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Molecular and Spatial-temporal Epidemiology of Highly Pathogenic Notifiable Avian Influenza (HPNAI) H5N1 in Nigeria

by

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Submitted in partial fulfillment of the requirements for the degree of Master of Science in Veterinary Science

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SUMMARY

MOLECULAR AND SPATIAL-TEMPORAL EPIDEMIOLOGY OF HIGHLY PATHOGENIC NOTIFIABLE AVIAN INFLUENZA (HPNAI) H5N1 IN NIGERIA

By

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Highly pathogenic notifiable avian influenza (HPNAI) is a disease caused by influenza A virus. It is frequently fatal in poultry. Since late 2003, disease outbreaks caused by the Asian strains of HPAI H5N1 virus have ravaged the poultry industry with the death of over 200 million birds. The epidemic has spread from Asia to Europe and more recently to Africa. To date, more than 200 human fatalities have occurred. A clear understanding of the full epidemiology of the disease at the genetic and spatial/temporal level is critical for the management, control and eventual eradication of the virus.

In this study, modern tools of molecular epidemiology (Reverse-transcriptase polymerase chain reaction (RT-PCR), molecular characterization and phylogenetic analyses), Geographical Information Systems (GIS) and remote sensing, and other epidemiological tools were used to explore the outbreak of HPNAI in Nigeria. The molecular and spatial analyses both concluded that Nigeria was infected with multiple infections. The spread of primary outbreaks, which affected mainly sectors 2 and 3 of the poultry industry as described by Food and Agricultural Organisation of the United Nations, were strongly linked to trades, live bird markets, inappropriate disposal of carcasses and poorly implemented control measures.

This work did not find a strong correlation between wild birds and HPNAI H5N1 in Nigeria. Some of the analyzed viruses showed genetic drift, and the implications of these for future epidemiology and ecology of avian influenza in Africa will need further evaluation. The

option of vaccination and its implications were adjudged good, and its shortcomings were highlighted. Community initiative at fighting emergency diseases like HPNAI H5N1 was similarly advocated.

The financial losses to the Nigerian poultry industry were estimated at around $680 million. The risk of the spread of infection was assessed using ecological niche modeling and the whole of West Africa is at risk of infection, should no concrete action be taken to halt the spread.

In conclusion, useful suggestions were proffered to affected countries like Nigeria, and unaffected countries that are at risk of infection, so that Africa can be safe from the scourge of HPNAI H5N1.

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"Praise to the Lord, who doth prosper thy work and defend thee;
Surely His goodness and mercy here daily attend thee:
Ponder anew, what the Almighty can do
If with His love he befriend thee"

-Joachim Neander (1650-1680)

Scientific publications arising from this Dissertation

1. **Fasina, F. O**., Joannis, T. M., Abolnik, C., McCrindle, C. M. E. and Bisschop, S. P. R. (2006). Community Measure: A Key to Highly Pathogenic Avian Influenza Control in Developing Nations. *International Journal of Applied Research in Veterinary Medicine*, **4:** 249-254
2. **Fasina, F. O**., Sirdar, M. M. and Bisschop, S. P. R. (2008) The Financial Cost Implications of HPNAI in Nigeria. *Onderstepport Journal of Veterinary Research* , **75**: 39-46
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4. **Fasina, F. O**., Bisschop, S. P. R. and Meseko, C. A. Avian Influenza Risk Perception among Nigerian Farmers and Farm Workers. *Emerging Infectious Diseases*. (Manuscript submitted and under review)
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6. **Fasina, F. O**., Bisschop, S. P. R. and Webster, R. G. Avian Influenza H5N1 in Africa: An Epidemiological Twist. *The Lancet Infectious Diseases.* **7:** 696-697.

Conference Proceedings

1. Highly Pathogenic Avian Influenza H5N1: The African (Nigeria) Experience. *3rd International Conference of the Society for Zoonotic Ecology and Epidemiology, Kalmar, Sweden. 03rd-05th May, 2006* (Oral Presentation)
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3. Poultry Production in Nigeria and Avian Influenza H5N1: An Epidemiological Analysis. *World Veterinary Poultry Association Congress. Beijing, China. 10th-15th September, 2007* (Oral Presentation)
4. Avian Influenza H5N1 in Africa: Infection, Spread and Potential Risks to the Consuming Public. *Vet Africa 2007 Congress, Johannesburg, South Africa. 27th-28th July, 2007* (Oral Presentation).
5. Diffusion patterns of the 2006 Nigerian Avian Influenza epidemic. *GIS Vet 2007 Conference*, *Denmark. 20th-21st August, 2007* (Oral Presentation).

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LIST OF ABBREVIATIONS

% : Percentage

^{O}C : Degree centigrade

AIHAF: Forward primer for avian influenza haemagglutinin gene

AIHAR : Reverse primer for avian influenza haemagglutinin gene

AIHAMid : Mid primer for avian influenza haemagglutinin gene

AIV : Avian influenza virus

BCR : Benefit cost ratio

CBA : Cost benefit analysis

cDNA : Complementary Deoxy ribonucleic acid

CIDRAP : Centre for Infectious Disease Research and Policy

CLUSTAL W : Multiple sequence alignment programme for DNA or protein

DEFRA : Department of Environment, Food and Rural Affairs

DFID : UK Department for International Development

DIVA : Differentiating infected from vaccinated animal

dNTP : Deoxynucleotide

DOC : Day-old-chicks

DTA : Decision tree analysis

ECTAD : The Emergency Centre for Trans-boundary Animal Diseases

EEC : European Economic Commission

ELISA : Enzyme-linked immunosorbent assay

EMBL : European Molecular Biology Laboratory

EMPRES : Emergency Prevention System for Transboundary Animals and Plant Pest Diseases

ESRI : Environmental Systems Research Institute

EU : European Union

FAO : Food and Agricultural Organisation of the United Nations

FAOSTAT : Food and Agricultural Organisation Statistical database

F_B : Future benefit

F_C : Future cost

FMA&RD : Federal Ministry of Agriculture and Rural Development

GDP : Gross domestic product

GIS : Geographical information system

GLIPHA : Global Livestock Production and Health Atlas

HA : Haemagglutinin

HA_0 : Haemagglutinin protein precursor
HI : Haemagglutination-inhibition test
HPAI H5N1 : Highly pathogenic avian influenza subtype H5N1
HPNAI : Highly pathogenic notifiable avian influenza
IBA : Important bird area
INFOSAN : The International Food Safety Authorities Network
IU : International unit
IVPI : Intra-venous pathogenicity index
LBM : Live bird market
LGA : Local government area
LPAI : Low pathogenic avian influenza
M1 : Matrix protein 1
M2 : Matrix protein 2
MEGA : Molecular evolutionary genetic analysis
M.MLV.RT : Moloney Murine Leukemia Virus Reverse Transcriptase
NA : Neuraminidase
NAI : Notifiable avian influenza
NAMRU : Naval Medical Research Unit
NCBI : The National Centre for Biotechnology Information
NS1 : Non-structural protein 1
NS2 : Non-structural protein 2
NVRI : National Veterinary Research Institute
OIE : World Organisation for Animal Health
PA : Protease
PAGE : Poly-acrylamide gel electrophoresis
PB2 : Polymerase B2
PB1 : Polymerase B1
PCR : Polymerase chain reaction
pMol : pico Mol
PSGA : Penicillin, streptomycin, gentamycin, amphotericin B combination
PV_B : Present value of benefit
PV_C : Present value of cost
RFLP : Restriction fragment length polymorphism
RNA : Ribonucleic acid
R_0 : Reproduction number

RT-PCR : Reverse transcriptase polymerase chain reaction
SAP : Structural Adjustment Programme
SPF : Specific pathogen free
SW Nigeria : South West Nigeria
U : Unit
UNDP : United Nations Development Programme
UK : United Kingdom
UNICEF : United Nations Children's Funds
USD : United States Dollars
USAID : United States Agency for International Development
USDA : United States Department of Agriculture
V : Volt
VRD : Viral Research Department
WHO : World Health Organisation

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CHAPTER ONE

LITERATURE REVIEW

AVIAN INFLUENZA

INFLUENZA {in"floo-en'zə}

Acute viral infection of the respiratory tract

Originated from the Latin word *influentia*, "to flow into"

Italian *influenza* referred to any disease outbreak thought to be influenced by stars.

In 1743, Italian *influenza di catarro* ("epidemic of catarrh") spread as an epidemic across Europe and the disease was subsequently named "influenza"

Past outbreaks have been recorded in 1889 (severe), 1899 (unrecognized), 1918 (Spanish flu), 1957 (Asian flu), 1968 (Hong Kong flu), 1977 (Russian flu) and currently 1988 (Avian flu) with varied fatalities.

Sources: Dorland's Illustrated Medical Dictionary. 30th ed. Philadephia:Saunders, 2003,
Merriam Webster Online Dictionary, {cited March 21, 2007}
Quinion M. World Wide Words. {cited Feb 27, 2007}
and Emerging Infectious Diseases, 12(1); 179

GENERAL INTRODUCTION

1.1 AFRICA AND FOOD DEMAND

The population of Africa is growing at an unprecedented rate. It currently has the highest annual population increase of any continent at 3.1% per annum. (Bulatao, Bos, Stephens and Vu, 1990) (Table 1.1). Africa's urban population is expected to reach almost 55% of the total by the year 2025 (United Nations, 1985) (Table 1.2). The rapid growth in sub- Saharan Africa's population is expected to continue to increase in the foreseeable future. This population growth and increasing urbanization puts ever-increasing pressure on the agricultural industry, since some additional 500 million people will have to be fed (especially food of animal origin), clothed, housed and educated with the declining finite land resources by the year 2025.

Table 1.1. Population Projection for sub-Saharan Africa, 1990-2025.

Year	Population (millions)	Annual Growth Rate(% 5-Year period)
1990	498	--
1995	580	3.1
2000	676	3.1
2005	784	3.0
2010	902	2.8
2015	1,028	2.6
2020	1,159	2.4
2025	1,294	2.2

Source: Derived from Bulatao *et al.*. 1990.

Table 1. 2. Urban Population as a Percent of Total, sub-Saharan Africa, 1960-2025.

Year	Urban (%)
1960	11.8
1965	13.7
1970	15.9
1975	18.8
1980	22.0
1985	25.4
1990	29.0
2000	36.6
2010	43.5
2025	54.2

Source: United Nations, 1985.

Urbanization particularly affects agriculture negatively in that less land becomes available and the pattern of food demand changes for people that produce little or none of their own food (city dwellers), have higher incomes and have tendencies to eat more animal protein.

Much of the African population lives in rural areas and engages mainly in farming. Agriculture contributes about 32% of the total GDP in Africa (Food and Agricultural Organisation Statistical Website (FAOSTAT), 2005). 25% of the contribution by the agricultural sector is of animal origin; excluding the non-monitised contributions. The African continent may therefore face a serious/massive deficit in livestock and livestock products needed to meet the expanding population and growing urbanisation by 2025 if the agricultural industry fails to develop faster than it is presently doing (Pritchard, Doyle, Fitzhugh, de Haan, Lynam, MacGillivray, Masiga, Peberdy, Sawadogo and Tacher, 1992).

Studies conducted by Gollin (1991) have shown that to meet the increasing demand for livestock and livestock products, efforts will need to be concentrated to develop the poultry and piggery sectors of the livestock production sector. This is in view of the fact that while ruminant population growth is rather inflexible considering the agro-ecological zones, maximum carrying capacities and pipeline technology, poultry and pig production are more responsive to demand (Pritchard *et al.*, 1992). Thus, poultry should be considered as a commodity to be rapidly developed.

1.2 POULTRY

Poultry is the class of fowl (birds) domesticated by man over the ages and reared for eggs, meat or feathers; and is principally of the order *Galliformes* (such as chickens and turkeys, guinea fowls and quails), *Anseriformes* (waterfowls, ducks and geese). Others birds whose definition as poultry is not yet generally acceptable include ostriches, pheasants, squab (pigeons) and other domesticated wild avian species. They are unarguably the most acceptable form of livestock; and are kept by all races throughout the world. They are especially kept for nutrition (eggs and meat) for the household, for socio-cultural and religious purposes and for the generation of income to meet other everyday needs of the family (Law and Payne, 1996). Mack, Hoffmann and Otte (2005), stated that poultry is the fastest growing component of global meat production, consumption and trade apart from opening up opportunities for export, increasing demand for feed and other inputs and investment options for the downstream sectors. The economic significance of poultry varies among the poultry producing countries of the world; it is enormous in

the developing countries where a larger percentage of the populations are basically rural and agrarian. In recent times in developing nations, poultry production as a socio-economic activity is moving from a mere subsistence form of agriculture to taking a more commercial oriented approach (FAOSTAT, 2005).

The increasing population pressure challenges for the developing economies to move from the era of food aid (a situation where developed nations dump their excess food annually on the developing countries as a form of assistance) to the era of food security (where every country is encouraged to use and develop its indigenous technologies and use its resources to provide good, nutritious and satisfying food for its populace in an environmentally sound and socially just way), should further justify the need for poultry industries to be encouraged. Similarly, the increasing need for every country to build capacity in poultry production cannot be over emphasized, since the challenges of urbanization come with less availability of land for livestock production. Poultry as a form of agriculture need little land, is highly prolific and has short life-cycles as opposed to most other farm animals that need large grazing areas.

The need for Africa to develop more peri-urban commercial livestock production centres to meet the above stated challenges of the future and the need to meet the increasing consumption of white meat also makes poultry development suitable, as it is well adapted to peri-urban, urban as well as the rural communities. Unfortunately, poultry production systems, especially in Africa, are faced with myriad of challenges, key amongst them are livestock diseases. As at 1990, livestock diseases alone accounted for an estimated annual loss of about $2 billion in direct losses (mortality) and another $2 billion in indirect losses (slow growth, lower productivity, increase morbidity, and lower fertility etc) in sub Saharan Africa (De Haan and Bekure, 1991). The effects of disease conditions are more severe in developing economies where modern technologies, advanced vaccines and medicaments and sound management practices are rarely available. Despite all the effort engaged to control or manage disease entities, viral diseases still present serious risks to commercial poultry production, since they remain almost impossible to treat, and strict biosecurity measures that can reduce their entrenchment are rarely compatible with poultry production in Africa.

Examples of viral diseases of poultry include amongst others Newcastle disease, Infectious Bursal (Gumboro) Disease, Infectious Bronchitis, Infectious Laryngotracheitis, Egg Drop Syndrome and Avian Influenza. These have varied degrees of endemicity within and between different geographical localities in Africa (Adene and Oguntade, 2006). Currently, the Asian

strains of Highly Pathogenic Avian Influenza (HPAI) H5N1 virus are ravaging the poultry industry. The disease has affected Asia, Europe, parts of the Middle East and Africa, causing the death of hundreds of millions of poultry and many migratory birds (Enserink, 2006).

1.3 THE NIGERIAN POULTRY INDUSTRY

The agricultural industry in general (particularly poultry) is second only to the oil industry in importance in the national economy (Ducatez, Olinger, Owoade, de Landtsheer, Ameerlaan, Niesters, Osterhaus, Fouchier and Muller, 2006). Poultry represents a major economic source of income in Nigeria. It provided 4.45% of the total animal contribution to agricultural gross domestic products (GDP) in 2004 (Central Bank of Nigeria, 2004). It outnumbers all other forms of livestock in Nigeria and is found throughout the country (Bourn, Wint, Blench and Wooley, 1992). It is the form of livestock that the poorest of the poor have within their households in Nigeria. The estimated poultry population in the country stood at over 150 million as at 2005 with about 60% in the backyard and rural sector (Food and Agricultural Organisation of the United Nations, 2006). The majority of the birds raised on commercial basis are from the southern states (Bourn *et al.,* 1992, Adene and Oguntade, 2006, Obayelu, 2007) and this was similarly represented in the poultry distribution within Nigeria (Fig. 1.1).

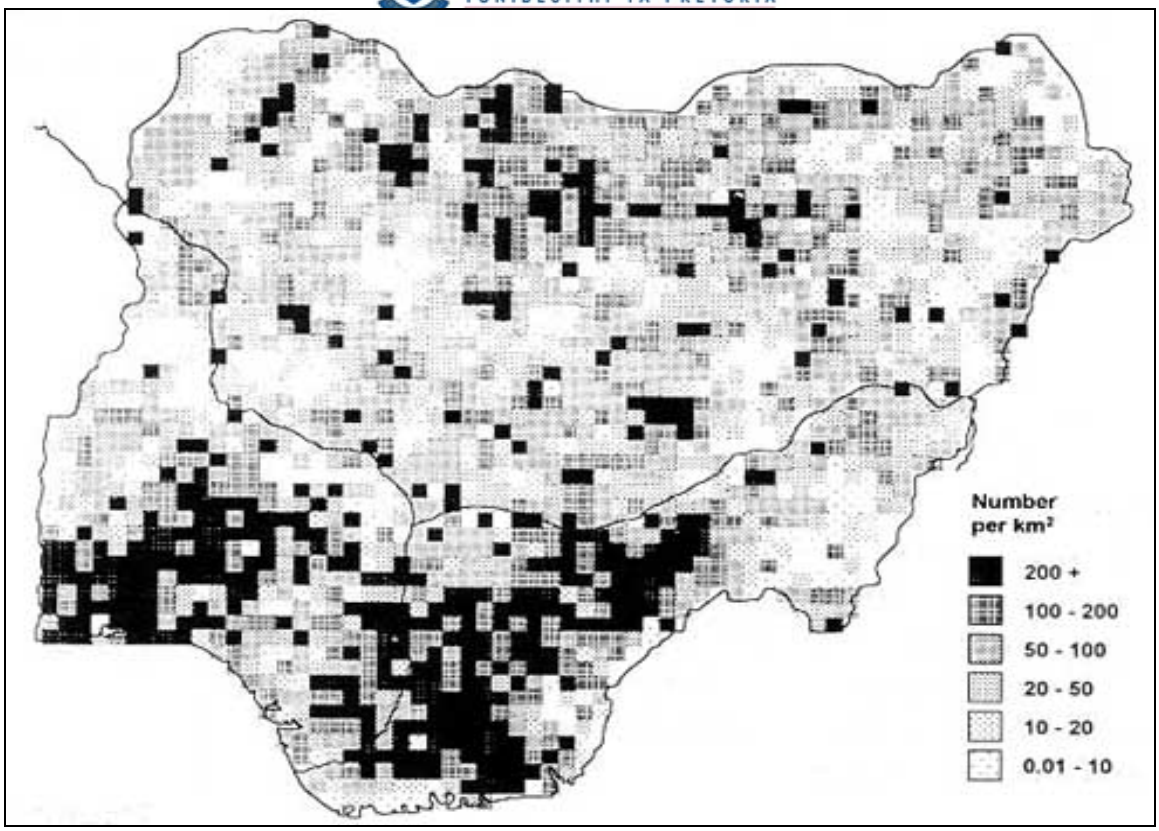


Figure 1.1. Poultry Density (poultry heads) and Distribution in Nigeria, 1992 ; *Source: Bourn et al., 1992*

The industry was solely Regional Government-driven in the early 1960s with the Western region taking the first initiative. The involvement of private investors in the industry started around late 1960s to early 1970s. This advent of private commercial poultry production marked a turning point for the industry as it rapidly grew from over 37 million in 1961 to almost 80 million as at 1980 (FAO, 2006). The early 1980s were also a boom period for the industry since the government subsidized training, inputs including day old chicks, vaccines, feed and diagnostic services. The gains associated with the petroleum economy also positively influenced the poultry industry at this time. In late 1980s, the federal government introduced the Structural Adjustment Programme (SAP) with the intention of boosting the agricultural and industrial sectors. While this programme was launched with these good intentions, it resulted in a period of downward trend for the poultry industry since subsidy removal and a ban on importation of some items negatively affected the industry (Adene and Oguntade, 2006).

Over the past two decades, the poultry farming industry is again experiencing growth due to the current regime's effort at encouraging the citizenry to invest in the industry. Several economic and

agricultural policy reforms aimed at boosting local production, especially the ban on importation of commercial day old chicks, eggs and frozen chickens/poultry and removal of import duties on agricultural products in Nigeria (United Nations Development Programme, 2006).

