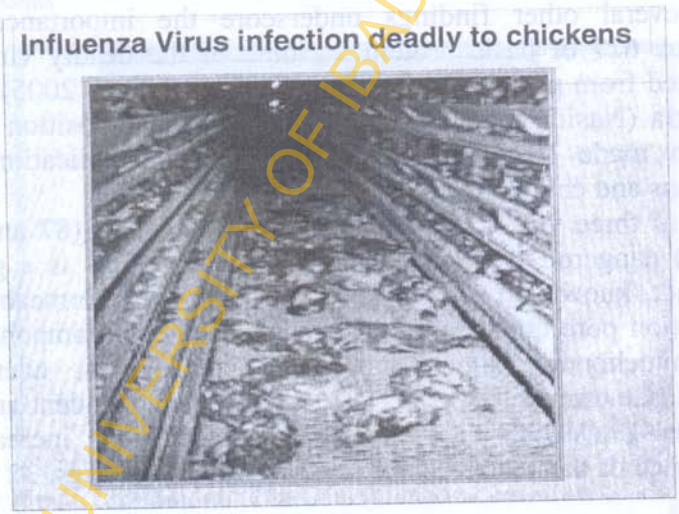


Fig. 32. High mortality due to H5 N1 flu virus

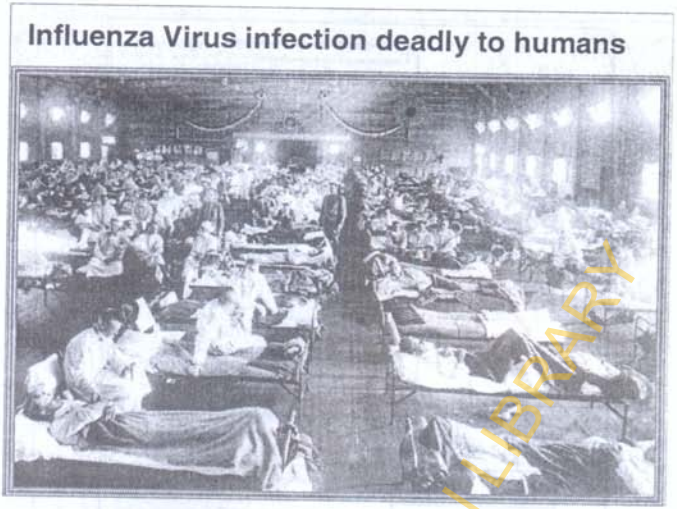


Fig. 33. High hospitalization rate in man due to pandemic H1N1 flu virus of 1918-1919

Phylogenetic analysis of the surface protein (the HA) and complete genome sequences of the deadly viruses (figs. 34 - 37) grouped our three viruses with other isolates from Europe, Middle East, Africa and other Nigerian strains.

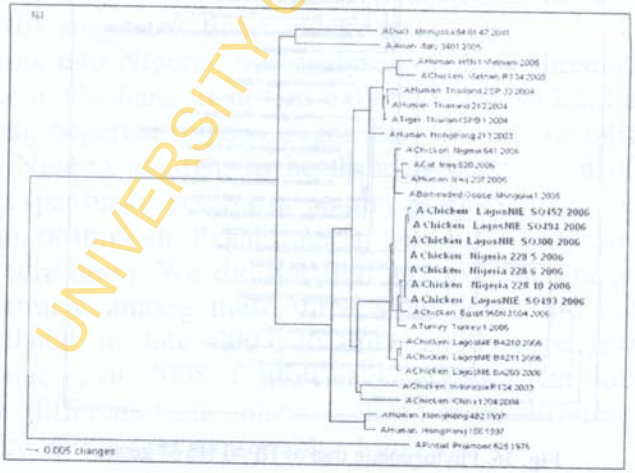


Fig. 34. Phylogenetic tree of HPAI H5 HA genes

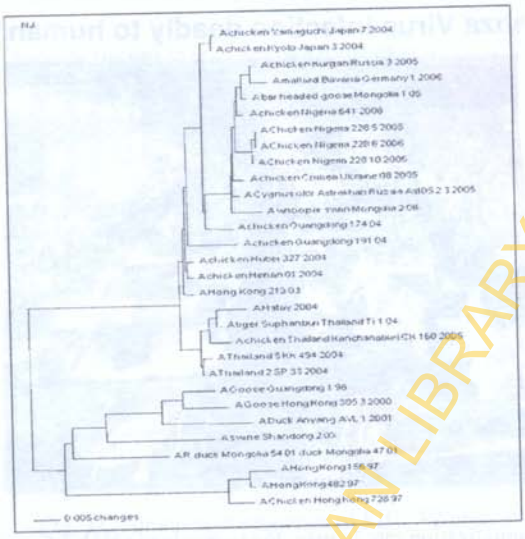


Fig. 35. Phylogenetic tree of HPAI H5 NP genes

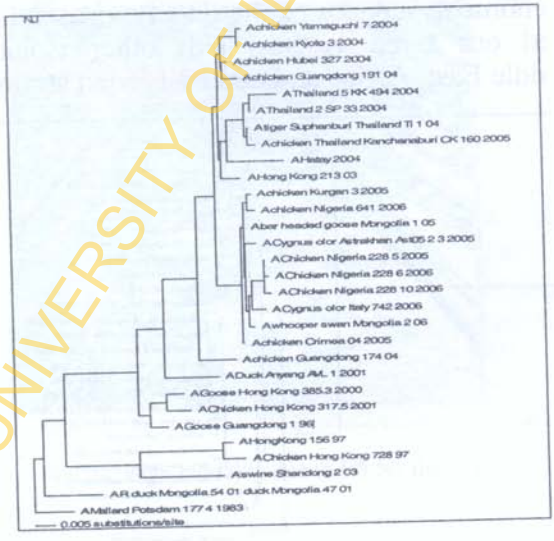


Fig. 36. Phylogenetic tree of HPAI H5 M genes

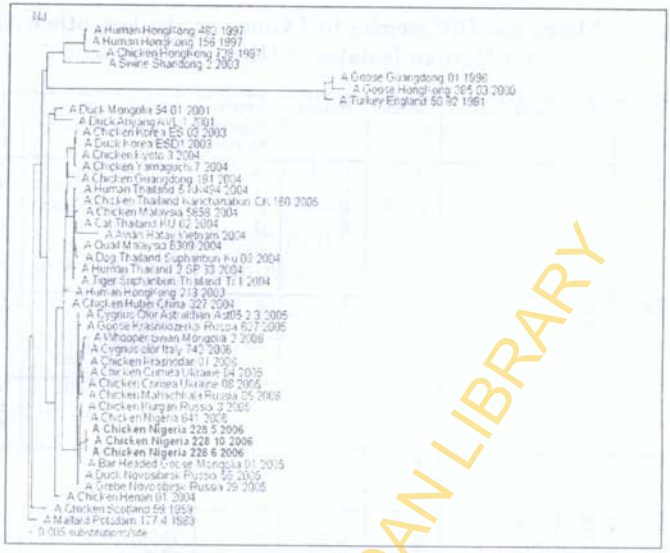


Fig. 37. Phylogenetic tree of HPAI H5 NS genes

According to a recent classification by the H5N1 Evolution Working Group, the viruses belong to clade 2.2.2 (previously referred to as European-Middle Eastern-Africa, EMA clade 1). Although previous reports (Ducatez *et al.* 2006, 2007, 2007b) suggested three independent introductions of these viruses into Nigeria, our analyses of these three strains and those in Genbank identified only two clades (2.2.2 and 2.2.3) among Nigerian isolates, suggesting two unique introductions into Nigeria, thought to be through importation of infected birds (pet birds, poultry or poultry products from Turkey into Kano, or through illegal trade in wild birds (one can never be too sure now). We did not find any evidence of genetic re-assortment among these three strains, but the viruses that circulated in late 2007 to July 2008 were re-assortants (Monne *et al.* 2008; Cattoli *et al.* 2009). The three viruses were different from others isolated from different parts of Africa in other respects (tables 1 and 2).