1.3.1 Sectors of the Poultry Industry

The poultry industry in Nigeria can be broadly classified into four sectors as described by the Food and Agricultural Organisation of the United Nations (2004). Sector 1 comprises of Industrial and Integrated farms which own the nation's grandparent and parent stock birds. This category of elite farms is in the minority in Nigeria with high level of strict biosecurity, and are fully commercialised. The hatcheries and grand parent/parent farms often also have commercial arms of their operations that raise hundreds of thousand of commercial birds.

Sectors 2 and 3 of the poultry industry usually have high to moderate and low to minimal level of biosecurity respectively. They are involved in commercial egg and meat production and are differentiated principally based on size of operations. While the sector 2 typically contains between 1000 and 4999 and sometimes far more chickens, sector 3 usually has between a few hundred to 999 birds. This sector 3 is particularly important in that it may ultimately lead to commercial operation and it serves as a means of assisting the rural and urban poor to get out of the cycle of poverty (Dolberg, 2001).

Sector 4 of the Nigerian poultry industry comprises the village and indigenous poultry stock. They are by far the largest population and the least productive of all the sectors. They are kept principally by the urban and rural poor and consist of a few to tens of poultry (often multi species) birds. Previous works have suggested that sectors 3 and 4 of the poultry industry stand a higher risk of infection by avian influenza viruses (Rushton, Viscarra, Guerne-Bleich and McLeod, 2005).

While it may be difficult to place a clear cut distinction on the different sectoral categorization since they transit from one to the other, it is important to know that of the around 150.68 million birds in 2005, approximately 60% belonged to the rural to backyard operations, 25% to the commercial and 15% to the group between backyard and commercial operations.

1.3.2 Poultry Distribution and Spread: Implication for Avian Diseases

The distribution of poultry in Nigeria follows a pattern that existed pre-independence. The majority (over 65%) of the commercial poultry are found in the South Western states of Lagos, Ogun, Oyo, Osun and Ondo (Adene and Oguntade, 2006). The remaining 35% is shared largely between the South-South, South-East and the Northern parts of the country. Similarly, eight of the nine parent stock facilities are found in the southern part of the country. In contrast, a larger number of the indigenous/rural poultry, pigeons, ducks and guinea fowls are found in Northern Nigeria. While live rural poultry and their products are preferred as special delicacies and moved southward, the exotic birds and their products move up north especially towards the Federal Capital Territory and other major cities to be processed and packaged.

In the same vein, the evolution of larger communities (mega-cities) and cities had led to the development of many peri-urban poultry farms and live bird/poultry markets (LBM) as evident in large cities such as Kano, Kaduna, Lagos, Jos, Ibadan, Abuja and Port Harcourt, among others. Such farms are closely grouped together and poultry birds of all species and from various sources are traded in the LBMs. These practices thus created an opportunity for many medium to large epizootiologic units, especially in the urban centres of Nigeria.

1.4 AVIAN INFLUENZA IN NIGERIA

Nigeria and seven other African countries viz. Sudan, Niger, Egypt, Cameroon, Burkina Faso, Cote d'Ivore and Djibouti; had reported outbreaks of H5N1 virus in 2006 (Enserick, 2006, Joannis, Lombin, de Benedictis, Cattoli and Capua, 2006, FAO, 2006a, Emergency Centre for Trans-boundary Animal Diseases, 2006). While the outbreak situations appeared to be localized to the areas of original introduction in other African countries, in Egypt (with the greatest number of human fatalities associated with the disease in Africa, n=15) and Nigeria, outbreaks appeared to be more refractory. The spread of the disease is also relatively fast within Nigeria and Egypt. Nigeria reported outbreaks in a number of poultry species including layers/pullets, broilers, breeders, Guinea fowl, ducks, geese, ostriches, turkeys, pigeons and wild birds, with varied clinical signs (Fig. 1.4 & 1.5, pages 33 & 34). Over 760 farms/premises were infected involving over 945 862 birds and spanning more than 51 local government areas (Fig. 1.2 & 1.3, pages 28 & 29) in the first twelve months after the disease's entry into the country (10th January, 2006 - 24th January, 2007) (ProMed, 2007, Nigerian Government, 2006, www.worldpoultry.net/news/id2205 12011/**bird**_flu_resurfaces_in_kano_nigeria.html).

Circumstantial evidence indicated that the disease may have been more widespread initially than reported. Poultry types affected include.

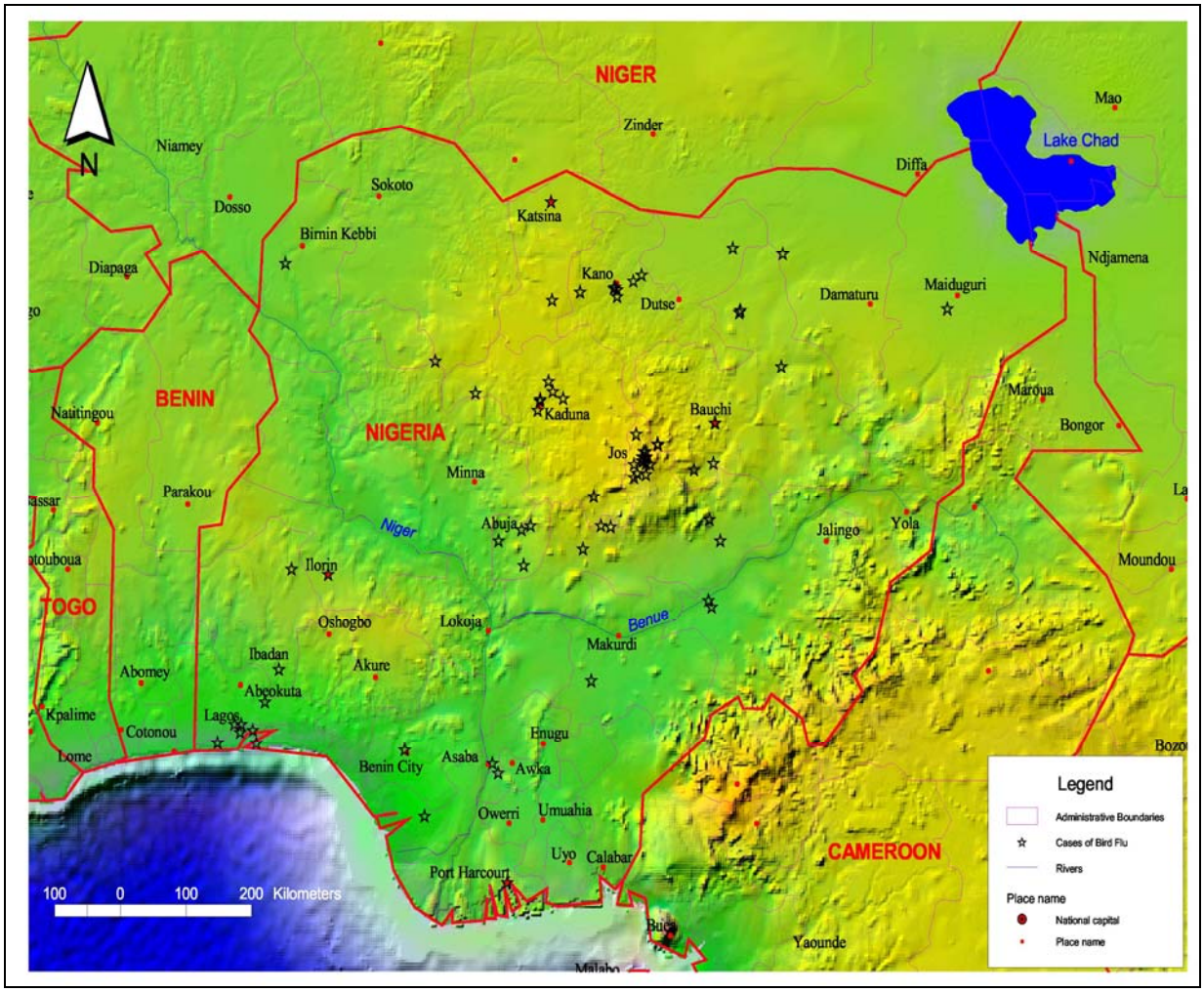


Figure 1.2. HPAI H5N1 Outbreak locations in Nigeria (January 2006-05 January, 2007)

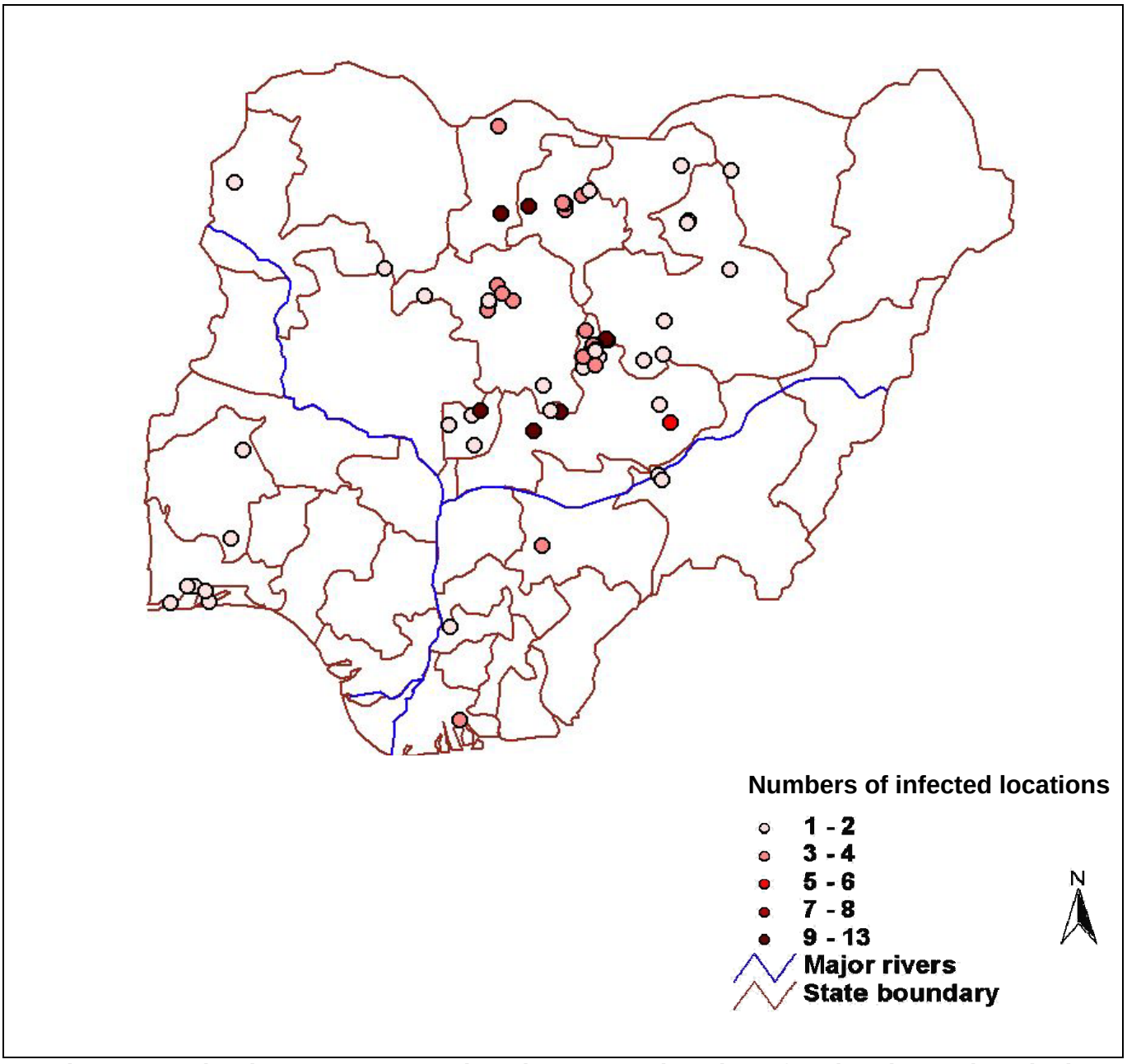


Figure 1.3. Nigerian HPAI H5N1 Epizootics In 2006 (number of outbreaks per location)

1.4.1 Public Reactions

The announcement of confirmation of the avian influenza H5N1 outbreak on 8th Feb., 2006 marked a significant point in history for the Nigerian poultry industry. Prior to this period, poultry meat was generally accepted as a luxury and occasional food item. Eggs were sometimes but not regularly eaten as a staple food items. These patterns of food consumption could not be associated with poverty alone as beef and other forms of red meat were generally regarded as regular meat. In view of the above, there was usually an increase in production of broiler chickens around the festive periods but eggs were regularly marketable with the exception of a few months that coincided with the annual laying season of guinea fowl. These patterns were beginning to experience a major shift with the establishment of more fast food outlets especially in the urban centres and poultry was becoming more regular on the table of average Nigerians (Adene and Oguntade, 2006).

With the HPAI announcement, the public became scared of eating infected meat and poultry eggs. Many individuals shifted their preference in favour of red meat and fish and this was evident in the sudden price changes that followed this outbreak report (Table 1.3). Small-scale operators from sectors 2, 3 and 4 who were afraid of losing their investment either disposed of their stock or reduced the size of their operations. This action had a major effect on the distribution of infections to new locations especially in situations where a farm was infected. Hatcheries faced an immense problem as bookings were not collected as scheduled, leading to the destruction of millions of day-old-chicks. Feed millers were forced to either close operations or reduce the size drastically. Larger farms were severely affected as there was a huge glut of eggs and poultry products. The worst affected individuals appear to have been the workers in the industry as most farmers either reduced staff or dramatically cut wages (UNDP, 2006).

Table 1.3. Percentage Prices of selected food items before, during and after Avian Influenza outbreaks.

Items	Before the outbreak	During the outbreak	After the outbreak
Pullet (DOC)	100%	50%	141.6%
Broiler (DOC)	100%	37.5%	250%
Chicken Eggs/Tray	100%	37.5-0%	155%
Staple Food items (yam, rice, beans, garri) excluding maize	100%	100.02-125%	100-122%
Maize (Corn)	100%	50%	75%
Beef	100%	171.4%	143%
Pork	100%	133%	133%
Fish	100%	285%	200%
Goat meat	100%	216%	150%
Broiler chicken (Whole)	100%	25%	120%
Spent layer hens	100%	35-0%	120%

* DOC = Day-old chick

Considering the prices before the outbreak to be the standard, all percentage prices were compared to prices before the outbreak. “During the outbreak” was taken as a period when HPAI outbreaks were at their peak in Nigeria in April, 2006 and “After the outbreak” was taken in October, 2006 when the report of outbreaks was minimal.

1.4.2 Socio-Economic Impact

The disease caused great socio-economic losses, compromised food security, major production losses (eggs and meat) and restriction of opportunities for upgrading the production potential of local livestock. It also added significantly to the cost of livestock production since costly disease control measures were applied, these seriously disrupted and inhibited trade in livestock and livestock products and caused major public health concerns (FAO, 2002). Individuals and communities directly impacted included but was not limited to poultry farmers (infected and un-infected farms), product sellers, input suppliers, feed millers, labour and the general public who had to pay higher prices for alternative food items. Of particular note were the affected rural farmers who lost all their birds but were not compensated since no birds were culled from such communities. It should be noted that compensation was paid for culled birds only.

1.4.2 Probable Introduction and Spread

The disease outbreak was suspected to have occurred as a result of much interplay of factors including legal and illegal importations of poultry and its products, intra-national poultry trades and movements, wild birds, poor surveillance and other related activities. Ducatez *et al.*, (2006) concluded that possibly three different strains of the virus were introduced into the country following the migratory pathway of wild birds, but did not clearly highlight the role of trade imports. Their study was restricted to the south-west region of the country that accounted for less than 10% of all outbreaks.

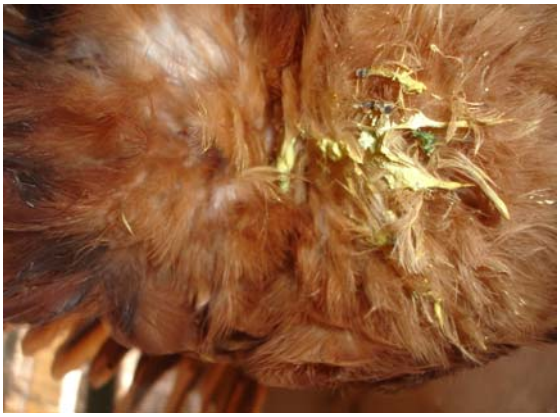
It is widely believed that individuals imported chickens from other countries including previously affected countries to meet the increasing demand for day old chicks in Nigeria. However, no due regard was given to biosecurity measures and this put undue pressure on the inadequate quarantine system in the country, possibly contributing to the outbreaks. It should be noted that the private veterinary sector in the country is more involved with drug supply chains and small animal clinical aspects of veterinary practice and plays virtually no role in the regulatory activities and control of infectious diseases in livestock (Emergency Centre for Trans-boundary Animal Diseases (ECTAD), 2006). There is therefore a need to more thoroughly investigate and verify the possible role of human activities, poultry movement within the country, trade imports and migratory birds in the epidemiology of highly pathogenic avian influenza H5N1 in Nigeria. Ducatez *et al.,* (2006) further inferred that slight nucleotides changes occurred amongst the 2 Lagos isolates (BA and SO) and the northern Nigerian isolates. They concluded through the use of genetic analysis that the isolates-BA, SO and Northern Nigeria were closely related to Astrakhan, Egyptian and Kurgan isolates (Ducatez *et al.*, 2006). This raised the question of the role of wild bird and trade in the outbreaks in Nigeria.

Although the government implemented different eradication and control measures to combat the continuing spread of the virus in the Nigerian poultry industry (Fig.1.6 & 1.7, page 35), the country continued to report outbreaks and at the time of writing, outbreaks were still spreading to new locations within the country. The majority of the affected farms to date belong to sectors 2 and 3 of the poultry industry.

Figure 1.4. High and rapid mortality due to Avian influenza H5N1 outbreaks in Nigeria, 2006

Clinico-Pathological Signs

A



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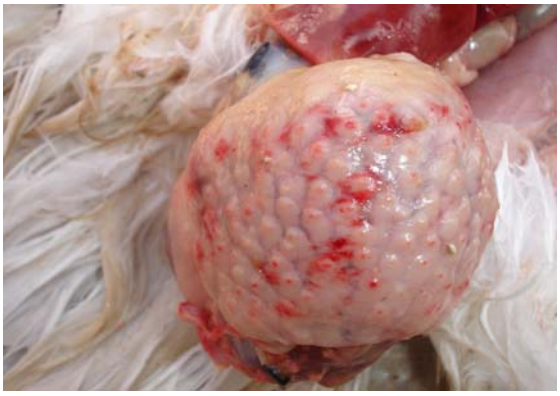

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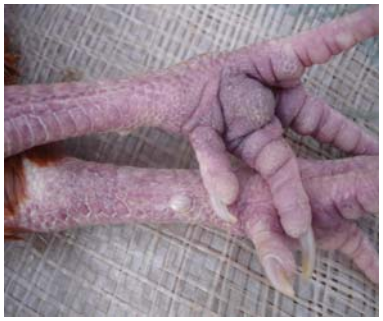


G

Figure 1.5: A. Prostrated bird with opisthotonus; B. Greenish white pasting of vent; C. Proventricular haemorrhages; D. Severe haemorrhagic tracheitis; E. Oophoritis; F. Cyanotic shriveled comb and wattles with opacity of the eyelids; G. Reddened feet and shanks

Figure 1.6. Control Effort (Policemen wearing protective clothing preparing to shoot infected ostriches)

A B

Figure 1.7. A. Removal of dead chickens for deep burial within the infected farm;
B. Preparation of dead infected chickens for burning and burial

1.5 AVIAN INFLUENZA

1.5.1 Introduction

Avian influenza viruses are known to cause two diseases on the basis of severity of the clinical signs in susceptible species: Highly Pathogenic Avian Influenza (HPAI) and Low Pathogenicity Avian Influenza (LPAI) (Kawaoka, Bean and Webster, 1987).
While HPAI is devastating with a short course, severe economic loss and a high potential to cause human pandemic (Claas, Osterhaus, van Beek, de Jong, Rimmelzwaan, Senne, Krauss, Shortridge and Webster, 1998; Webster, Peiris, Chen and Guan, 2006; Webster, 2004), LPAI is milder causing less severe economic losses with a longer course. However, some LPAI viruses, and in particular the H5 and H7 subtypes, may have the potential to mutate and become HPAI viruses (Kawaoka *et al.*, 1987; Eckroade and Silverman-Bachin, 1987; Campos-Lopez, Rivera-Cruz and Irastorza-Enrich, 1996).