Table 1: Molecular Differences in 7 Genes of Ibadan, other Nigerian and Human Isolates of HPAI H5 Viruses

Protein	Amino acid position		Avian	Human	3 early Nigeria Strains	Strains analyzed by Cattoli *et al.*, 2009	Mutation In Human
PB2	661 199 627 73		A A E K	T S K R	A A K Q	Ch/Ghana/2534/07 dk/Egypt/5169-6/07 All Strains analysed by Cattoli et al., 2009 All IV Sublinage of EMA viruses	A-T A-S E-K K-E
PBI-F2	82 79		L R	S Q	L R	dk/Egypt ch/Egypt Ch/Egypt Ch/Nig/1071-3/07	L-S R-Q
PA	100 400 356		V Q/T/S K	A L R	V S K	Ch/Nig/AB 13/06 dk/Eg, Ch/Eg ch/Buk Faso	V-A S-L K-R
NP	33 109		V I	I V	I I	78/81 viruses Ch/Nig/1047-8/06 Ch/Sud/1784-8/06	V-I I-V
M2	55 27 30 31	Associated with adamantane resistance	L V A S	F A/T/E V/A/I N	L V A S	Ch/Sudan/1784-10/06 Ch/Buk Faso/13.1/06 Avian/Togo/3618-10/07 Ch/Togo/4106-1/07 Ch/Egypt/1709-5/08	L-F V-I A-S S-N
NSI	227		E	R/K	E	Ch/Nig/FA4/06	E-G
NS2	70		S	G	S	Ch/Nig/FA4/06 Ch/Nig.Fa7/06	S-G
NA	294	associated with resistance to NA inhibitors	N	S	N	A/Egypt/14725	N-S

Table 2: Molecular Changes in the HA Genes of Ibadan, other Nigerian and Human Isolates of HPAI H5 Viruses

HA	133	Deleted	Deleted	I	A/Eg, ch/Eg	S-L
	155	I	T	G	Ch/Nig./1071-10/07	I-T
	186	N	S	E	Ch/Nig/1071-22/07	N-S
	227	S	N	P	A/Egypt/2947/06	S-N
	230	M	IV	A	About 32 Egyptian strains	M-V/I

The sequence variation observed in the three viruses isolated within one to three months compared to others would not be unconnected with the high mutation rate of influenza A viruses ($7.6x10^{-5}$) as well as the transmission chain in

different poultry populations in which outbreaks were reported, and the possibility of later re-assortment which is a hallmark of influenza A viruses.

The virulence of the three viruses were also assessed by the intravenous pathogenicity test in groups of 4-week old specific pathogen free (SPF) white Leghorn chickens, in which they produced a mean death time of 1.0-1.4 days. Infectivity and pathogenicity were also determined using simulated natural respiratory route of exposure with intra-nasal inoculation with infectious allantoic fluid (10 $MEID_{50}$). All the chickens died within three days with mean death time of 2.1 days. The viruses produced a systemic infection characterized by virus replication and associated inflammatory and necrotic lesions in critical organs such as the heart, brain, lungs, gastro-intestinal tract, kidneys and gizzards. A prominent vascular tropism was evident by widespread viral replication in blood vessel endothelium throughout the body, typical of the deadly bird flu viruses of Asian lineage (fig. 38).

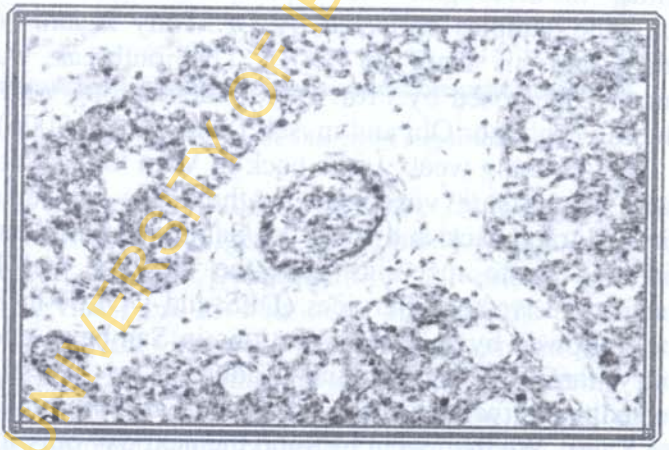


Fig. 38. Flu virus antigen in pulmonary and blood vessel endothelial cells

The nucleotide and amino acid sequences of the three viruses have been deposited at the Flu virus sequence data

base in NCBI and the Genbank under accession numbers EU697214-EU697237.

The results of our studies were presented at a number of international conferences and seminars for criticisms and possible re-evaluation as it is the practice in Pirbright before their publication. Such conferences include the 23rd World Veterinary Congress in Glasgow in July, 1993, American Society for Virology conference in Wisconsin in 1994, First International Consortium for Antivirals (ICAV), Avian Influenza conference in Abuja in May, 2007, and ViralCheck International workshop in Dakar, Senegal in June, 2008, to mention a few.

I participated actively in the January, 2006 outbreak investigations before the Federal Government of Nigeria made the announcement on 8 February, 2006. Professor Obi and I were called on 10 January, 2006 by the Executive Director of National Veterinary Research Institute (NVRI), Vom, Dr. (Mrs.) Lamin Lombin (DVM, Ibadan) to the Institute to investigate the Sambawa Farm outbreak by testing the samples collected by Dr. Tony Joannis (DVM, Ibadan) and his team in NVRI. This outbreak was first clinically diagnosed by Prof. D. F. Adene along with others including Professor Obi and myself (Adene *et al.* 2006).

The following week, I was back in Vom to lead a team of experts comprising virologists, pathologists and epidemiologists to trace back and forth the Sambawa Farm outbreak, which led us to meetings with the Director and Deputy Director of Livestock Services (DLS and DDLS) in Kaduna State, followed by sample collections in Sambawa and other poultry farms in and around Kaduna. Our investigations revealed that affected birds in Sambawa Farm originally came from Kano. We then went to Kano the next day only to arrive on poultry farms having very high death rates, culling off their live birds and slaughtering and dressing the remaining for human consumption. These birds were destined for the southeast and the southwest through Jos/Enugu and Abuja/Lokoja axes respectively. By the following week, of

course, outbreaks were reported in Plateau State and as far south as Enugu State, and in the southwest States as far as Ogun and Lagos.

Prior to these outbreak events, late in December 2005, a team of experts led by Professor Obi produced an influenza pandemic emergency preparedness plan (fig. 39).

Fig. 39. Flu virus pandemic emergency preparedness plan

This was presented to all the State DLS by the Chief Veterinary Officer of Nigeria, Dr. Junaidu Maina, who was the Director, Federal Department of Livestock and Pest Control Services, on 13 February, 2006 at the Veterinary Council of Nigeria Corporate Headquarters in Abuja. Later that day, I attended, with Professor Obi, a meeting with international donor agencies where requirements and strategies for the control of the outbreaks were mapped out. Later that night, we worked on the emergency preparedness plan with a team of experts from the African Union-International Bureau for Animal Resources (AU-IBAR) till far into the night to perfect the preparedness plan and the requirements for controlling the disease in Nigeria.

All these efforts led to a working document on an EU-sponsored, FAO- administered national active surveillance of deadly bird flu H5N1 in the entire country prepared by Professor Obi who by now had been requested by FAO office in Abuja to oversee the control efforts in the country as Team Leader. The project proposal draft, symbol OSRO/NIR/602/EC was titled "Active Avian Influenza Surveillance Study in Nigeria". I was among a select committee of five experts invited on 19 September, 2006 to vet and approve the document and its financial implications for eventual execution of the project slated to commence later that same month. For this active surveillance, an inception workshop was held in Abuja on 21 September, 2006, at the United Nations House. A training workshop on RT-PCR in the diagnosis of the deadly bird flu was held from 6-9 March, 2007, in the molecular biology lab in NVRI for virologists from the five Veterinary Schools with established Veterinary Teaching Hospitals including Ibadan, ABU, Nsukka, Maiduguri and Sokoto. I was a resource person in this training and Dr. Oluwayelu of the Dept. of Vet. Micro. & Parasit. attended this workshop.