1.5.2 Notifiable Avian Influenza (NAI)

The recent update (8th July, 2005) of the World Organisation for Animal Health *(OIE)* Manual of Diagnostic Tests and Vaccines for Terrestrial animals defined notifiable avian influenza (NAI) as all influenza A viruses of the H5 and H7 subtypes that are highly pathogenic for domestic poultry (HPNAI). However, because of the risk of H5 and H7 viruses of low pathogenicity becoming virulent by mutation in poultry hosts, all H5 and H7 viruses have been classified as NAI viruses (OIE, 2005).

The Terrestrial animal health code (OIE, 2006c) also described HPNAI viruses as all Influenza A H5 and H7 subtypes, as well as all other AI virus with an IVPI of greater that 1.2 (or causing death of at least 75% of 4-8 week old susceptible birds within 8 days). At the molecular level, all H5 or H7 viruses, regardless of their virulence, that possess a cleavage site HA0 (Wood, McCauley, Bashiruddin and Alexander, 1993) resembling those of the HPNAI viruses are regarded as **HPNAI** viruses while all other H5 and H7 viruses that do not possess amino acid cleavage site HA0 similar to those of HPNAI are classified as **LPNAI**. All the remaining AI viruses that are not highly pathogenic for poultry are regarded as **LPAI** (OIE, 2005)

1.5.3 Highly Pathogenic Avian Influenza (HPAI)

The highly pathogenic form of avian influenza, also called “fowl plague” was firstly described by Edoardo Perroncito in the late 19th century in Italy. Highly pathogenic avian influenza (HPNAI/HPAI) is an infection with virulent strains of influenza A virus (OIE, 2005). It is highly contagious and rapidly fatal in susceptible avian species especially chickens and turkeys (Capua and Marangon, 2000; Banks, Speidel, Moore, Plowright, Piccirillo, Capua, Cordioli, Fioretti and Alexander, 2001; Stegeman, Bouma, Elbers, de Jong, Nodelijk, Koch and van Boven, 2004; Henzler, Kradel, Davison, Ziegler, Singletary, Debok, Castro, Lu, Eckroade, Swayne, Lagoda, Schmucker and Nesselrodt, 2003).

HPAI is caused by virulent strains of Influenza A virus and up until now, only strains of the H5 and H7 subtypes are known to produce HPAI disease. Two H10 viruses however also fulfilled the criteria for classification as HPAI viruses (OIE, 2005).

Signs of infection vary from acute death with no premonitory signs to respiratory signs, excessive lacrimation, sinusitis, oedema of the head, cyanosis in unfeathered skin, drop in egg production, diarrhoea and severe nervous signs although none of the signs can be defined as pathognomonic since factors such as host, age, presence of other exacerbating factors or organism and environmental condition may all play a role in the severity of clinical signs.

Basically, HPAI viruses usually arise in the poultry population by mutation in the cleavage site of the precursor of the virus external protein haemagglutinin (HA0) and mutations elsewhere in the genome.

The first record of HPAI infection in the poultry population was documented in 1959 in Scotland (Capua and Alexander, 2007), and since then, occurrence of highly pathogenic avian influenza has been recorded twenty-five times (Table 1.4).

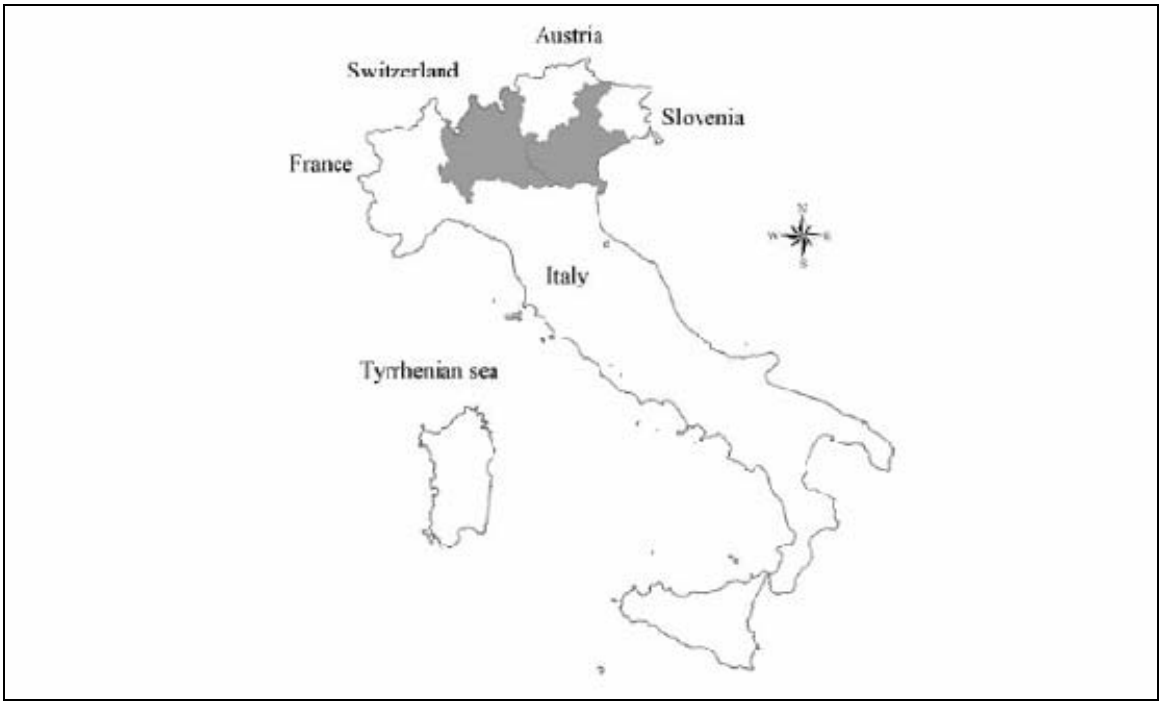
Table 1.4 Reported highly pathogenic avian influenza (HPAI) primary outbreaks in poultry since 1959

	HPAI virus causing outbreaks	Subtype	Approximate numbers of poultry involved	Extent of spread
1	A/chicken/Scotland/59	H5N1	Not known	One small farm
2	A/turkey/England/63	H7N3	29 000	Three small farms
3	A/turkey/Ontario/7732/66	H5N9	8 000	One farm
4	A/chicken/Victoria/76	H7N7	58 000	Chicken& duck farms
5	A/chicken/Germany/79	H7N7	Not known	Chicken& goose farms
6	A/turkey/England/199/79	H7N7	9 000	Three small farms
7	A/chicken/Pennsylvania/1370/83	H5N2	17 000 000	356 HPAI and 90 LPAI farms
8	A/turkey/Ireland/1378/83	H5N8	307 000	One duck farm (270 000), three turkey/chicken farms
9	A/chicken/Victoria/85	H7N7	120 000	One farm
10	A/turkey/England/50-92/91	H5N1	8 000	One house in one farm
11	A/chicken/Victoria/1/92	H7N3	18 000	One chicken farm& one duck farm with H7 antibodies
12	A/chicken/Queensland/667-6/94	H7N3	22 000	One farm
13	A/chicken/Mexico/8623-607/94	H5N2	Unknown-millions	Many farms
14	A/chicken/Pakistan/447/94	H7N3	>6 000 000	Many farms
15	A/chicken/NSW/97	H7N4	310 000	Two chicken& one emu farms
16	A/chicken/Hong Kong/97	H5N1	3 000 000	All poultry in Hong Kong culled
17	A/chicken/Italy/330/97	H5N2	8 000	Eight farms
18	A/turkey/Italy/99	H7N1	14 000 000	413 farms
19	A/chicken/Chile/2002	H7N3	700 000	Two farms
20	A/chicken/Netherlands/2003	H7N7	30 000 000	241 farms-Netherlands, 8 farms-Belgium, 1 farm Germany
21	A/chicken/Eurasia&Africa/2003-06*	H5N1	Over 200 000 000	Over 26 European countries, nine African countries
22	A/chicken/Texas/2004	H5N2	6 600	One farm
23	A/chicken/Canada-BC/2004	H7N3	17 000 000	42 commercial, 11 backyard-all poultry in Fraser valley area culled
24	A/ostrich/South Africa/2004	H5N2	30 000	Many
25	A/ostrich/South Africa/2006	H5N2	Unknown	Many

*26 European, 9 African and 10 Asian countries. Note that several other outbreaks have been recorded since this table was first used. *Source: Capua and Alexander, 2007*

In the last decade, 10 epizootics of HPAI have been recorded with increasing impact on animal health and production, involvement of a number of species and human health issues. In 1997, the northern part of Italy, where industrial poultry production is concentrated (Fig. 1.8) was affected by an HPAI H5N2 virus subtype, that infected eight backyard and semi-intensive flocks (Capua *et al.*, 2003). The disease was quickly eradicated. In 1999, the same area of the country experienced another HPAI epidemic, which involved the industrial poultry production and in about six months caused 413 outbreaks and the death of over 13,000,000 birds.

This particular epidemic started as a LPAI H7N1 around the end of March 1999, and the virus circulated uncontrolled in the industrial poultry farms until December 1999, when it became an HPAI virus (Capua *et al.*, 2003; Mannelli, Ferre and Marangon, 2006,). The epidemic of HPAI H7N7 that occurred in Netherlands in 2003 was also of particular interest. It affected 255 flocks in densely populated poultry regions of Gelderse Vallei and Limburg leading to the culling and death of over 30,000,000 birds; moreover, during this epidemic about eighty human cases (one fatal, a veterinarian) were recorded (Koopmans, Wilbrink, Conyn, Natrop, van der Nat, Vennema, Meijer, van Steenbergen, Fouchier, Osterhaus and Bosman, 2004; Stegeman *et al.*, 2004).



Source: Mannelli et al. 2006

Figure 1.8. Map of Italy showing areas affected by HPAI H5N2 and H7N1 in between 1997 and 2000.

1.5.4 Asian strain of HPAI H5N1

The virus HPAI H5N1 that is presently circulating worldwide was first reported in 1997 in Hong Kong (Xu, Subbarao, Cox and Guo., 1999), it has become endemic in the poultry population in South East Asia. The precursor virus was isolated in China in 1996, (Influenza A/Goose/Guangdong/1/96) from geese (Xu, *et al.*, 1999). The continuous circulation of the virus in poultry and possibly other species has given the virus the unique opportunity to evolve leading to multiple reassortments. This also presents opportunities to expand knowledge in the area of avian influenza research. (Wood *et al.*, 1993; Claas *et al.*, 1998; Xu *et al.*, 1999; Alexander, 2000; Banks *et al.*, 2001; Webster *et al.*, 2002; Liu *et al.*, 2003).

The genetic pool has given rise to several re-assortant viruses, (Li *et al.,* 2004) that have became established in terrestrial poultry populations and the genotype Z re-assortant has became the predominant circulating virus since then leading to the death of well over 200 million chickens in South East Asia and other parts of the world from December 2003 till date. (Peiris, 2005; Melville and Shortridge, 2006).

Studies comparing virus samples over time show that H5N1 has become progressively more pathogenic for mammals and waterfowl, and is now hardier than in the past, surviving several days longer in the environment. Evidence further suggests that H5N1 is expanding its range of susceptible mammalian species to include large felines (tigers and leopards), domestic cats (Thornley, 2004; Quirk, 2004; Keawcharoen, Oraveerakul, Kuiken, Fouchier, Payungporn, Noppornpanth, Wattandorn, Theamboonlers, Tantilercharoen, Pattanarangsan, Arya, Ratanakorn, Osterhaus and Poovorawan, 2004), mink and marine mammals (van Gils, Munster, Radersma, Liefhebber, Fouchier and Klaassen, 2007) - species not previously considered susceptible to disease caused by any influenza A virus.

Although several mutations have been detected in the H5N1 viruses (Enami, Luytjes, Krystal and Palese *et al.*, 1990; Garcia, Crawford, Latimer, Rivera-Cruz and Perdue, 1996; Perdue, Crawford, Garcia, Latimer and Swayne, 1998; Blick *et al.*, 1998; Matrosovich *et al.*, 2000; Mitnaul *et al.*, 2000; Banks *et al.*, 2001), the significance of these mutations in terms of virulence and transmissibility in humans are being studied (Walensten *et al.*, 2007).

Outbreaks and losses in the poultry industry associated with these viruses have been the most widespread and devastating. It has spread from South East Asia (Liu, He, Walker, Zhou, Perez, Mo, Li, Huang, Webster and Webby, 2003; Lee, Suarez, Tumpey, Sung, Kwon, Lee, Choi, Joh, Kim, Lee, Park, Lu, Katz, Spackman, Swayne and Kim, 2005) to Europe and Africa (Enserink, 2006). To date, the H5N1 viruses has been reported in poultry in over 10 Asian countries including Mongolia, Indonesia, Vietnam, Thailand, Lao PDR, Cambodia, Japan, South Korea, Hong Kong SAR and central China. Outbreaks were also reported in Albania, Austria, Azerbaijan, Denmark, France, Germany, Hungary, Romania, Russian Federation, Serbia, Turkey, United Kingdom and Ukraine.

African countries of Burkina Faso, Cote d'ivoire, Djibouti, Egypt, Ghana, Niger, Nigeria Togo and Sudan have been reporting infection since 2006. Outbreaks in wild birds has also been documented in Bosnia-Herzegovina, Bulgaria, Croatia, Czech Republic, Georgia, Greece, Italy, Poland, Slovakia, Spain, Slovenia, Switzerland and the United Kingdom (Emergency Prevention Systems *(EMPRES)* Watch, 2006; OIE, 2006b). The viruses had not only expanded their geographical territories but also the species spread to include previously unaffected animal species (Rappole and Hubalek, 2006, van Gils *et al.*, 2007). To date, almost 60 countries have reported infection with H5N1 Avian Influenza with about 50 of them reporting infection and sometimes re-infection with HPAI H5N1 viruses. (OIE, 2007).

Unarguably, the most highlighted effect of the disease is the public health concern that the H5N1 virus or other avian influenza viruses may mutate and cause a human pandemic (Subbarao, Klimov, Katz, Regnery, Lim, Hall, Perdue, Swayne, Bender, Huang, Hemphil, Rowe, Shaw, Xu, Fukuda and Cox, 1998; Banks, Speidel and Alexander, 1988; Claas *et al.*, 1998; Horimoto and Kawaoka, 2001; Li, Guan, Wang, Smith, Xu, Duan, Rahardjo, Puthavathana, Buranthai, Nguyen, Estoepangestie, Chaisingh, Auewarakul, Long, Hanh, Webby, Poon, Chen, Shortridge, Yuen, Webster and Peiris, 2004; Ferguson, Fraser, Donnelly, Ghani and Anderson, 2004; Monto, 2005; Ferguson, 2005).

These concerns about the potential mutation were based on past pandemic outbreaks of influenza viruses in man thought to have originated from avian hosts (Fig. 1.9). Confirmed laboratory and clinical reports indicated that the H5N1 virus had affected over 306 persons with death in 185 cases till date-May 16, 2007 (WHO, 2006a; OIE, 2006b). Webster and Govorkova,

(2006) further expatiated on the continuing evolution and spread of the H5N1 virus (Fig. 1.10), and advised that all countries must prepare for the possibility of an influenza pandemic

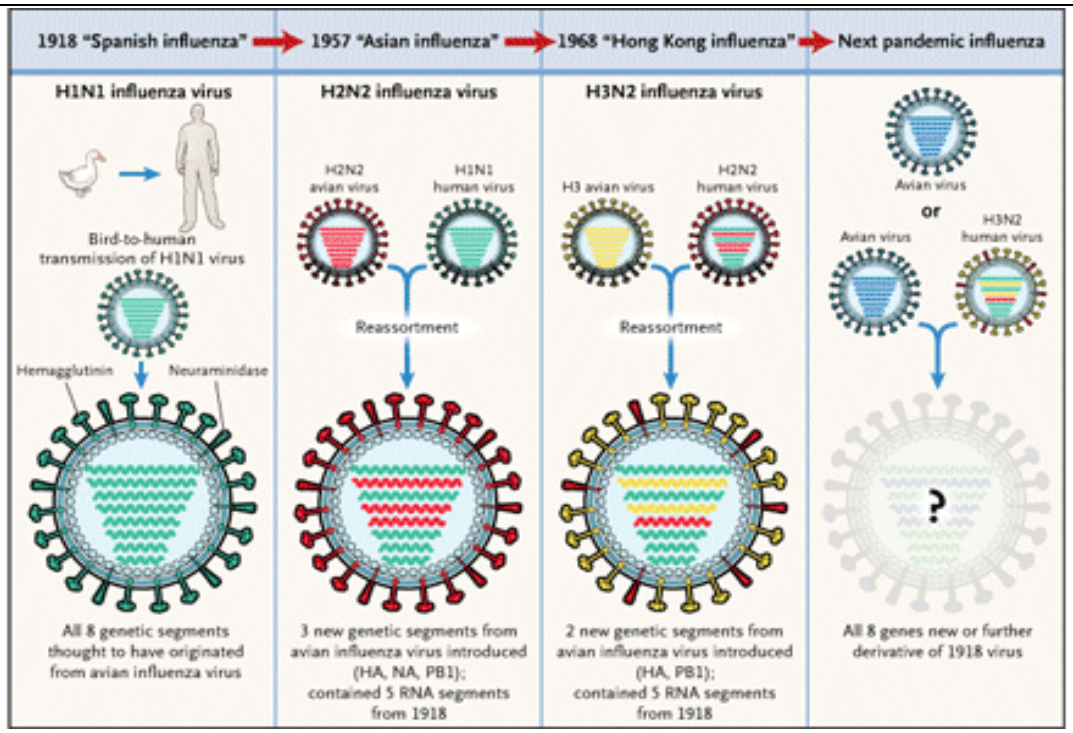


Figure 1.9. Past influenza outbreaks in man and virus re-assortments

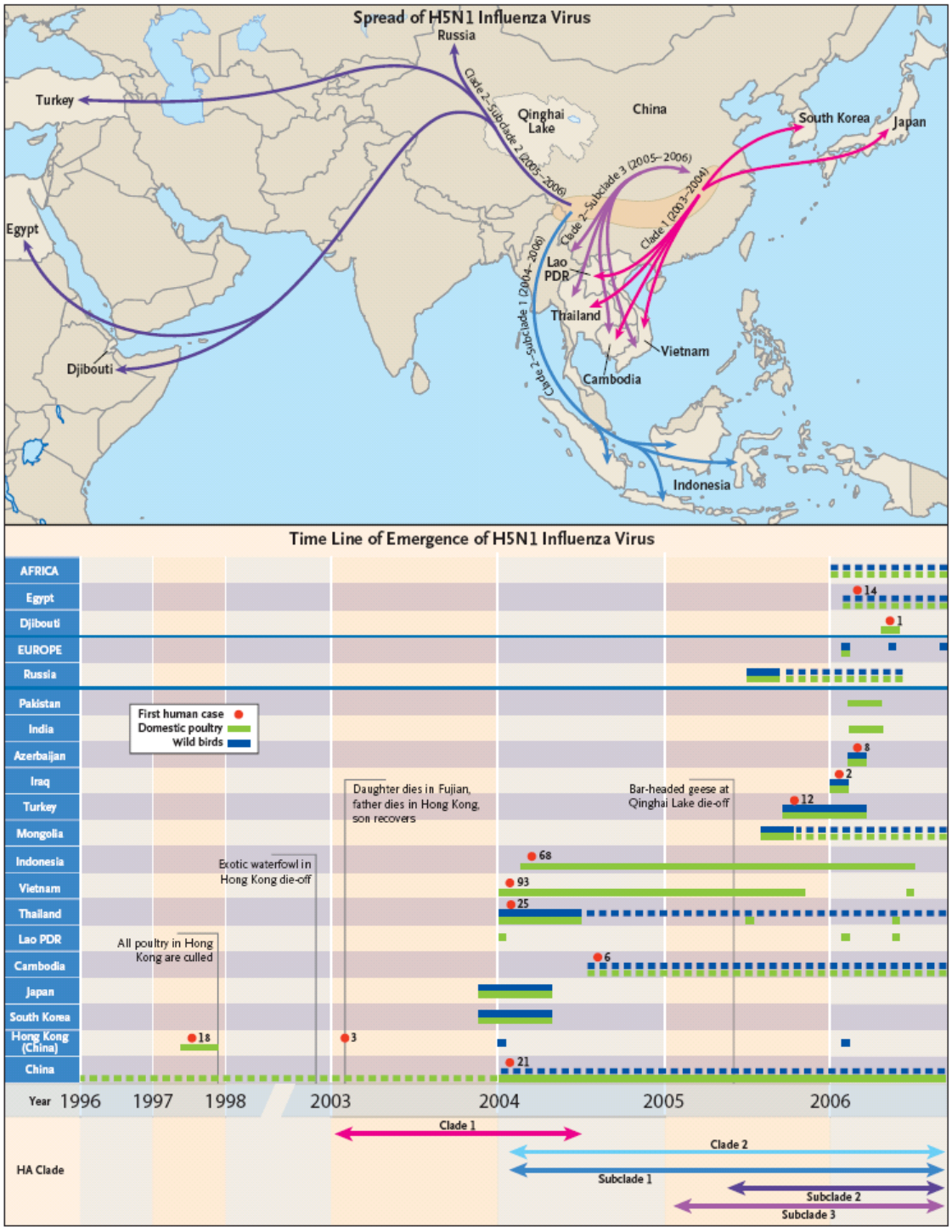


Figure 1.10. Timeline of HPNAI H5N1 (1996-2006). *Source: Webster and Govorkova, 2006.*

The avian influenza H5N1 virus poses a long-term threat to both human and animal health globally and this threat of further spread is clear and could occur from the legal or illegal movement of poultry or poultry products, migratory birds, farm practices and marketing structures and other human activities including transportation and tourism. The argument on whether

migratory birds are involved in the spread or not is essentially unimportant at this time, they should also be considered as posing a serious threat.

1.6 AETIOLOGIC AGENT: AVIAN INFLUENZA VIRUS (AIV)

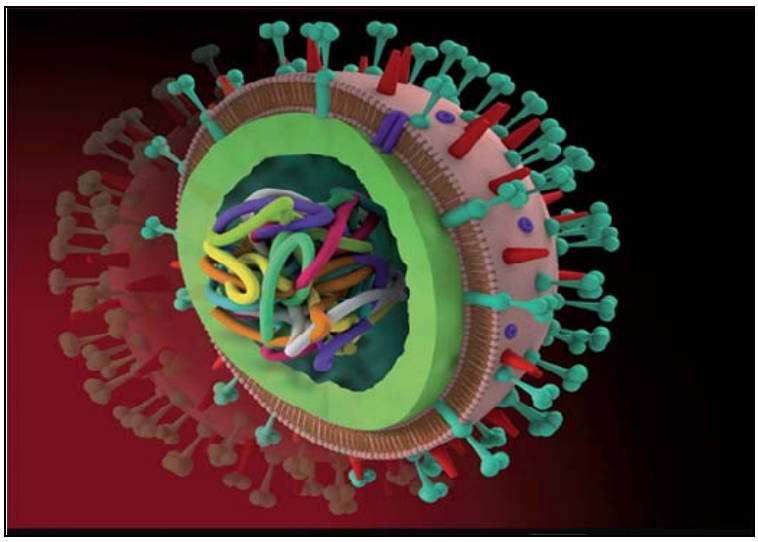


Figure 1.11. Avian influenza virus

Influenza viruses belong to the family ***Orthomyxoviridae***. There are three types viz- Influenza A, B and C. Of all the types, only influenza A virus is known to infect birds (Swayne and Halvorson, 2003; OIE, 2005).

Influenza A viruses are sub-typed based on the antigenic properties of the surface proteins/spikes (antigens); sixteen subtypes of the rod-shaped trimer Haemagglutinin (HA) (H1-H16) and nine of the mushroom shaped tetramer Neuraminidase (NA) (N1-N9) are known.

These surface proteins undergo frequent antigenic change termed antigenic shift or antigenic drift that are responsible for evasion of host immunity and serve as a major obstacle to vaccine composition and disease control (Motta, Rosado and Siqueira. 2006).. Antigenic shift is a sudden and drastic change due to re-assortment of virus genes within a cell co-infected with 2 different sub types of influenza A virus. Antigenic drift on the other hand refers to a gradual change caused by cumulative mutational events.

While the HA is responsible for the attachment to cell surface receptors (sialyloligosaccharides) and haemagglutinating activity of the virus, the NA is responsible for the release of new virus by action on neuraminic acid in the receptors (Fig. 1.12, 1.13, 1.14 & 1.15, pages 46 & 47) (Swayne and Halvorson, 2003). All influenza A viruses have eight segments of negative single stranded RNA (ssRNA) with highly conserved 5′ and 3′ terminals. Virions are typically spherical to pleomorphic but can be filamentous with a diameter of 80-120nm. For purposes of genetic and molecular biological studies, the ssRNA must first be converted to complementary DNA (cDNA) since the polymerase enzyme will act on DNAs and not RNAs.

The two surface polypeptides, in addition to another six namely Transcriptases Polymerase B1 (PB1) and Polymerase B2 (PB2), Nucleoprotein (NP), Matrix protein (M1) and Matrix protein (M2) together form the constituents of the spherical to pleomorphic virions (80-120nm in diameter). The virus also possesses two other viral proteins namely Non-structural protein 1 (NS1) and 2 (NS2). Both NS genes are encoded on a single RNA segment. Recently, another influenza A viral protein was described and named PB1-F2 by Chen, Yang, Tsao, Huang, Lee, Yang, Huang, Lin and Shin, (2004) and further characterized by Zell, Krumbholz and Wutzler, (2006).

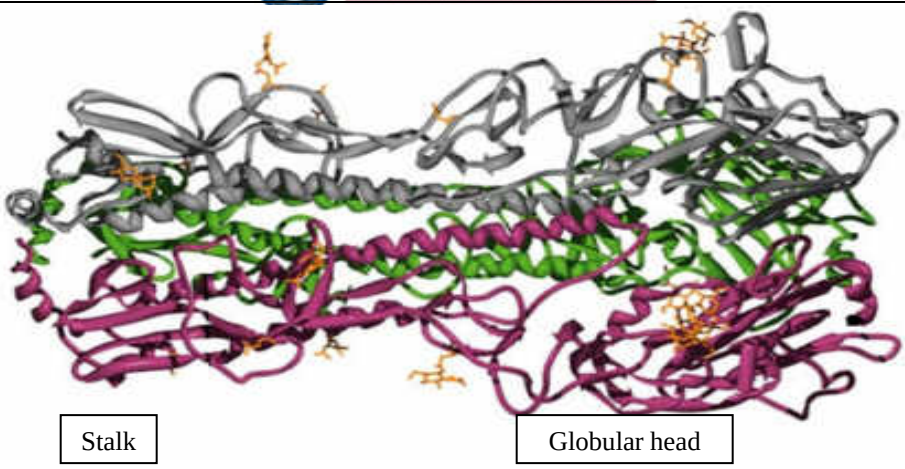


Figure 1.12. Diagramatic representation of Haemagglutinin gene of influenza A virus. *The stalk and globular head both carry N-linked oligosaccharide side chains. Those attached to the stalk region are highly conserved but those at the tip of the molecule show considerable variation in structure and number among different influenza A viruses*

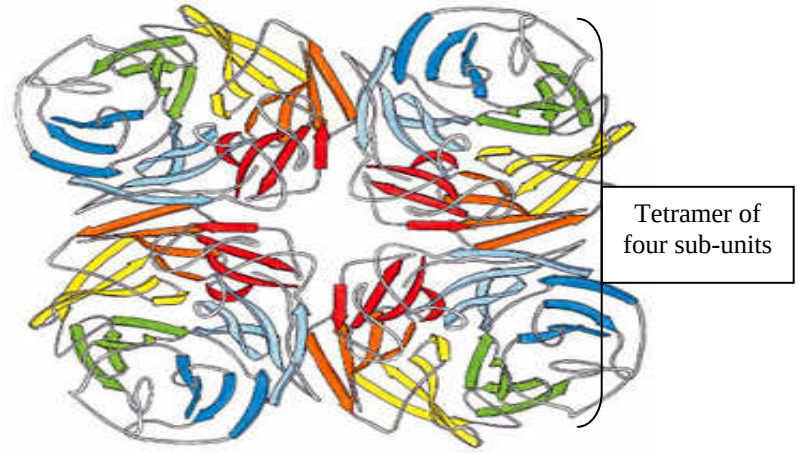


Figure 1.13. Diagramatic representation of Neuraminidase gene of influenza A virus

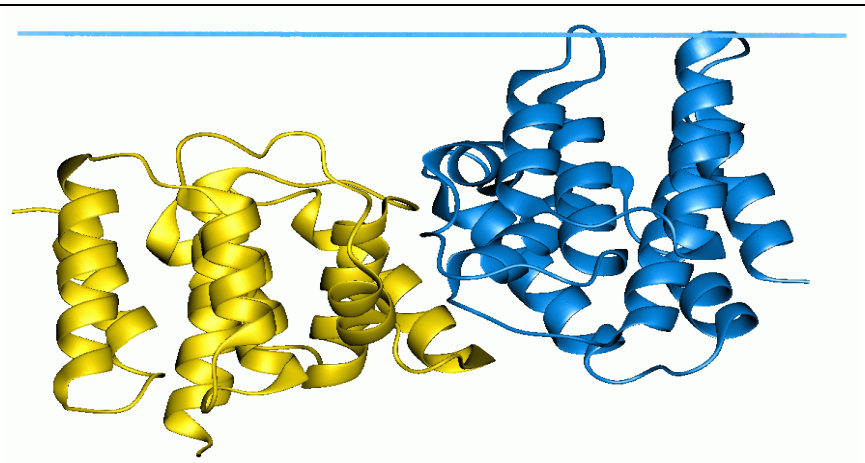


Figure 1.14. Diagramatic representation of Matrix gene of influenza A viruses

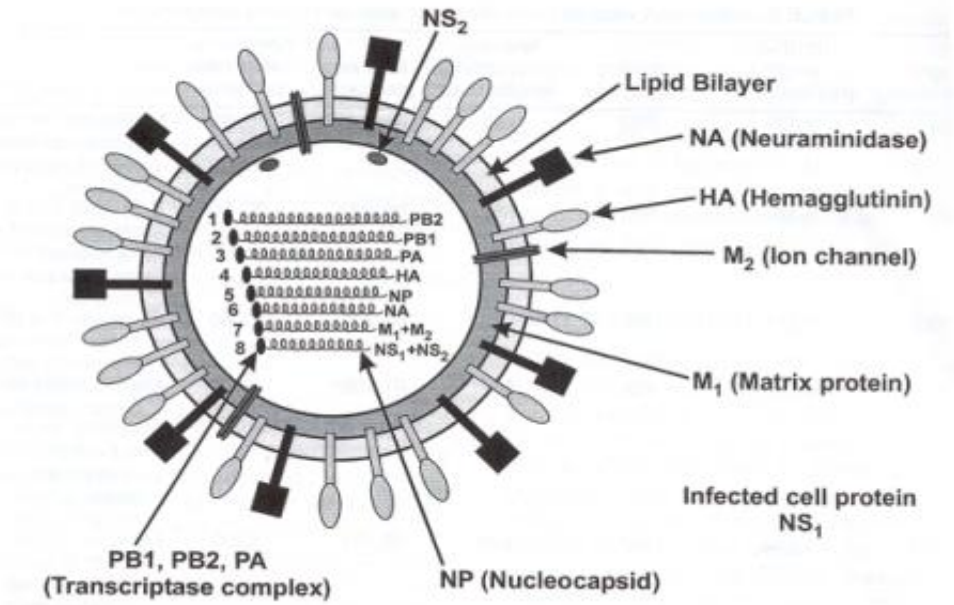


Figure 1.15. Position of the polypeptides found in influenza virus

The virus replicates in the host system by adsorbing it's HA to host cell receptors containing sialic acids bound to glycoproteins. This leads to receptor-mediated endocytosis which is then followed by a complex biochemical (un-coating and replication) processes (Fig. 1.16) (Lamb and Krug, 1996, Swayne and Halvorson, 2003).

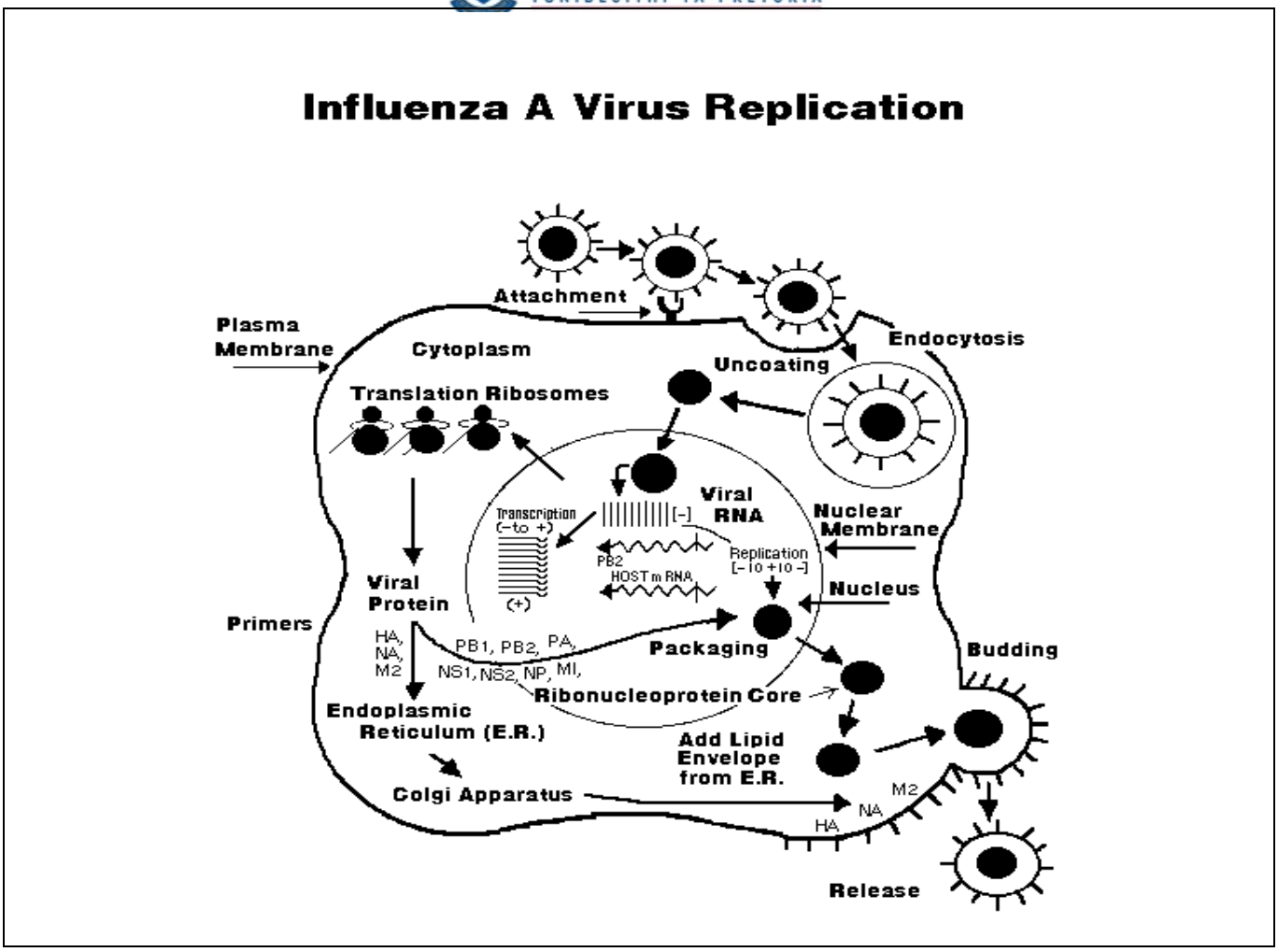


Figure 1.16. Influenza A virus replication

Antibodies against HA are neutralizing and protect the host from infection. Antibodies against NA can also neutralize virus but because there are fewer copies of the NA spikes, they are less effective and they play a more important role in restricting virus spread. The HA, NA and M2 are embedded in the lipid envelope derived from the lipid membrane of the host cell, four other proteins (NP, PB1, PB2 and PA) form the major structural protein that surrounds the RNA molecules while the matrix protein coat the inside membrane (Swayne and Halvorson, 2003).

1.6.1 Virus stability

The virus is inactivated by organic solvents and detergents, chemical agents including aldehydes, beta-propiolactones, ethlyenimine, phenols, quaternary ammonium, hypochlorite, acids and hydroxylamine (Swayne and Halvorson, 2003; De Benedictis, Beato and Capua, 2007). It is relatively unstable in a harsh environment. Physical conditions such as heat, high acidic/basic pH,

non-isotonic conditions and desiccation can inactivate the virus. Conventional cooking at temperatures of 70°C or above in all parts of a food item will similarly inactivate the influenza virus but refrigeration or freezing will not kill the virus (WHO, 2005. Sick birds, the shell and content of eggs can contain the virus and although no epidemiological evidence has indicated that people have been infected with the H5N1 virus following consumption of properly cooked poultry or eggs; there is, however, a high risk of contamination/infection for the handlers and slaughterers/butchers of sick birds (WHO, 2006).

1.6.2 Host and Transmission

Wild birds of the orders Anseriformes (particularly ducks, geese and swans) and Charadriiformes (particularly gulls, terns and waders) are the natural reservoir of all influenza A viruses (Webster and Bean, 1998; Horimoto and Kawaoka, 2001

However, a wide variety of wild and domesticated species (humans, pigs, horses, felids, marine mammals and birds have been affected (Keawcharoen, *et al.*. 2004; Ellis, Bousfield, Bissett, Dyrting, Luk, Tsim, Strum-Ramirez, Webster, Guan and Peiris, 2004; van Gils *et al.*. 2007). Evidence suggests that Live Bird Markets (LBMs), which serve as a portal of pooling different birds from different sources together, have played important roles in the transmission and spread of epizootics (Webster, 2004, Henzler *et al.* 2003). Birds that survive infection have been shown to excrete virus for up to 10 days, orally and in faeces, thus facilitating further spread (EEC, 2004; Olsen, Laosiritaworn, Pattanasin, Prapasiri and Dowell, 2005). Unlike in chickens where infection with the highly pathogenic form of the virus is rapidly expressed with severe consequences; domestic ducks have been known to be resistant to the viruses and can serve as asymptomatic carriers, thus acting as a “silent reservoir” that perpetuates transmission (Ellis *et al.*, 2004; Tumpey, Suarez, Perkins, Senne, Lee, Lee, Mo, Sung, and Swayne, 2002).

Recent surveillance in Central Asia, Europe and Africa has shown evidence that at least some species of migratory birds may be directly involved in the spread of HPAI H5N1 virus to new regions (Alexander, 2000; Simpson, 2002; Kilpatrick, Chmura, Gibbons, Fleischer, Marra and Daszak, 2006, Feare and Yasue 2006; van Gils *et al.* 2007), as some countries along major migratory pathways like Turkey and Romania became infected and isolates have also been recovered from some dead migratory birds. Farming practices without giving consideration to

good levels of biosecurity have also been found to contribute to spread of the virus (Birdlife International., 2006a).