We also held training workshops for field officers in Abuja who would distribute sampling materials, and on 20-23 November, 2006 in UI and ABU Veterinary Schools simultaneously for Federal, State and private veterinarians as field surveillance officers who would collect clinical samples from poultry farms (rural, backyard and commercial) across the country. However, because of logistics and the oncoming fasting and *Sallah* festival in 2006, the active surveillance could not take place until 23 March to 13 April, 2007, and sample analysis by RT-PCR and serological methods in NVRI, Vom until late May to mid-August, 2007. I was among the team that eventually carried out the molecular analysis in Vom in 2007 along with Dr. Oluwayelu. Dr. Omolade Oladele of the Department of Veterinary Medicine, University of Ibadan and Prof. Paul Abdu of the Department of Veterinary Medicine and Surgery, ABU, were engaged for

six months by FAO Office, Abuja, from February to July, 2007 as National consultants to assist Professor Obi in Abuja on the surveillance project, a task Dr. Oladele performed very creditably. On 12-14 August, 2007, a closing national consultative forum workshop on the active surveillance was held in Hamdala Hotel, Kaduna to present the overall results of the surveillance effort to SDLS and other stakeholders. It was largely successful as the status of these deadly bird flu viruses in Nigeria as at that time became known. We analysed 4,065 tracheal, 3,914 cloacal and 3,166 serum samples from 36 states and the FCT.

Although none of the samples tested was positive by RT-PCR or serology, we gained an experience in the use of geographic information system (GIS) and a randomized geographic coordinate sampling system (RGCCS) which were used to locate sampling sites. We also gained an insight into challenges that could militate against the success of such an endeavor. Strengthening and upgrading of the five VTH labs to a level 2 Biosafety (P2 Lab) facility was emphasized to complement the central national and regional reference lab in NVRI, Vom, The P2 laboratory is now being constructed in the Department of Veterinary Microbiology and Parasitology, University of Ibadan, a gain for the University from deadly bird flu outbreaks. Mr. Vice-Chancellor Sir, it is also noteworthy that the generality of Nigerian veterinarians successfully rose to the occasion of controlling the deadly bird flu disease in the country and veterinary scientists from the University of Ibadan took the front seats in these efforts.

This active surveillance was, however, followed by targeted surveillance in live bird markets (LBM) in October/November, 2007 in 54 markets from 26 States where outbreaks were reported. The targeted surveillance yielded some positive results with 6.5% prevalence indicating that live bird markets could be a source of outbreaks of disease. Pooled samples from Borno, Lagos, Ogun, Nasarawa, Rivers, Ekiti, Delta and Kwara States had the deadly bird flu virus. Thus, on November 10, 2008, an international consultative forum was organized at the ECOWAS Secretariat, Abuja by

Federal Department of Livestock, Federal Ministry of Agriculture, in collaboration with NVRI, FAO and USAID. I was a resource person to present Nigeria's deadly bird flu status to the international community approximately three years after the initial outbreaks with a view to sharing Nigeria's experience in the control of this deadly bird flu with the international community and preparing a "Road Map" for future prevention. The results presented indicated a framework for the preparation of a road map for the control of HPAI in Nigeria that included continuous active and passive surveillance particularly in live bird markets, backyard/rural poultry comprising indigenous stock as well as commercial poultry farms and in humans. It included bio-security, compensation, capacity building and a workable Federal Government policy implementation approach and instruments which include complete eradication of disease and infection from poultry in the shortest time possible, minimizing risk of human exposure, informed risk-based decision making, improved data collection, collation, analysis and transparency in disease reporting as well as a holistic and coordinated veterinary service delivery. Other aspects include ban on importation of poultry and their products, multi-sectorial approach to transboundry animal disease (TAD) control, public sensitization and advocacy, revamping the poultry industry and payment of compensation. This as at early November, 2008 stood at N631m (US$5.43m) paid to 3,037 farmers/farms based on total number of suspected cases (1,525), and confirmed cases (300). The last outbreaks were in Kano/Katsina states in July, 2008. States that had suspected outbreaks were 32+ FCT, and states that had confirmed cases were 25+ FCT, from 97 LGAs. Total number of birds depopulated were 1,264,911.