1.6.3 Pathogenesis

The pathogenesis of HPNAI in poultry usually begins with the inhalation or ingestion of HPNAI virions. The trypsin-like enzymes in the respiratory and digestive epithelium result in the cleavage of surface HA with resultant multiple replication cycles and release of more infectious virions (Brown, Olander and Senne, 1992, Hillerman, 2002). The virions later invade the sub-mucosa and enter the capillaries, replicating in the endothelia cells and spreading through the lymphatic and vascular systems to affect cells of other organs. The virus may become systemic before its extensive replication (Swayne and Halvorson, 2003).

Pathologically, AIV negatively impacts on cells of the avian tissues by two means viz -necrosis and apoptosis: These effects are caused directly by virus replication in cells, tissues and organs, and the indirect effect from cellular mediator production like cytokines and ischaemia that results from vascular necrosis of tissues. Death and other clinical signs are usually due to multiple-organ failure (Brown *et al.*, 1992; Swayne and Halvorson, 2003).

AIV and other RNA viruses have high mutation rates. Though the host resistance mechanism eliminates “non-self” by producing neutralizing antibodies against the two major surface proteins of AIV *viz* HA and NA, the rate of mutation assists the virus to maintain itself within the host system (Wardley, Martin and Saif, 1996). AIV opportunistically changes its genetic constituents by antigenic shift or drift, to fit best into its new host environment.

In summary, the following factors may be said to be responsible for AIV’s successful maintenance in the poultry populations:

1. environmental stability,
2. successful transmission,
3. evasion of surface defense mechanisms,
4. localization and successful cell entry near portal of entry,
5. primary replication,
6. evasion of non specific immunity,
7. ability to spread from primary site,

8. cellular and tissue tropism,
9. secondary replication,
10. specific immune mechanism and
11. release for transmission to a new host (Wardley *et al.*, 1996).

1.7 CLINICAL AND PATHOLOGICAL SIGNS

Clinical signs in domesticated poultry may vary from acute death with no premonitory signs to mild/severe respiratory signs, excessive lacrimation and salivation, paralysis, sinusitis, opisthotonus or torticollis, oedema of the head, shriveled cyanotic combs and wattles, cyanosis in un-feathered skin, corneal opacity, and a mild to severe drop in egg production. Other common signs include reddened feet and shanks as well as diarrhea often together with pasted vents. In wild birds, similar signs to a lesser degree have been found in addition to paresis, with or without tremor. None of the signs can be said to be pathognomonic since factors such as host, age, presence of other exacerbating factors, the pathogenicity of the infecting organism and environmental condition may all play a role in the severity of clinical signs (Ellis *et al.*, 2004).
At autopsy, investigation usually reveals birds in good body condition with varying pathological changes including congestion of the lungs, liver, intestine, trachea, brain and kidneys. The lung is usually oedematous and spleen may be swollen, mottled or congested. There is presence of increased pericardial fluid, petechiation (haemorrhage) of pericardial fat, mottled pancreas, haemorrhagic duodenum, thickened airsacs, haemorrhagic proventriculus and scanty feed materials in the digestive system (Ellis *et al.*, 2004, Joannis *et al.*, 2006).

Histopathological examination often reveals marked congestion and oedema of the lung and trachea, mucous glands and loss of epithelial cells of the trachea, brain congestion with or without multiple focal necrosis, mild gliosis, multi focal non-suppurative meningo-encephalitis, peri-acinar to diffuse vacuolar degeneration and hepatocyte necrosis of the liver, congestion and focal necrosis of the small intestine and caeca, acute necrotic pancreatitis, thymic congestion and haemorrhagic oophoritis (Ellis *et al.*, 2004). These are usually associated with the multiple organ failure that ultimately results in the death of the bird (Wardley *et al.,* 1996).

1.8 DIAGNOSIS

The clinical and pathological signs are often not sufficient to diagnose avian influenza since these various sign are also expressed by various other infectious diseases like Newcastle disease,

other paramyxoviral diseases, infectious laryngo-tracheitis, infectious bronchitis, chlamydiosis, mycoplasmosis, avian pasteurellosis, salmonellosis, and others. The final diagnosis of HPNAI is usually made in the laboratory. Presumptive diagnoses are made by detection of virus antigens or antibodies according to the methods listed in the OIE manual (OIE, 2005).

1.9 MOLECULAR EPIDEMIOLOGY

Epidemiology is the study of disease in populations and of factors that determine its occurrence. When such disease involves only animals, it is referred to as epizootiology. It has five main objectives including:

- Determination of origin of diseases whose cause is known
- Investigation and control of diseases whose cause is unknown or poorly understood
- Acquisition of information on the ecology and natural history of a disease
- Planning, monitoring and assessment of disease control programmes and
- Assessment of the economic effect of a disease and analysis of the cost and economic benefit of alternative control programmes (Thrushfield, 2005).

Molecular epidemiology is a branch of science that fulfills the above objectives using the diagnostic tools of molecular biology in studying infections, diseases and outbreaks. It is used in viral epidemiology by typing of viruses through genetic determination and comparison of the nucleotide sequences of fragments of the viral genome. It has been used extensively in foot and mouth disease (FMD) studies in Europe and in West Africa (Thrushfield, 2005). Lubisi, (2005) similarly used molecular epidemiology in studying African swine fever virus in the East African sub-region and Abolnik (2007) used it to study Newcastle disease and Avian influenza in South Africa.

1.9.1 Application of Biotechnology to Disease Diagnosis

Several diagnostic tests for avian influenza have been outlined in the previous section (see Section 2.4.1.). To date, for avian influenza virus characterization (identification and typing) it is necessary the preliminary isolation in embryonating chicken eggs (OIE, 2005).

However, the test is time consuming, labour intensive and only detects viable viruses; it has the shortcoming of not being suitable to handle large numbers of samples, as is common with

emergencies of HPNAI. This has led to the development of applied molecular biology techniques that operate by diagnosing genetic and molecular differences, and detect nucleic acids (OIE, 2005; Slomka *et al.*, 2007).

Molecular biology methods have over the years become increasingly applicable in infection diagnoses. Tests are highly discriminatory between isolates of closely related pathogens that regular serology and virus isolation may not be able to distinguish. Molecular biology tests have been modified over time to conform to technical ease, safety, reproducibility and eventual automation that every conventionally accepted test possesses.

1.9.2 Phylogenetics

Phylogeny is the study of relationship and differences between and within species including their evolutionary relationship. Molecular phylogenetics can be said to predate DNA sequences. It originated from the traditional methods of classifying organisms according to their similarities and differences as practiced by Linnaeus in the 18th century (Brown, 2002). The main objective of phylogenetics is to reconstruct the tree-like pattern that describe the evolutionary relationship between the organisms being studied. Phylogenetic analysis is done by:

- Aligning the DNA sequences and obtaining comparative data that will be used to construct a tree
- Converting the comparative data into a reconstructed tree
- Assessing the accuracy of the reconstructed tree
- Using a molecular clock to assign dates to branch points within the tree

It has been used extensively to study disease origin, occurrence and spread as well as origin of man and other animals; and the relationships that exist between the two (Brown, 2002).

1.10 GEOGRAPHIC INFORMATION SYSTEMS

Geographic information system (GIS) is a computer-aided database management and mapping technology that organizes and stores large amounts of multi-purpose information. GIS adds the dimension of geographic analysis to information technology by providing an interface between the

data and a map. This makes it easy to present information to key decision-makers quickly, efficiently and effectively.

Geographic information systems and remote sensing (RS) from earth observing satellites are sophisticated and powerful technologies that are finding applications far beyond those originally intended. Both GIS and RS are initial products for military purposes. Together, they allow near real-time access to data on temperature, soil, elevation, patterns of land use, and phases of vegetation in addition to the precise geographic location of water bodies, population centres, buildings, roads, and other infrastructure. Their use for purposes ranging from the search for natural resources to transportation engineering, urban design, and agricultural planning was quickly recognized and exploited (WHO, 2006b).

Geographic information systems and remote sensing have capabilities that are ideally suited for spatial epidemiology in the use in infectious disease surveillance and control, particularly for the many vector-borne neglected diseases that are often found in poor populations in remote rural areas. They are also highly relevant to meet the demands of outbreak investigation and response, where prompt location of cases, rapid communication of information, and quick mapping of the epidemic's dynamics are vital.

However, until recently, the use of these tools in human and animal health were largely limited in use due to two major problems: the prohibitive cost of hardware and the great complexity of GIS software that made it extremely time-consuming as well as costly to extract information relevant to the practical demands of disease prevention and control.
Recent developments have forced the situation to change dramatically over the past few years. Hardware prices have plummeted, simple new devices are available, and new generation of user-friendly satellite information are readily accessible.

Similarly, simple and low-cost geographic information and related data management and mapping systems for disease surveillance are now readily available. These technologies are making it possible to acquire high-quality epidemiological data with precision and speed (WHO, 2006b).

1.10.1 Ecological Niche Modeling

Ecologic niche modeling is a growing subject in the field of spatial modeling of disease. It involves the use of GIS software to predict the potentials of a pathogen to spread in the presence of exacerbating factors, other favourable environmental conditions, geography and ecology of the pathogen (Peterson, 2006).

Since molecular and spatial epidemiology are different sub-disciplines of Epidemiology, and molecular epidemiology studies genetic clusters mostly while spatial epidemiology studies spatial/map clusters, the integration of the two (molecular and spatial epidemiology) will result in a fuller epidemiological description of this particular disease situation.

1.11 ECONOMIC COST MODELING

Animal epizootic diseases have enormous consequences which can be direct and indirect. While direct impacts are easier to assess, indirect impacts have proved to be difficult (Rushton, Thornton and Otte, 1999). Animal health economics is a relatively new discipline that involves the use of concepts, procedures and data to support decision making process with the aim of optimizing management and profitability in animal farming systems. Over the years, the economic implications of animal diseases in particular, have received little attention. However, since farmers engage in animal production mainly for the purpose of economic benefits, it is necessary to thoroughly evaluate all issues that limit the maximum productivity of animals.

Several models for evaluating the economic implications of disease and those of interventions that may halt disease entities have been assessed and used by previous workers (Rushton *et al.*, 1999, Ramsay, Philip and Riethmuller, 1999, Marsh, 1999, Horst, de Vos, Tomassen and Stelwagen, 1999). These models become particularly useful in convincing policy makers in taking necessary control and eradication steps which come at additional costs in view of other competing interests. They also assist in making informed decisions at farm level for the choice of intervention.

1.12 OBJECTIVES OF THE STUDY

The study is therefore aimed at:

1. Identifying the possible origins of HPAI H5N1 infection and route(s) of spread in Africa with regards to Nigeria through the use of available technologies of molecular biology, sequencing, phylogenetic tree analyses of isolates from Nigeria and geographical information system (GIS).

2. Assessing the impact of the HPAI H5N1 on agricultural production in Nigeria.

3. Evaluating costs associated with the HPNAI H5N1 outbreaks and analysing options aimed at the control of highly pathogenic avian influenza H5N1 in Nigeria.

The findings will contribute to the understanding of the epidemiology and ecology of HPAI in Africa, greatly assist in the planning and design of effective control measures; and serve as a pedestal for a comprehensive estimation of the socioeconomic impact of the virus in the poultry industry. It will also aid the understanding of the evolution of the virus variants in Nigeria and assist in monitoring for antigenic drift of HPAI viruses.

CHAPTER TWO

MATERIALS AND METHODS

2.1 DETERMINATION OF POULTRY DENSITY AND DISTRIBUTION IN NIGERIA

The search for poultry production figures and data for Nigeria revealed little information. The most accurate and recent of the information gathered was a study conducted by Bourn, *et al.*, (1992) (Fig. 1.1, page 5). This situation therefore necessitated more recent information on poultry density and distribution in Nigeria if a good assessment of the epidemiology will be done since the knowledge of population at risk is important.

Data was collected from the Federal Ministry of Agriculture and Rural Development, Abuja, Nigeria, some of the States' Ministry of Agriculture, the Food and Agricultural Organisation of the United Nations Statistical Website and recent literature on the poultry population in Nigeria and used to produce a map of the country with poultry densities and distribution (Fig. 3.1, page 56).

2.2 MOLECULAR EPIDEMIOLOGY OF THE HIGHLY PATHOGENIC AVIAN INFLUENZA H5N1 IN NIGERIA, JANUARY 2006-JANUARY, 2007

2.2.1 Sample collection

Tissue samples (lung, liver, spleen, heart, trachea and intestine) were collected by the National Veterinary Research Institute (NVRI) outstation laboratories and state veterinary offices from the field by visiting outbreak locations and harvesting the samples from dead carcasses. Alternatively, such samples were submitted by farmers to the National Veterinary Research Institute Epidemiology Department and transferred to the Viral Research Department for processing. All remaining carcasses were disposed by burning and deep burial.

2.2.2 Viruses

Pooled tissue homogenates were treated with isotonic phosphate buffered saline previously subjected to antibiotic treatment-Penicillin, Streptomycin, Gentamycin and Amphotericin B (PSGA) according to standard protocol (OIE, 2005).

35 isolates of HPAIV H5N1, covering the period under investigation (January, 2006-January, 2007) and the different regions of Nigeria were selected and grown in 9-11 day old specific pathogen free (SPF) embryonating chicken eggs (NVRI Vaccine Birds Unit, Nigeria) using standard procedure (OIE, 2005), at the Viral Research Laboratory, National Veterinary Research Institute, Vom, Nigeria (Table 2.1). Only 32 of the isolates could be sequenced, as virus titres in the remaining three were too low - the 3 isolates which were incompletely characterized are identified in the table below with green marks.

Infective allantoic fluids were harvested and tested using monospecific anti-sera for AIV H5 group and Newcastle viruses. The fluids were later aliquoted into cryo-vials and cryo-preserved at -196^{O}C. Portions of the aliquots were treated with lysis buffer (MagNA Pure LC, Roche Applied Sciences, Mannheim, Germany) and transported to the Onderstepoort Veterinary Institute, South Africa.

Table 2.1. Nigerian HPAI H5N1 virus isolates analysed in this study

	Name	Species affected	Location, State	Date of report/collection
1	A/chicken/Nigeria/VRD35/2006	Commercial layer chicken	???	02/02/2006
2	A/chicken/Nigeria/VRD42/2006	Commercial layer chicken	Katako Area, Plateau	09/02/2006
3	A/chicken/Nigeria/VRD44/2006	Commercial layer chicken	Rikkos, Plateau	09/02/2006
4	A/chicken/Nigeria/VRD49/2006	Commercial layer chicken	Dahol Giring (Forest), Plateau	14/02/2006
5	A/chicken/Nigeria/VRD83/2006	Chicken	Naraguta, Plateau	17/02/2006
6	A/chicken/Nigeria/VRD91/2006	Commercial layer chicken	University Quarters	18/02/2006
7	A/chicken/Nigeria/VRD130/2006	Commercial layer chicken	Apollo Cresent, Plateau	24/02/2006
8	A/chicken/Nigeria/VRD130b/2006	Commercial layer chicken	Apollo Cresent, Plateau	24/02/2006
9	A/chicken/Nigeria/VRD145/2006	Pullet chicken	Sabon Barki, Plateau	26/02/2006
10	A/chicken/Nigeria/VRD146/2006	Commercial layer chicken	Katako Market, Plateau	26/02/2006
11	A/chicken/Nigeria/VRD111/2006	Commercial layer chicken	Katako Market, Plateau	27/02/2006
12	A/chicken/Nigeria/VRD157/2006	Commercial layer chicken	Zaria Road, Plateau	27/02/2006

13	A/chicken/Nigeria/VRD165/2006	Commercial layer chicken	Chwelnyap (Congo), Plateau	28/02/2006
14	A/chicken/Nigeria/VRD184/2006	Vulture	Vom, Plateau	04/03/2006
15	A/chicken/Nigeria/VRD193/2006	Commercial layer chicken	Mando, Kaduna	08/03/2006
16	A/chicken/Nigeria/VRD200/2006	Pullet chicken	Jos, Plateau	07/03/2006
17	A/chicken/Nigeria/VRD203/2006	Commercial layer chicken	Kaduna, Kaduna	08/03/2006
18	A/chicken/Nigeria/VRD216a/2006	Commercial layer chicken	Cooperative Farm, Agege, Lagos	10/03/2006
19	A/chicken/Nigeria/VRD218/2006	Commercial layer chicken	Rikkos, Plateau	11/03/2006
20	A/chicken/Nigeria/VRD219/2006	Commercial layer chicken	Old Airport Junction, Plateau	13/03/2006
21	A/chicken/Nigeria/VRD244/2006	Commercial layer chicken	Bukuru, Plateau	30/03/2006
22	A/JWP/Nigeria/VRD252/2006	Wild species	Jos Wildlife Park	03/04/2006
23	A/turkey/Nigeria/VRD262/2006	Turkey	Ungwan Dosa, Kaduna	06/04/2006
24	A/chicken/Nigeria/VRD284/2006	Commercial layer chicken	Apata, Plateau	13/04/2006
25	A/chicken/Nigeria/VRD286/2006	Commercial layer chicken	Jos, Plateau	16/04/2006
26	A/chicken/Nigeria/VRD311/2006	Commercial layer chicken	Bukuru, Plateau	26/04/2006
27	A/chicken/Nigeria/VRD340/2006	Commercial layer chicken	Zaria Road, Plateau	05/05/2006
28	A/turkey/Nigeria/VRD345/2006	Turkey	Rukuba, Plateau	08/05/2006
29	A/chicken/Nigeria/VRD368/2006	Commercial layer chicken	Dogon Dutse, Plateau	20/05/2006
30	A/pigeon/Nigeria/VRD370/2006	Pigeon	Vom, Plateau	20/05/2006
31	A/chicken/Nigeria/VRD403/2006	Commercial layer chicken	Molete Market, Oyo	06/06/2006
32	A/duck/Nigeria/VRD418/2006	Duck	Dogon Karfe, Plateau	08/06/2006
33	A/chicken/Nigeria/VRD419/2006	Local and commercial layer chicken	Dogon Karfe, Plateau	08/06/2006
34	A/chicken/Nigeria/VRD457/2006	Commercial layer chicken	Ijegun, Lagos	28/06/2006
35	A/guinea fowl/Nigeria/VRD005/2007	Guinea fowl	Kebbe, Sokoto	05/01/2007

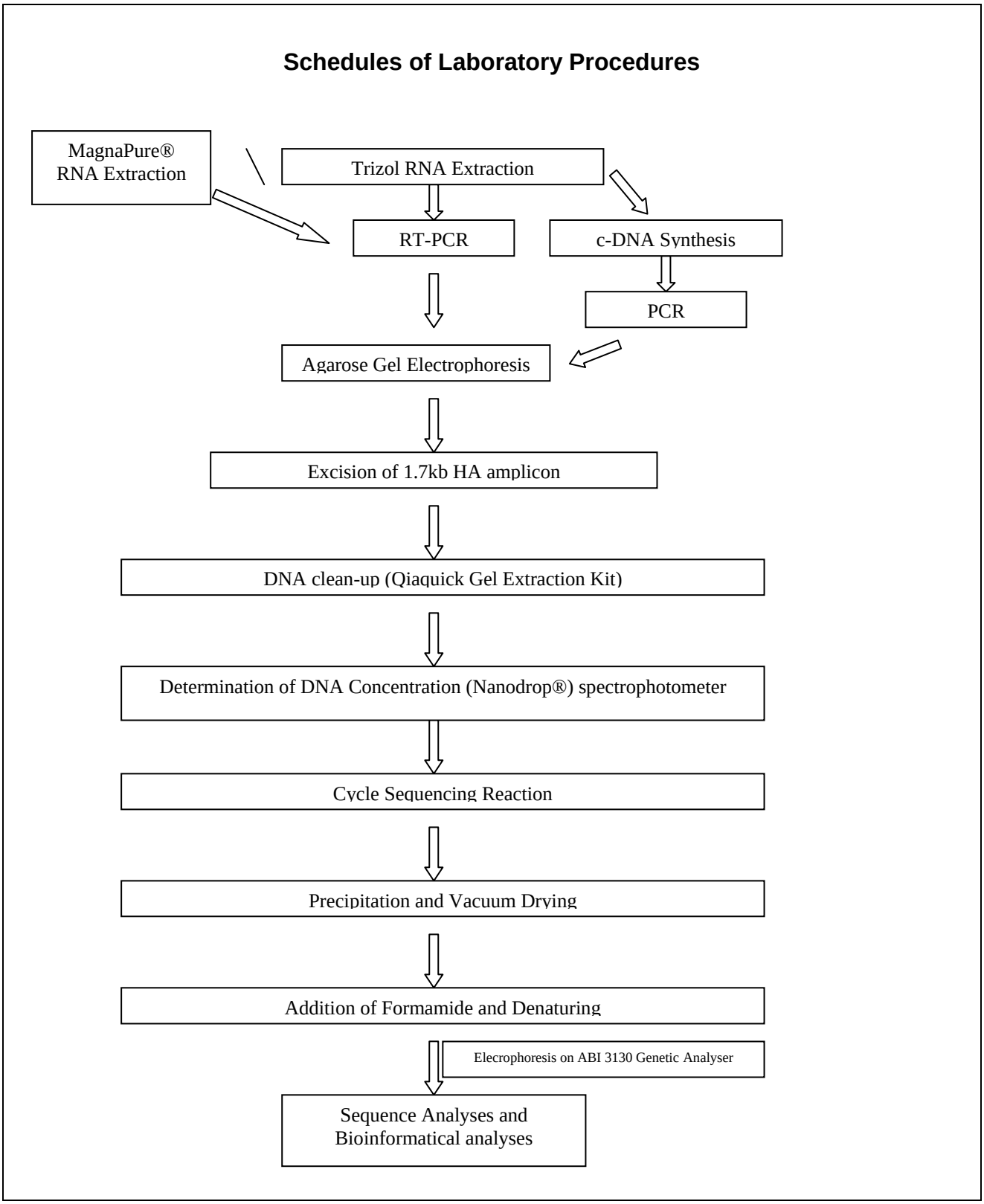


Figure 2.1. Schedule of Laboratory activities

2.2.3 RNA extraction and RT-PCR

Viral RNAs were extracted from lysates using Trizol LS® reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) or MagNA Pure® LC total nucleic acid isolation reagent (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturers' instructions. Bontsi Mochotlhoane performed the MagnaPure® extractions.

2.2.4 First strand cDNA synthesis

Reverse Transcription was performed with 60 IU of M-MLV reverse transcriptase (Promega, Madison, USA) and 8 IU of ribonuclease inhibitor (Amersham, Biosciences, Piscataway, NJ, USA) at 42^{O}C for 90 minutes on 5 µl of extracted viral RNA. 3pMol of the vGEN oligonucleotide, that anneals to the 5′ terminal sequence of the HA gene segment was used for chain extension.

In other cases, one-step RT-PCR was performed by adding a 20-minute 42^{O}C incubation step to the thermocycling profile prior to PCR

2.2.5 PCR

The full length HA gene was amplified using 20pMol each of forward 5′(AGCAAAAGCAGGGGW)3′ and reverse 5′(AGTAGAAACAAGGGTG)3′ primers; 5U/µl Ex Taq polymerase (Takara Biotech, Shiga, Japan) and 2.5mM de-oxy ribonucleic acids (dNTPs) using an Eppendorf Mastercycler® (Eppendorf AG, Hamburg, Germany). A partial HA gene region was amplified for some Nigerian H5N1 isolates for which full-length gene could not be obtained due to RNA degradation.

RT-PCR amplicons were electrophoresed on 1% Agarose gel with Ethidium Bromide at 120V, and excised from the agarose gel (Hispanagar, Burgos, Spain). The DNA products were extracted with QIAquick® Gel Extraction Kit (QIAGEN, Hilden, Germany).

Table 2.2. Thermal cycling conditions used to amplify the H5N1 HPAIV gene

Target	Initial Denaturation	Denaturation	Annealing	Elongation	Cycles	Final Elongation
HA (touchdown)	95^{O}C (5 minutes)	95^{O}C (30 s)	51^{O}C(30 s)	72^{O}C (2minutes)	3	72^{O}C (2minutes) 4°C (∞)
		95^{O}C(30 s)	48^{O}C(30 s)	72^{O}C (2minutes)	3	
		95^{O}C(30 s)	45^{O}C(30 s)	72^{O}C (2minutes)	3	
		95^{O}C(30 s)	42^{O}C(30 s)	72^{O}C (2minutes)	3	
		95^{O}C(30 s)	41^{O}C(30 s)	72^{O}C (2minutes)	30	

∞= Hold at this temperature until removed

2.2.6. DNA sequencing and phylogenetic analysis.

Template DNAs were quantified using a NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Inc, USA). Cycle sequencing reactions were performed using the forward and reverse primers used for PCR, and a third oligonucleotide (CCACCTATATTTCCGTTGGGAC) designed to span the mid region of the gene. The BigDye® Terminator V3.1 chemistry kit (Perkin Elmer/Applied Biosystems) was used according to manufacturer's instructions. Reactions were electrophoresed on a 3130 Genetic Analyzer (Applied Biosystems). Sequences were edited and assembled using Chromas Lite and BIOedit.

Blast homology searches (http://www.ncbi.nlm.nih.gov/blast) were used to identify 76 closely related sequences representing wide species, geographical and spatial distributions and including all of the HPAI H5N1 sequences available from Africa. Multiple alignments were performed using CLUSTAL W (http://www.ebi.ac.uk/clustalw/index.html, Thomson, Higgings and Gibson, 1994). Pair-wise nucleotide sequence identities were also calculated using Bioedit (Hall, 1999).

The region of the HA genes of Nigerian viruses phylogenetically analyzed corresponds to nucleotides 92 to 1 633 of the complete 1 730 nucleotide protein encoding region of the HA gene of HPAI H5N1 viruses.

Phylogenies were reconstructed (Fig. 3.3a & b, pages 84 & 85) using Neighbour-joining tree method using MEGA 3.1 software (Kumar, Tamura and Nei, 2004) with the Kimura 2-parameter sequence evolution model, and 1 000 bootstrap replicates were performed to assign confidence levels to branches.

All of the processes above were repeated for viruses (underlined in Fig. 3.3b) whose short sequences were generated and these were analysed alongside other viruses in Fig. 3.3b.

2.2.7. Accession Numbers

Gene sequences of selected viruses were deposited in the Genbank under the accession numbers EF631164-EF631187.

2.3. APPLICATION OF GEOGRAPHICAL INFORMATION SYSTEMS (GIS) IN THE DETERMINATION OF EPIDEMIOLOGY OF HPNAI H5N1 IN NIGERIA

Outbreak locations were visited and geo-referenced using a global positioning satellite system (GPS) (Garmin nuvi 370® GPS, Garmin, Olathe, KS, USA). Locations difficult to access were geo-referenced using TADinfo® version 1.101 software (a pre-georeferenced package customised for Nigeria) (EMPRES-FAO, 2006). Records of history, numbers and species affected, dates and other epidemiological data supporting the outbreaks were collated and confirmed using data deposited at the National Veterinary Research Institute, Vom, Nigeria.

Data

Spatial-temporal data on 113 poultry farms (appendix E), infected with Avian Influenza (AI) virus strain H5N1, as well as the poultry population data of each Nigerian state, were facilitated by the National Veterinary Research Institute, (NVRI) Vom, Nigeria and the FAO paper (http://www.fao.org/docs/eims/upload//214281/poultrysector_nga_en.pdf). The Nigerian major road network map was created from WHO country situational basemaps (http://www.reliefweb.int/rw/fullMaps_Af.nsf/luFullMap/B6887A6EB2E41E1FC125723400298710/$File/who_REF_nga060208.pdf?OpenElement).

Geographical Information System methods

Using the *analysis* menu and *find distance* command on the major road shapefile (*ArcGIS 3.3* and *ArcView 8.0*, both from ESRI, Redlands, CA), the distance from each infected farm to the nearest point on the major road network (DNR) was calculated by generating a point layer of all road points. This “nearest major road point” layer was used in the "POINT DISTANCE" command to create fields within the target layer's attribute table that contained the farm identifier (FID) or farm distance (DIST) to the nearest road point (NEAR), so that the distance to the nearest road point (NEAR_DIST) and the X,Y coordinates of the road point (NEAR_X,NEAR_Y) were produced. The same procedure calculated the distance of each infected farm to the nearest major road intersection (DNI).

A layer on road density was produced by *intersecting* the "major road network” layer with the "states" layer. From the resulting attribute table, a *summary* by "state" was generated, which provided the length of roads (kilometers) within each state. The road density layer was created by dividing the road length by the state area (km/sq km). The same procedure generated the state poultry population/sq km.

Randomized spatial points along the major road network

In spite of lack of information on the actual location of susceptible farms and the limited data available by epidemic day four, the question of whether infected farms were observed predominantly near major road intersections, was assessed by measuring the distance between farms infected by epidemic day four and the nearest major road intersection (DNI). That distance was compared to that of a sample of spatial points (assumed to represent the population of susceptible farms). This comparison was based on a very conservative assumption viz. that all susceptible farms were considered to be located within 10 km from the major road network.

The major roads layer was buffered at 10 kilometers, creating a polygon within which all points were at a distance ≤ 10 km from the major road line. Using the "CREATE RANDOM POINTS" command, an output file of 10 000 random points was generated using the 10-km buffer layers as a constraining feature. Then, using the procedure indicated above to randomly generate 10 000 points, the DNI of each point was generated.

Transmissibility assessment

The reproductive number (Rø) was estimated by applying a susceptible-exposed-infectious-removed (SEIR) transmission model to weekly numbers of avian influenza

cases, with a Bayesian estimation framework used previously to estimate the reproductive number for pandemic avian influenza (S1). The latency period was fixed to 1.9 days and the infectious period to 10 days (S2). The Bayesian framework of this model estimated the effective reproduction number over time, as well as its confidence intervals (S3) (Bettencourt, Ribeiro, Chowell, Lant and Castillo-Chavez, 2007; Chowell, Nishiura and Bettencourt, 2007).

Statistical analyses

Comparison of medians, correlation analysis and generation of random samples were conducted with *Minitab 14* (Minitab, State College, PA).

Data analysis strategy

To develop a decision-making oriented analysis, data were assessed both retrospectively and prospectively. The retrospective analysis was meant to assess methods that can monitor epidemic evolution and/or evaluate the efficacy of control measures. The goal of the prospective analysis was to test a method to be used in real time and within the critical response time (the time assumed to result in least costs and/or optimal benefits or first infectious period [S4]).

The retrospective analysis assessed 3 factors in relation to time: 1) the farm distance to the nearest major road (DNR), 2) the number of infected farms, and 3) transmissibility. Together, they were expected to answer whether the dispersal of AI occurred at random and, if not, whether the structure of major roads facilitated its dispersal.

2.3.1 Ecological Niche Modelling

The field data were collected using TADinfo® v 1.101 (EMPRES-FAO, 2006) and Garmin nuvi 370® GPS, (Garmin, Olathe, KS, USA). The georeferenced data collated were confirmed using Alexandria Digital Library Gazetteer (http://middleware.alexandria.ucsb.edu/client/gaz/adl/index.jsp), GEOnet Names Server (http://gnswww.nga.mil/geonames/GNS/index.jsp) and Rand McNally New Millennium World Atlas Deluxe (www.randmcnally.com). Stored data were electronically exported into Modelling by Desktop GARP (Genetic Algorithm for Rule-set Prediction) (http://www.nhm.ku.edu/desktopgarp/) and viewed using ArcGIS 9.2® software (ESRI, Redlands, CA, USA)

The following factors were considered in data analyses:

1. Virus biological and pathogenic characteristics,
2. Associated case occurrences of HPNAI H5N1 in relation to raster GIS layers that summarise variations in the ecological and environmental parameters (sets of conditions that govern the virus maintenance of its population and expansion of its geographical territories).

This process led to the generation of training data which were used for the predictions of risk of continued spread. The high predictivity of HPNAI H5N1 case distribution was based on the fact that one or more elements in the transmission cycle have strong ecological determination.

***Input data*.**

The principal suite of occurrence information for this study was HPNAI H5N1 case-occurrence data for January-April 2006 from the National Veterinary Research Institute, Nigeria, which consisted of 72 unique locations (including isolations from 2 wild birds, the remainder from poultry). Textual descriptions of occurrence localities were converted to geographic coordinates accurate to the nearest 0.001° using the Alexandria Digital Library Gazetteer; GEOnet Names Server; and other sources (Rand McNally 1998); duplicate occurrences at the same localities were discarded. Although the geographic coordinates assigned may not always fix the exposure point precisely, they represent a best guess as to its position, and likely are representative of the coarse-scale ecologic conditions under which the HPNAI H5N1 transmission occurs; if error in geo-referencing exists, the methods used are able to detect and ignore such problems.

Environmental data sets included 24 monthly composite remotely-sensed data layers for April 1992-March 1993 and February 1995-January 1996, in each case presenting values of the Normalized Difference Vegetation Index (NDVI; native spatial resolution 1 km). NDVI is derived from reflectance in the visible and near-infrared domains, and as such is sensitive to photosynthetic activity and is closely correlated with photosynthetic mass (Tucker 1979)—the time series of NDVI values used here thus profile differences in land cover and plant phenology across landscapes. This model also included 4 data sets summarizing aspects of topography-elevation, slope, aspect, and compound topographic index (which summarizes tendency to pool water)— from the U.S. Geological Survey's Hydro-1K data set, native resolution 1 km). Climate data were not included in these analyses for lack of sufficiently high-resolution data sets across the region of interest but

previous datasets were included to take into account any effects that these global climate phenomena might have on West African landscapes.

The test was developed based on subsets of available occurrence information set aside prior to model development. Of data provided to GARP, the program divides occurrence data randomly into 3 subsets: training data (for rule development), intrinsic testing data (for evaluation of rules), and extrinsic testing data. Spatial predictions of presence versus absence can include two types of error: false negatives (areas of actual presence predicted absent) and false positives (areas of actual absence predicted present) (Fielding and Bell 1997)—rule performance in each of these dimensions is evaluated via the intrinsic testing data set. Change in predictive accuracy from one iteration to the next are used to evaluate whether particular rules should be incorporated into the model or not, and the algorithm runs either 1000 iterations or until convergence (Stockwell and Peters 1999). The final rule-set is then used to query the environmental data sets to identify areas fitting the rule set predictions to produce a hypothesis of the potential geographic distribution of the species

Since GARP includes several random-walk elements, each replicate model developed produces distinct results, representing alternative solutions to the optimization challenge. Following proposed best-practices approaches (Anderson, Lew and Peterson, 2003), 100 replicates of each model were developed and filtered based on their error characteristics, retaining the 20 with lowest omission error, and out of the 20, the 10 closest to the median of proportional area predicted present were retained as an index of false-positive error rates (Anderson, *et al*., 2003). A consensus of these “best subset” models was then developed by summing values for each pixel in the map to produce final predictions of potential distributions with 11 thresholds (integers from 0 to 10).

The customary approaches to spatial model validation (e.g., receiver operating characteristic, kappa statistics) are not applicable to situations in which presence-only data are the only information available (Fielding and Bell 1997; Manel, Dias, Buckton and Ormerod, 1999). As such, the models were validated using simple calculations of binomial probabilities that coincidence of predictions and independent test data are no better than random, with the probability of k successes in n trials depending on p, the probability of success in any one trial. The p was estimated as the proportion of the testing area

predicted present, and *k* as the number of the *n* testing points that were successfully predicted (Anderson, *et al*., 2003). Binomial probabilities were calculated for each of the 10 thresholds representing predictions of presence (1 = broad, 10 = narrow), in each case testing whether predictivity is better than that expected by chance. In one case, the effects of spatial uncertainty regarding the localization of outbreak sites was explored by calculating success in predicting areas of presence within 100 m of known occurrence sites, adjusting *p* appropriately to reflect the broader area of potential presence.

Modelling approach*.*

This study focuses on the question of whether HPNAI H5N1 occurrences in West Africa follow a consistent and predictable environmental regime. As such, a series of tests of model predictivity were developed and in each case, the independent suites of occurrence data was used as bases with models developed and predictions tested. Model tests were based on subsets of the 2006 Nigerian occurrence data described above, as well as on 12 additional occurrences from November 2006 - January 2007 in Nigeria. The Nigerian models were also tested with occurrence data assembled from the archives of the International Society for Infectious Disease (ProMED Avian Influenza archive) for West Africa (14 occurrences; Figure 1): Burkina Faso (4 points), Ivory Coast (3), Ghana (2), Niger (2), and Cameroon (1), excludes 2 duplicated localities and 4 localities (3 from Niger, 1 from Ivory Coast) for which it was difficult to locate coordinates of the reported site. The basic design of testing was as follows.

1. *Predictivity across training landscape*: The 72 Nigerian occurrences in 2006 were randomly divided into 2 equal groups, one group was used for model development and the other for model testing (hereafter referred to as “RND” tests). The ability of 2006-based ENMs to predict the spatial distribution of cases from November 2006 - January 2007 (hereafter referred to as “YEAR” tests) was also tested. This scheme assesses the ability of the modelling approach to anticipate the spatial distribution of HP-H5N1 cases were sampling density to be increased, but across a region in which samples are already available.
2. *Predictivity across space (medium scale)*: The 72 Nigerian occurrences in 2006 were stratified spatially into quadrants above and below the median longitude and median latitude of the occurrence data. From this spatial stratification, 3 pairs of quadrants were developed: west versus east of the median longitude (hereafter referred to as “EW” tests), north versus south of the median latitude (hereafter

3. *Predictivity across space (broader scale)*: Nigeria-trained ENMs was projected onto the rest of West Africa, to test their spatial predictions via their coincidence with the 14 cases for which geographic coordinates were available in other West African nations (hereafter refered to as “WA” tests). These tests evaluated the ability of the ENMs to predict into even broader unsampled areas.

2.4 FINANCIAL COST IMPLICATIONS

For the financial cost evaluation of HPNAI in Nigeria, the actual situation and scenarios of mild (10%) and severe (70%) generalized outbreaks in the commercial flocks were selected.

In Nigeria, the commercial layer production is very important, accounting for almost 90% of all egg production (Adene and Oguntade, 2007). Similarly, ~99% of all infected poultry populations were commercial layers and layer breeders (Data retrieved from National Veterinary Research Institute, Nigeria). Our estimates deal only with this segment which often operates with little to no biosecurity.

A number of assumptions were made:

1. HPNAI caused 100% mortality in affected flocks either through pathologic death or control measures by destruction.
2. 100% cessation in egg production was assumed based on published reports (Capua and Marangon, 2000).
3. HPNAI caused a loss of 6 months in layer/layer breeder systems (downtime and raising new stock to point of lay).
4. Laying birds were in full production and would lay 284 eggs (80% production) for 1 laying cycle, and layer breeders would lay 265 eggs (75% production). 50% of the breeders’ offspring would have market value (pullet) and 50% would be cockerels with zero value. 200 chicks per breeder hen are expected

5. All deaths in the poultry population in Nigeria occurring during the period of study (January – August, 2006) arose from HPNAI or factors associated with it.

Other baseline data were obtained from Resource Inventory Management, Nigeria National Livestock Resource Survey and FAOSTAT-GLIPHA (FAO 2006a, b & c)

It is difficult to place an economic value on human beings affected by HPNAI. The affected human population was not economically assessed. Prevention of the spread of the disease in livestock would prevent its introduction in the human population.