In 2008, my research team comprising Prof. D. F. Adu, Drs. Oyekanmi Nash, Omolade A. Oladele, and Comfort O. Aiki-Raji applied for and got the group Senate research grant SRG/COM/2008/14C of N500,000.00 for further characterization of bird flu viruses from southwestern Nigeria including, Oyo, Ogun, Lagos, Ondo, Osun, Ekiti and Kwara

states. It was used to train, mentor and supervise at least 3 Master's degree candidates (Francis Enibe, Adetola Adedeji and M. B. Ogunji) initially and currently a Ph.D student. We isolated an avian flu virus which was not the usual deadly bird flu virus from a reported outbreak of disease and high death rate in February, 2008, from a farm in Lagun village, some 20km from Ibadan on the way to Iwo in Osun State. The clinical and pathological signs as well as a rapid diagnostic test strongly suggested bird flu for which I received a panel of six deadly bird flu monoclonal antibodies from Professor Webster's laboratory in Memphis and multiplex PCR primers from Dr. John McCauley now of N.I.M.R., Mill Hill, London for our work on these viruses. These viruses were, however, unfortunately lost due to prolonged power failure in the University at the time. We worked in collaboration with NVR1 virology scientists in Vom where some of our studies were carried out and these biologicals are stored. These biologicals are now used for analysing samples collected from time to time.

Conclusion and Recommendations

Flu virus research for me has been very exciting and rewarding because of its influence on me which I had passed on to others (I hope) whom I have mentored. However, it has been fairly difficult to recruit as many students as possible at both undergraduate and postgraduate levels into molecular biology/virology research as it is perceived to be difficult because their knowledge of cell and molecular biology as well as biochemistry at the molecular level is limited and the will and interest to learn is just not there. Unfortunately too, the cost of the reagents and biologicals required is very high.

Available body of evidence indicates that there is no longer any boundary in science-based disciplines as biotechnology is the in-thing and health and disease are now viewed from the molecular perspective. It is therefore desirable that these aspects be incorporated in our curricular at undergraduate and postgraduate levels. It is anticipated that the central multidisciplinary research laboratory on campus and the proposed zonal molecular biology laboratory will

fulfill their purpose in training these students as well as junior faculty and technologists. It is heartwarming that the south-west geopolitical zonal molecular biology laboratory is also to be built in the University of Ibadan from ETF BLINT funds.

Flu viruses have a great potential for rapid global spread and infect a wide range of host species in wide geographical locations. They also cause serious economic losses in affected hosts particularly land-based poultry and increased hospitalization rate in man. The most logical thing is to prevent the disease through vaccination which is now available although because of antigenic drift occasioned by constant point mutations particularly in man, pig and horse viruses, it is advisable to constantly upgrade existing vaccines. The key to tracking the disease is through increased surveillance, both passive and active, in all host species. In the light of pandemic threats occasioned by genetic shifts through re-assortment and RNA recombinations, the Nigerian Government is advised to stockpile vaccines and antiviral drugs. It is also desirable to carry out extensive research in antiviral drug and vaccine development. The disease is treatable with such drugs as amantadine, rimantadine, zananivir, oseltamivir and ribavirine, all of which are effective.

The good news here is that it is now possible to use reverse genetics technology for artificial generation of flu viruses (fig. 40). These systems are highly efficient and they have revolutionized flu virus research in that they allow researchers to study the functions of viral proteins, their contributions to the viral life cycle, and their role in pathogenesis and host range restriction. Moreover, these systems are invaluable tools for the generation of flu virus vaccines and vaccine vectors. In fact, reverse genetics has permitted the generation of inactivated and live vaccine strains for deadly bird flu viruses of H5N1 subtype that could not have been produced by conventional approaches.