Table 2.3. Types and number of birds affected between 10 January and 31 August, 2006

SPECIES AFFECTED	NUMBER	PERCENTAGE
Chicken: Layer/Pullet §	770,826	98.12
Chicken: Broiler/Cockerel	2,755	0.004
Chicken: Breeder	11,501	0.015
Guinea Fowl/quail	19	0.000024
Duck/Goose	148	0.000188
Ostrich*	218	0.000278
Turkey	101	0.000129
Wild Bird(multi species)	2	0.0000025
TOTAL	**785, 570**	**~100**

- § Include local , backyard and free range laying hens
- * Ostriches numbers were estimated based on available data

Table 2.4. Parameters used in assessing the economic impacts

s/no.	Description	Symbol	Basic Data	Actual Scenario	Mild Scenario	Severe Scenario
1	Population size at Risk (layers and breeders)	P	42,000,000	0.0056% (758,570)	10 % (4,200,000)	70% (29,400,000)
2	Susceptible population	S	100%	100%	100%	100%
3	Mortality/disposal		100%	100%	100%	100%
4	Commercial Layer population affected		37,800,000	774,069	3,780,000	26,460,000
5	Layer Breeder population affected		4,200,000	11,501	420,000	2,940,000
6	Total market value of adult birds {commercial layer at ~$7 and Layer Breeders at ~$27.30}	Layer	$264,600,000	$5,418,483	$26,460,000	$185,220,000
		Breeder	$114,660,000	$313,977	$11,466,000	$80,262,000
		Total	$379,260,000	**$5,732,460**	**$37,926,000**	**$265,482,000**
7	Value of eggs at ~$2.18 {layers only} & meat {old lay value-at ~$4.36/bird} per annum	Eggs	$601,549,200	$15,974,720	$60,154,920	$421,084,440
		Meat	$164,808,000	$3,374,941	$16,480,800	$115,365,600
		Total	$944,899,200	**$19,349,661**	**$76,635,720**	**$536,450,040**
8	Value of chicks expected		$294,163,424	$1,074,023	$29,416,342	$205,914,397
9	Proportion in production		75%	75%	75%	75%
10	Mean Egg price per tray*		N280 ($2.18)	N260 ($2.02)	N200 ($1.56)	≤N150
11	Delay in next production		Pre outbreak period	6 months	6 months	($1.16) 6 months

*Average egg price derived from field data collected before, during and after the crises period of outbreak. Note that the egg price per tray of 30 eggs was progressively dropping as the outbreak situation worsened.
Layer represents commercial layers, Breeders represent Layer breeders. Other data were derived from UNDP, 2006.

Table 2.5. Budgets and allocations for 2005 fiscal year

Department	Classification number	Expenditure items	2005 allocation	% estimated to be spent on HPNAI
FMA&RD	06200002501004	Publicity &advertisement	$22,757	50%
FMA&RD	02500002000240	(a)	$77,821	50%
FMA&RD	02500002000241	Animal disease control (b)	$77,821	50%
NVRI	02500002000202	National veterinary quarantine services (c)	$38,911	50%
NVRI	02500002000205	Strengthening of Central & outstation laboratories (d)	$155,642	100%
		Research and studies (avian influenza) (e)		
Total:			**$372,952**	**$264,297**

FMA&RD: Federal ministry of Agriculture and Rural Development, NVRI: National Veterinary Research Institute
Source: Nigerian Government (2006a & b)

Note that details of all calculations are inserted as Appendix C below

2.5 OPTION OF VACCINATION AS AN ADDITIONAL CONTROL MEASURE AGAINST AVIAN INFLUENZA H5N1

A two-times vaccination strategy combined with test and slaughter policy over a three year period was decided upon by using a decision tree analysis (DTA) (Figure 2.5, page 76). A choice of the most effective vaccination strategy for the country was based on national peculiarities (poor biosecurity, unorganized farming systems, poor veterinary infrastructures). Nobilis® Influenza H5 vaccine (H5N2) A/Chicken/Mexico/232/94/CPA (Intervet International, Boxmeer, The Netherlands) was selected as the model vaccine. The unit cost per dose of the vaccine for poultry was $0.06 based on data collated and figures available from the producer's website (http://www.medicalnewstoday.com/medicalnews.php?newsid, http://www.avian-influenza.com)

The final cost of vaccination was based on the total number of animals to be vaccinated, frequency of the vaccination, labour and distribution costs. Vaccination administration cost (labour) of $0.04/bird/dose (N5.00) was based on the prevailing market price of vaccination (for the procedure) in Nigeria. Other costs for distribution and administration of $156,128 (N20 million) per annum were based on the 2005 budget of the Federal Ministry of Agriculture and Rural Development, Nigeria (Animal Disease Control and National Veterinary Quarantine services).

Laboratory costs were based on the 2005 avian influenza research and studies budget of National Veterinary Research Institute, Nigeria while compensation and eradication estimates were based on adjusted compensation paid out to affected farmers by the government in 2006 (Nigerian Government website, www.nigeria.gov.ng/dbudget2005.pdf).

It was assumed that a 50% reduction in affected poultry population would be achieved in each year of the programme.

Graduated values for poultry population were arrived at over a three year period based on trends of production data available from the FAO statistical website (GLIPHA) (FAO, 2006b). It was estimated that 70% of the poultry population would covered at each vaccination (100% of commercial poultry population and approximately 50% of family poultry).

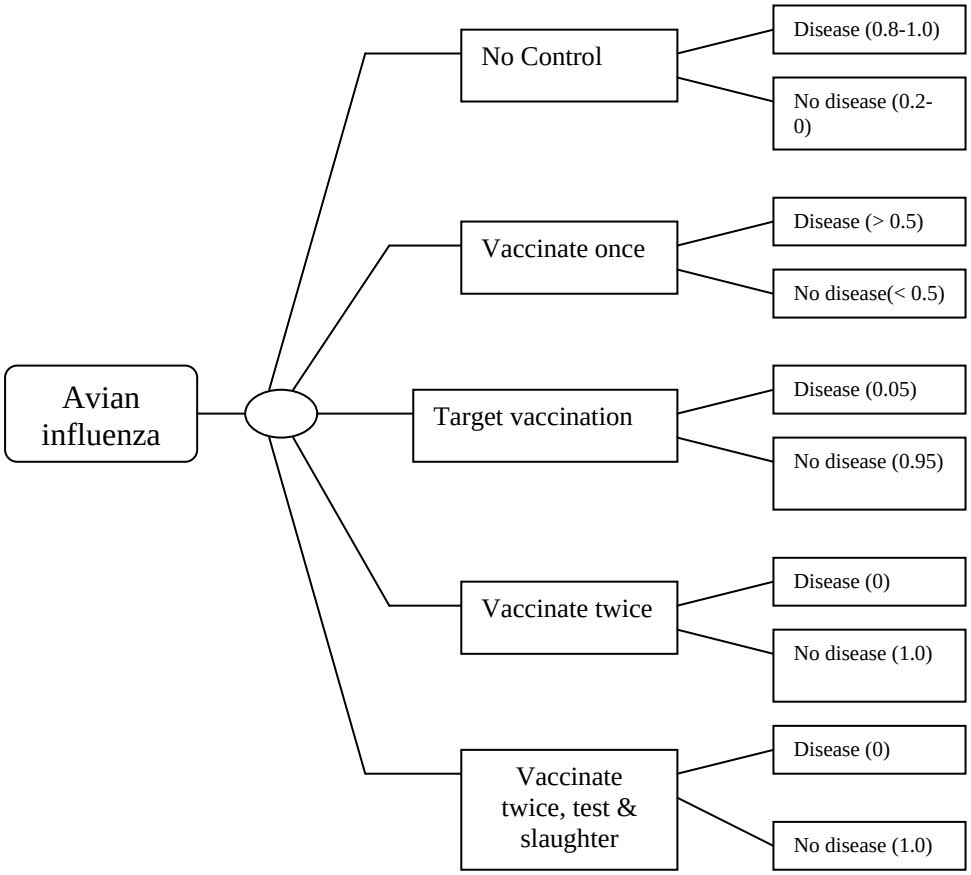
Associated benefits were evaluated by using established production indices, poultry population figures in Nigeria and other available records. It included reduced compensation per annum, prevention of egg production losses, regaining of regional trade in poultry meat, normalization of egg prices, evaluation of salvaged birds and prevention of redundancy of poultry facilities (Fasina, *et al.*, unpublished data). Most data were concentrated on layers (commercial and rural) since 98.1% of all HPAI affected birds in Nigeria were laying birds and approximately 85% of the national poultry flocks are layers.

Three years for effective control was chosen based on the Vietnamese example as reported by CIDRAP News Special Report 1 and 2 (http://www.cidrap.umn.edu/cidrap/content/influenza/avianflu/news/oct2506vietsuccess/html).

All currency conversion was done using Currency Converter on-line (http://finance.yahoo.com/currency/convert?amt= Accessed on 6th March, 2007).

The robustness of the values used was subjected to experts' opinion.

Details of all calculations are inserted as appendix D below



Decisions were taken based on the works of Ellis *et al.*, (2005) Gao *et al.*, (2006) and Hoelscher, *et al.*. (2006)

Figure 2.2. Decision Tree Analysis (DTA) for choice of intervention

Present Value of Benefit (PV_B) = $\frac{\text{Future Benefit (FB)}}{(1+r)^n}$

Present Value of Cost (PV_C) = $\frac{\text{Future Cost (FC)}}{(1+r)^n}$

Where “n” is number of period (years) and “r” is the periodic interest

Gross Benefit Cost Ratio (BCR) = $\frac{PV_B}{PV_C}$

CHAPTER THREE

RESULTS

3.1 DETERMINATION OF POULTRY DENSITY AND DISTRIBUTION IN NIGERIA

Based on the field data collected, Fig.3.1 represents the poultry population in Nigeria in 2005. Poultry densities and distributions in the different geographical locations had no obvious correlation with the dispersal of HPAI H5N1 in Nigeria from January 2006 to January 2007.

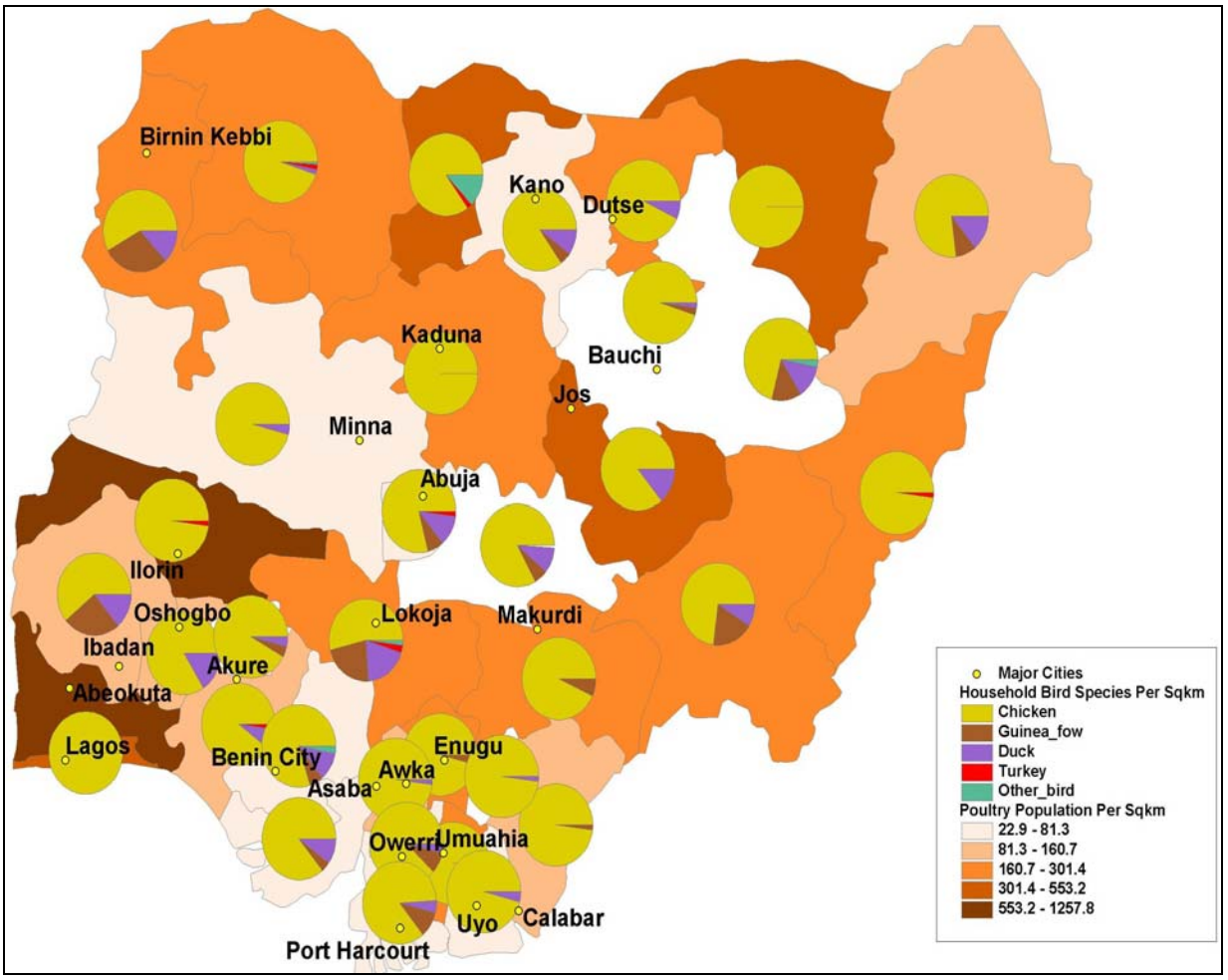


Figure 3.1. Poultry Density and Distribution in Nigeria as determined from collected field data, 2005

3.2 MOLECULAR EPIDEMIOLOGY

Six sub-lineages were identified amongst the Nigerian H5 genes based on branching order, molecular markers (nucleotide and amino acid) and bootstrap values. These six sub-lineages (A, D, E, F, G & H) (Fig. 3.3a, 3.3b & 3.3c, pages 61-63) were distributed in three previously described clades which circulated during the outbreaks of HPAI H5N1 in Nigeria in 2006 (Ducatez *et al*., 2006). Further characterisation using the 3′ end (478-nt base pair fragment 1156-1633) of the viruses resulted in three different groups (i, v and vii) (Fig. 3.3b, page 62).

The Nigerian viruses in sub-lineage A (n=3) were isolated around the 24th February, 2006 in the early course of HPAI H5N1 infection in Nigeria. This group of viruses showed a close genetic similarity to the Egyptian viruses which were isolated at about the same time viz 17th February, 2006. Whereas Nigerian viruses differ in positions K^{30} and E^{200}, Egyptian viruses contained a unique S^{250} residue (Fig.3.4a, pages 64-68).

The viruses in sub-lineage D (n=6) were all isolated in the first and second weeks of February 2006 at three different locations within the same Local Government area (LGA) (Jos North). This phylogenetic grouping is supported by a shared R^{339} residue in the partial amino acid alignment (Fig. 3.4a & b, pages 64-68, 69-70). These viruses were amongst the first isolated from the outbreaks in northern Nigeria. Although isolate A/chicken/Nigeria/VRD286/2006 (H5N1) grouped in sub-lineage D and was also found in the same LGA, it contained an L^{225}→M substitution and precipitated outbreaks some 8 weeks after the related viruses.

These earlier sub-lineages of Nigerian viruses (sub-lineage D) together with viruses from Burkina Faso, Cote d'Ivoire, and Sudan represent the widest diversity in term of geographical locations and host species among all of the viruses studied. This sub-lineage spread in the West African sub-region and up towards North Africa (Sudan). It is interesting to note that Sudanese viruses are not phylogenetically closely related to viruses from the infected geographical neighbour, Egypt (sub-lineage A). Ducatez *et al.* (2007) indicated that A/chicken/Sudan/1784/2006 has the maximum percentage nucleotide difference-(1.8% when compared to A/chicken/Egypt/5611NAMRU3-AN/2006)-

among the African viruses but the significance of this is unclear. Species affected by this sub-lineage D include commercial chickens, ducks, hooded vultures, turkeys and guinea fowl.

The isolates in sub-lineage E (n=2) (fig. 3.3a) were isolated from SW Nigeria and had also been previously reported (Ducatez *et al.*, 2006). These strains precipitated outbreaks around the first week of March, 2006. This sub-lineage was characterized by the following amino acid markers: T^{256}, I^{273} and A^{279}(Fig. 3.4, page 64-70).

Sub-lineage F (n=14) caused severe outbreaks in Northern Nigeria. These viruses were isolated between 9th February and 6th June, 2006. Most of these viruses shared 100% nucleotide sequence identities, except for A/chicken/Nigeria/VRD218/2006 (H5N1) and A/chicken/Nigeria/VRD219/2006 (H5N1) which shared an I^{205} residue in the partial amino acid alignment (Fig 3.4a, pages 64-68). Sub-lineage F strains viz A/chicken/Nigeria/VRD42/2006 (H5N1), A/chicken/Nigeria/VRD200/2006 (H5N1), A/chicken/Nigeria/VRD146/2006 (H5N1), A/chicken/Nigeria/VRD91/2006 (H5N1), A/chicken/Nigeria/VRD157/2006 (H5N1), A/chicken/Nigeria/VRD130/2006 (H5N1), A/chicken/Nigeria/VRD130b/2006 (H5N1), A/chicken/Nigeria/VRD244/2006 (H5N1) all have $V^{190}\rightarrow I$ and $A^{201}\rightarrow T$ substitutions. This group of viruses had a strong link to markets and major poultry trade routes within the country and shared common recent ancestors with viruses found in domestic ducks from Niger Republic, which had its first outbreak around the same time viz 13th February, 2006 (OIE, 2006b). There were previous reports by FAO (2006c) linking outbreaks in northern Nigeria to those reported in Gallaya Riga village, Niger Republic. The data presented here confirms this report.

Sub-lineage G (n=4) was involved in outbreaks in local turkeys, free range chickens, and commercial birds. These outbreaks occurred between 13th April in commercial birds and 8th June in local turkeys and free range chickens respectively. These viruses were phylogenetically related, sharing a G^{286}, although A/chicken/Nigeria/VRD284/2006 (H5N1) has $V^{40}\rightarrow D$ and $A^{351}\rightarrow T$ substitutions. They occurred within the same region in Plateau state.

Sub-lineage H (n=18) contains viruses isolated from market areas, distant locations and a diverse species of birds (chicken, vulture, wild life, guinea fowl, pigeon and local breeds of domesticated birds). They are most probably linked to the movement of poultry and poultry

products, as most of the outbreaks occurred when movement restrictions were absent or relaxed. Infected hatcheries and markets may also have played a role in the dissemination of these viruses. The amino acid sequences at the HA cleavage site viz ^{337}PQGERRRKKRG^{347} was identical to that of viruses from Western Europe, Western Asia and Africa (Egypt, Burkina Faso, Cote d'Ivoire, and Niger) with only a G^{339}→R substitution in: A/chicken/Nigeria/VRD42/2006(H5N1), A/chicken/Nigeria/VRD44/2006(H5N1) and A/chicken/Nigeria/VRD83/2006(H5N1).

Another African virus in group D A/chicken/Sudan/1784-7/06(H5N1) also had a G341→R substitution at this position (Fig. 3.4, page 64-70).
While Salzberg *et al*. (2007) indicated that A/chicken/Nigeria/1047-62/2006 was a reassortant generated in Africa; we identified four similar strains (according to homology with the haemagglutinin proteins): A/chicken/Nigeria/VRD35/2006, A/chicken/Nigeria/VRD44/2006, A/chicken/Nigeria/VRD83/2006 and A/chicken/Nigeria/VRD286/2006. The further spread of this group of viruses in poultry population and possibly mammals may increase the pandemic alert level as suggested by the World Health Organisation.