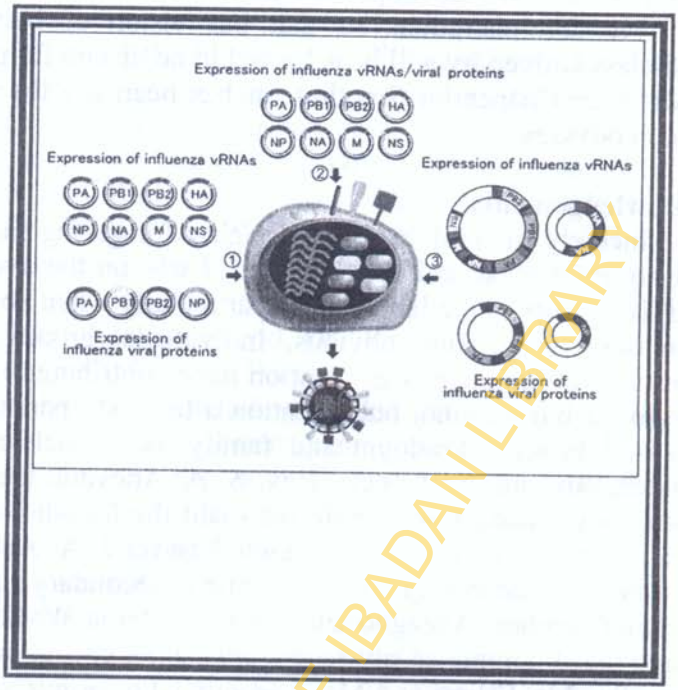


Fig. 40. Schematic diagram of flu virus reverse genetics

There should therefore be more collaboration between veterinarians and their medical counterparts in flu virus research and the concept of one health should be enthusiastically embraced by veterinary, medical, agricultural, pharmaceutical, nutritional and animal scientists to promote and produce a healthy nation.

It has been repeatedly stated in inaugural lectures here that facilities and the learning environment should improve in this University. The McArthur, ETF BLINT and other funds (Internally Generated Revenue (IGR), Capital Development Fund, Consultancy, endowment fund, gifts and grants) that accrued to the University came handy to actually transform our teaching, research and learning environment and to build capacity. We appreciate the immediate past and current vice-chancellors and their teams for this visible transformation in

our University. We do hope this trend will continue under successive administrations so that our vision of being a world-class University will be achieved in no distant future.

Mr. Vice-Chancellor Sir, thus far has been my flu virus research odyssey.

Acknowledgements

I am sincerely grateful to God Almighty for giving me the grace to get this far and do this much. I was on the average doing 18+ hours in the laboratories particularly when abroad; the reasons were quite obvious. Individuals, groups, and institutions too numerous to mention have contributed to my story so far but I cannot but mention a few: My parents Pa Eddy and Felicia Omidoyin and family, both nuclear and extended, our late great uncle, Rev. S. A. Adeyefa, the first University graduate from Ile-Ife who laid the foundation for education in the family, my late uncle Lawyer J. A. Adeyefa with whom I lived during holidays while in secondary school, my senior brother, Adeagbo and his wife Mama Wale, who ensured that I completed my secondary school education, my late uncles, Mr. Faleye and Mr. Adeogun who set my feet on the path of veterinary medicine through the Animal Health School, Moore Plantation, my cousins, Sister Adeola and her husband, Professor Fabunmi, Dr. Adeniran Adeyefa, Cannon (Dr.) Tunde Adeyeoluwa who ensured that I gained admission into our great University of Ibadan, his mother, late Mrs. Beatrice Adeyefa, who at my tender age of 10 inculcated in me that I had the potential to become somebody, Kola Omisore, Jire Abimbola, and friends from Ilesha period till date, Ayo Fatunashe, Ralph Odalume, Tokunbo Adeola who assisted me in settling down in Zik Hall on entry into UI for prelim, Banky Oke, Tunde Otesile, Kunle Fagbemi, Niyi Okewole, Femi Obasaju, Laitan Balogun, Stephen Akpavie, Muyiwa Oladimeji, Victor Taiwo (*meren meren*) and Israel Ajimoko just to mention a few.

I also sincerely thank Mr. John Omorogbe, my computer operator, and Adenike Ogunye by whom the manuscript and

slides were prepared. I acknowledge with pride and profound gratitude Dr. John W. McCauley, my mentor and friend, and members of the Flu group in Pirbright Laboratory; Professors Webster, Tomori, Obi, Oduye, and all my former teachers; members of my various research teams and my project students past and present; Drs. Totherpilly, Jacky Huges and the Mol. Biology scientists in the Biotechnology Laboratory, IITA, Ibadan; Drs. William Smith and Larbi of ILRI in IITA, Ibadan from 1993-2003; staff of Virology laboratory, College of Medicine, UCH, from 1988 till date; Prof. Mark Nwagwu, Folake Akintelure and Dr. Rose Angela Nwuba in Prof. Nwagwu's Cellular Parasitology laboratory; Ina Busby and the Skinners including Helen in Pirbright; Kunle and Tokunbo Daniel formerly in London now in Dublin; Deji Akintola and his wife in the UK, the British Council, Commonwealth, IFS and TWAS for their various contributions to my research efforts.

It was not all work and no play. We had occasional social gatherings and outings. I therefore wish to thank all my folks in Pirbright, UK and USA, all my folks in UI Senior Staff Club, *Abe Igi*, and *Spices* in *Abadina*, *Klassics*, *Cosmos* and *Metro* in *Akobo* and other places, particularly those behind the scenes, that make life worth living, home and abroad as well as everybody and every event that have contributed positively to my life, and finally to every one here present for your patience and attention.

Thank you all for listening.

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BIODATA OF PROFESSOR ADEYINKA O. ADEYEFA

Professor Christopher Adeyinka Olugbenga Adeyefa was born on 26th May, 1949 in Ile-Ife into the great Adeyefa family. He had his secondary education at Oyemekun Grammar School, Akure where he obtained his West African School Certificate in 1967. He proceeded to Animal Health School, Moore Plantation, Ibadan in October 1968 for the Veterinary Assistant Course which he passed with distinctions in June, 1970.

He gained admission into our prestigious University of Ibadan in 1971 and graduated with a DVM degree in 1977. For his undergraduate studies he won the Cocoa and Western State Bursaries in 1974. He worked in the Nigerian Institute for Tryponosomiasis Research in Kaduna from July 1977-July 1978 as a Research Fellow and then as a Veterinary Officer in Federal Department of Livestock in charge of Ogun State from August 1978-Septemebr, 1979.

Professor Adeyefa started his teaching career as Lecturer II in the Department of Veterinary Medicine from October 1979 and rose to become a Professor of Veterinary Medicine and Molecular Virology in 1997. He gained the British Overseas Development Administration Fellowship for his Master's degree in Equine Studies, at the University of Wales, Aberystwyth from September 1981-December 1982 but did his laboratory work in the Immunoparasitology Laboratory, Cambridge Veterinary School, United Kingdom. He later proceeded to the Agriculture and Food Research Council (AFRC) Institute for Animal Health, Pirbright Laboratory, United Kingdom for his training in Molecular Virology where he worked on influenza A Viruses in 1992 under the Commonwealth Academic Staff Fellowship.

He is an erudite scholar, having mentored many postgraduate and undergraduate students in the fields of Orthomyxo and Paramyxo-virus research. He has close to 50 publications in highly reputable peer-reviewed international journals to his credit and has attended many national and

international conferences, seminars and symposia in Africa, Europe and America where he presented a number of papers. He was appointed a Consultant Scientist in 2007 by FAO to work on the national samples collected from poultry during the 2006-2007 outbreaks of highly pathogenic avian influenza H5NI in Nigeria. He has served as external examiner and external assessor to many Universities. His current research focus is in the area of molecular basis of Flu virus pathogenicity and cytocine responses. His research has equally been supported over the years by internal and external funds such as University of Ibadan Senate Research grants, International Foundation for Science (IFS) and Third World Academy of Sciences (TWAS) research grants.

He has served the Faculty and the University in many capacities over the years as Member, Faculty of Veterinary Medicine Admission Committee, Chairman, Eruwa Veterinary Field Station Committee, Member, Faculty of Veterinary Medicine Board of Studies, Member, Faculty Board, Member, Department of Veterinary Medicine Postgraduate Committee and Finance Sub-committee, Acting Head Department of Veterinary Medicine and Member, University of Ibadan Senate since 1997. He is currently the Director of the Veterinary Teaching Hospital.

Professor Adeyefa is a member of many learned societies including Nigerian Veterinary Medical Association, Society for General Microbiology and American Society for Virology. He is also a Consultant to the Nigerian Polo Association. He is a registered Veterinarian.