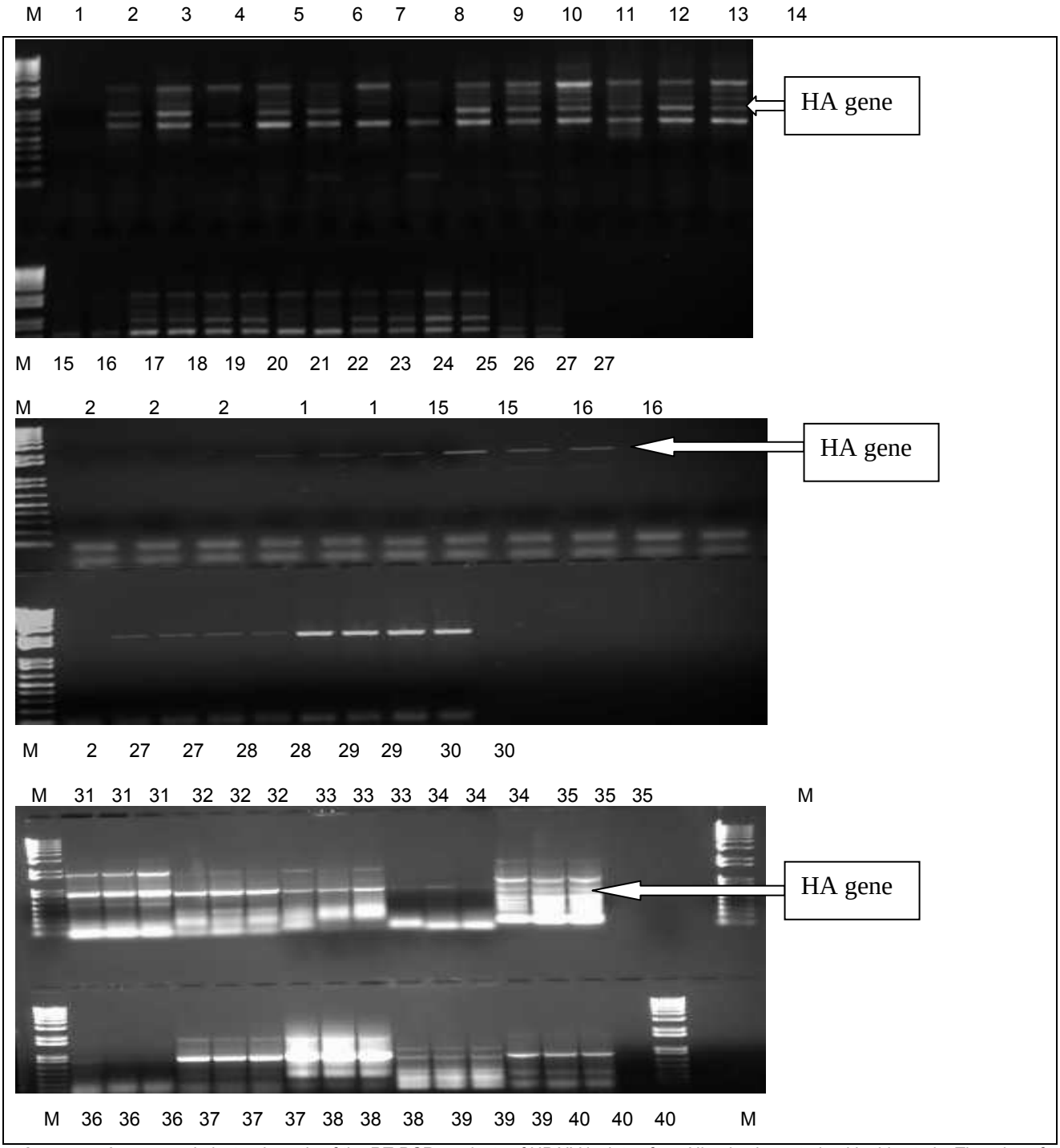


Figure 3.2. Agarose gel electrophoresis of the RT-PCR products of HPAIV isolates from Nigeria characterized in this study. The size of the target gene is 1633 *(~1700bp):VRD83/06; 2:VRD60/06; 3:VRD35/06; 4:VRD42/06; 5:VRD44/06; 6:VRD49/06; 7:VRD165/06; 8:VRD184/06; 9:VRD252/06; 10:VRD284/06; 11:VRD286/06; 12:VRD340/06; 13:VRD345/06; 14:VRD370/06; 15:VRD311/06; 16:VRD368/06; 17:VRD418/06; 18:VRD146/06; 19:VRD218/06; 20:VRD419/06; 21:VRD200/06; 22:VRD145/06; 23:VRD91/06; 24:VRD111/06; 25:VRD157/06; 26:VRD130/06; 27:VRD130b/06; 28:VRD244/06; 29:VRD184/06 repeat; 30:VRD219/06; 31:VRD262/06; 32:VRD005/07; 33:VRD158/06; 34:VRD203/06; 35:VRD193/06; 36:VRD496/06; 37:VRD216a/06; 38:VRD457/06; 39:VRD403/06; 40:VRD250/06). VRD is the unique identification number from the Viral Research Department, National Veterinary Research Institute, Nigeria.*

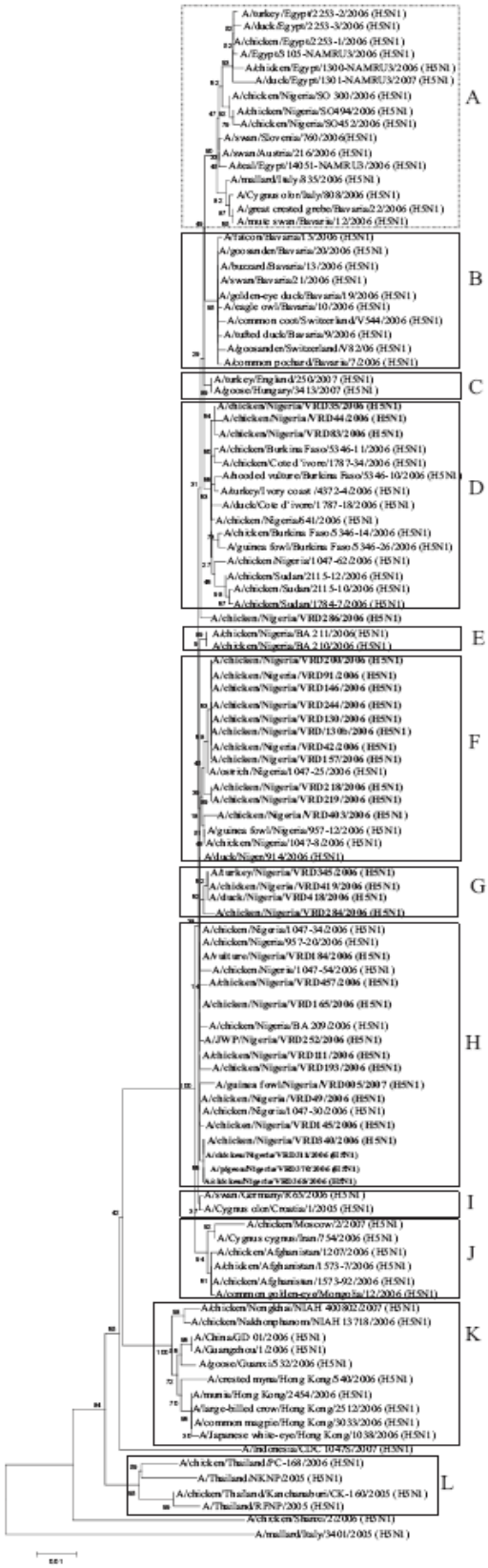


Figure 3.3a. *Dendogram of the neighbour joining tree of Nigerian isolates and reference viruses. The region analysed was a 1104 base pair fragment (nt 92-1196). This region was analysed separately to include all of the available African viruses. A separate phylogenetic analyses of a 478 base pair fragment (1156-1633) was done to study the 3' end of the haemagglutinin protein*

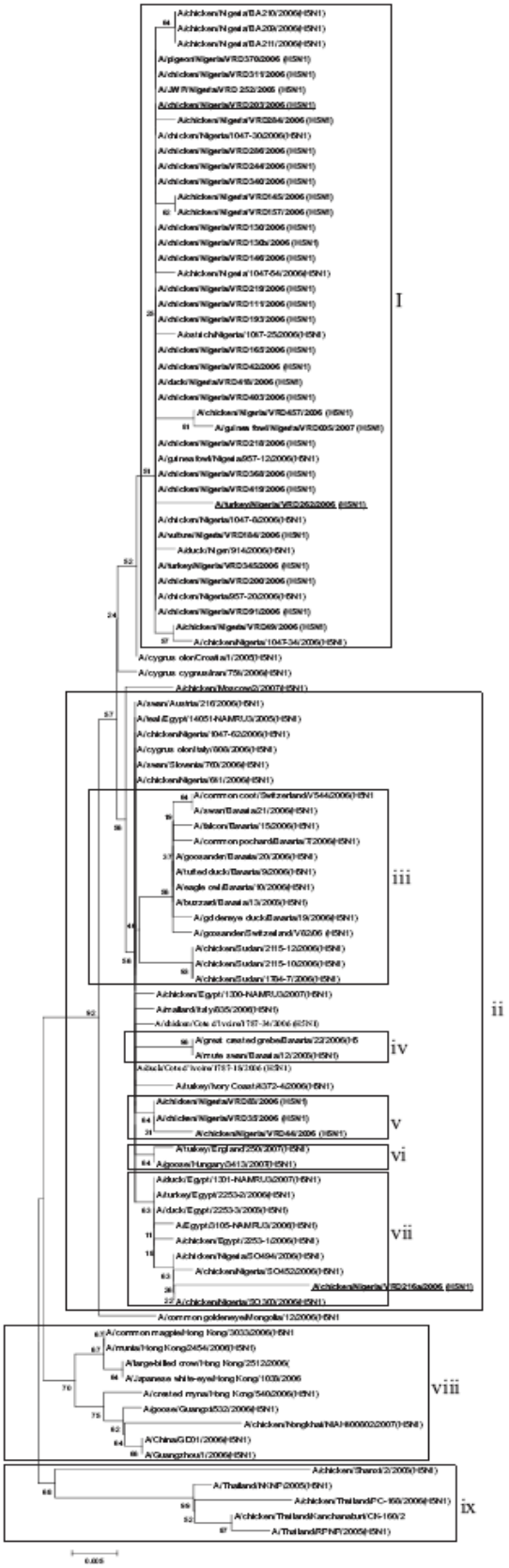


Figure 3.3b. *Dendogram of the neighbour joining tree of Nigerian isolates and reference viruses. The region analysed was a 478 base pair fragment (1156-1633) to study the 3' end of the haemagglutinin protein.*

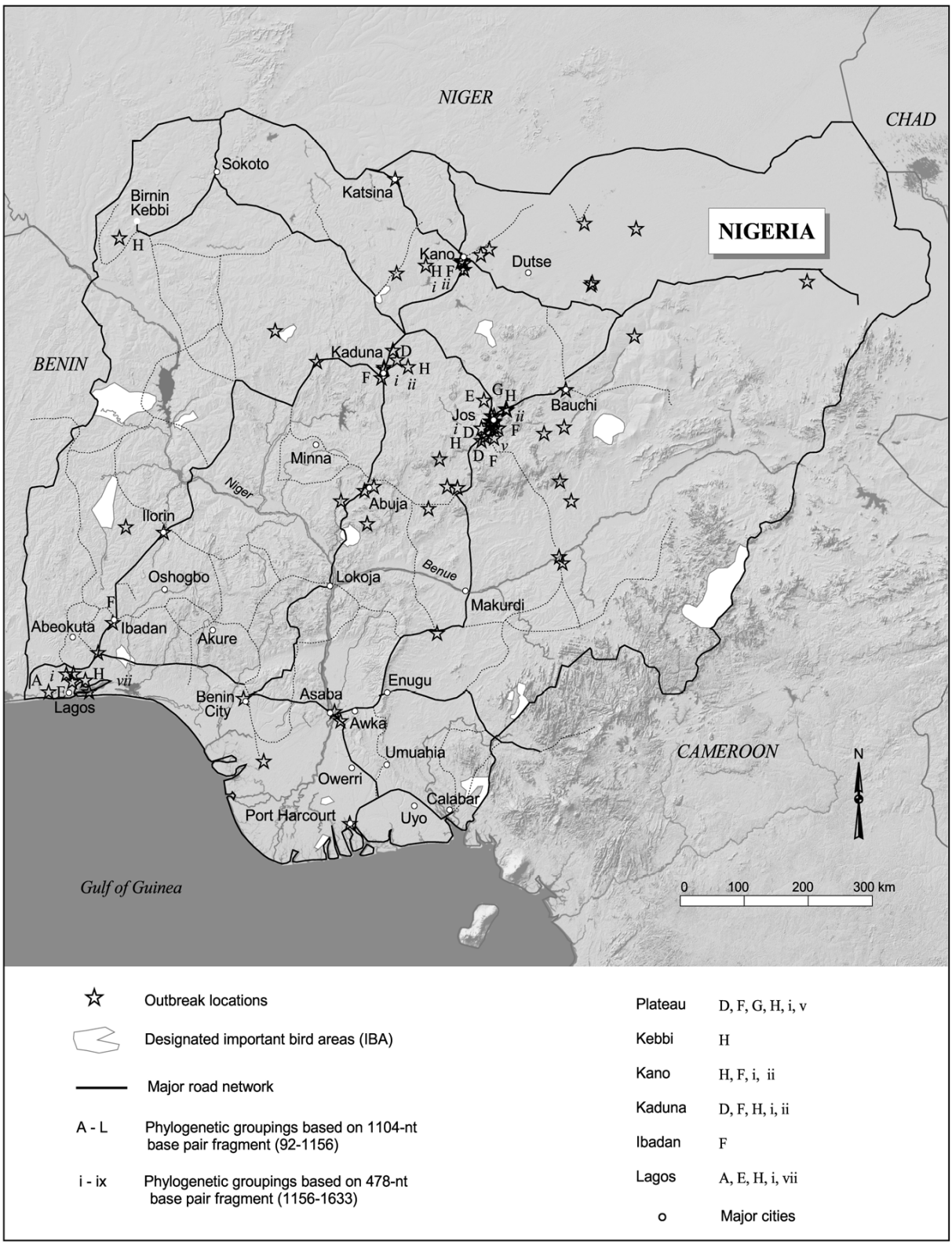


Figure 3.3c Nigerian map showing the Important Bird Areas (IBAs), origins of viruses used in the study, road networks and all outbreak areas.

Figure 3.4 a. Amino acid alignment of full-length H5 genes. The haemagglutinin peptide cleavage site (H_0) at position 337-347 is identified in box. Highlighted alignments indicate Nigerian viruses contributed by this study.

```
                                  30        40        50        60        70        80        90        100
                                  ..|....|....|....|....|....|....|....|....|....|....|....|....|....|..
A/chicken/Ng/VRD457/06 (H5N1)     YHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCDLDGVKPLILRDCSVAGWLLGNPMCDEFLNVPEWSYIVEKINPA
A/G fowl/Ng/VRD005/07 (H5N1)      ................................................................................
A/chicken/Ng/VRD83/06 (H5N1)      ................................................................................
A/chicken/Ng/VRD35/06 (H5N1)      ................................................................................
A/chicken/Ng/VRD42/06 (H5N1)      ................................................................................
A/chicken/Ng/VRD44/06 (H5N1)      ................................................................................
A/chicken/Ng/VRD49/06 (H5N1)      ................................................................................
A/chicken/Ng/VRD165/06 (H5N1)     ................................................................................
A/vulture/Ng/VRD184/06 (H5N1)     ................................................................................
A/JWP/Ng/VRD252/06 (H5N1)        ................................................................................
A/chicken/Ng/VRD284/06 (H5N1)     ................D...............................................................
A/chicken/Ng/VRD286/06 (H5N1)     ................................................................................
A/chicken/Ng/VRD340/06 (H5N1)     ................................................................................
A/turkey/Ng/VRD345/06 (H5N1)      ................................................................................
A/pigeon/Ng/VRD370/06 (H5N1)      ................................................................................
A/chicken/Ng/VRD311/06 (H5N1)     ................................................................................
A/chicken/Ng/VRD368/06 (H5N1)     ................................................................................
A/duck/Ng/VRD418/06 (H5N1)       ................................................................................
A/chicken/Ng/VRD146/06 (H5N1)     ................................................................................
A/chicken/Ng/VRD218/06 (H5N1)     ................................................................................
A/chicken/Ng/VRD419/06 (H5N1)     ................................................................................
A/chicken/Ng/VRD200/06 (H5N1)     ................................................................................
A/chicken/Ng/VRD145/06 (H5N1)     ................................................................................
A/chicken/Ng/VRD91/06 (H5N1)      ................................................................................
A/chicken/Ng/VRD111/06 (H5N1)     ................................................................................
A/chicken/Ng/VRD157/06 (H5N1)     ................................................................................
A/chicken/Ng/VRD130/06 (H5N1)     ................................................................................
A/chicken/Ng/VRD130b/06 (H5N1)    ................................................................................
A/chicken/Ng/VRD244/06 (H5N1)     ................................................................................
A/chicken/Ng/VRD219/06 (H5N1)     ................................................................................
A/chicken/Ng/VRD193/06 (H5N1)     ................................................................................
A/chicken/Ng/VRD403/06 (H5N1)     ................................................................................
A/chicken/BFaso/5346-14/06(H5N    ................................................................................
A/chicken/BFaso/5346-11/06(H5N    ................................................................................
A/hvulture/BFaso/5346-10/06(H5    ................................................................................
A/g fowl/BFaso/5346-26/06(H5N1    ................................................................................
A/mallard/Italy/3401/05(H5N1)     ..........................A.....S.N.....................................D..I
A/chicken/Sud/2115-10/06(H5N1)    ..................Q.............................................................
A/chicken/Afgh/1573-7/06(H5N1)    ................................................................................
A/turkey/ICoast/4372-4/06(H5N1    ................................................................................
A/chicken/Sud/2115-12/06(H5N1)    ................................................................................
A/turkey/Egy/2253-2/06(H5N1)      ................................................................................
A/chicken/Egy/2253-1/06(H5N1)     ................................................................................
A/chicken/Afgh/1573-92/06(H5N1    ................................................................................
A/C olor/Italy/808/06(H5N1)       ................................................................................
A/chicken/Moscow/2/07(H5N1)       ..........................A.....................................................
A/turkey/Eng/250/07(H5N1)         ................................................................................
A/duck/Egy/1301-NAMRU3/07(H5N1    ................................................................................
A/chken/Egy/1300-NAMRU3/07(H5N    ................................................................................
A/swan/Germany/R65/06(H5N1)       ................................................................................
A/Indonesia/CDC1047S/07(H5N1)     ..........................................K.................I...........A..T
A/China/GD01/2006(H5N1)           ..........................................................I...........A...
A/c coot/Switz/V544/06(H5N1)       ................................................................................
A/goosander/Switz/V82/06 (H5N1    ................................................................................
A/gc grebe/Bavaria/22/06(H5N1)    ................................................................................
A/swan/Bavaria/21/06(H5N1)        ................................................................................
A/tuft duck/Bavaria/9/06(H5N1)    ................................................................................
A/c pochard/Bavaria/7/06(H5N1)    ................................................................................
A/chicken/Thai/Ka/CK-160/05(H5    ..........................................................I...........A...
A/c g eye/Mongolia/12/06(H5N1)    ................................................................................
A/Guangzhou/1/06(H5N1)           ..........................................................I...........A...
A/teal/Egy/14051-NAMRU3/05(H5N    ................................................................................
A/chicken/Shanxi/2/06(H5N1)       ..........................................K.................I..S........AS..
A/J w-eye/H Kong/1038/06(H5N1)    ..........................................................I...........A...
A/c myna/H Kong/540/06(H5N1)      ..........................................................I...........A..V
A/g fowl/Nigeria/957-12/06(H5N    ................................................................................
A/Egy/3105-NAMRU3/06(H5N1)        ................................................................................
A/chicken/Ng/SO494/06(H5N1)       .......K........................................................................
A/chicken/Ng/SO452/06(H5N1)       .......K........................................................................
A/chicken/Ng/SO300/06(H5N1)       .......K........................................................................
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A/chicken/Ng/BA209/06(H5N1)       ................................................................................
A/duck/Niger/914/06(H5N1)         ................................................................................
A/chken/Cd'Ivoire/1787-34/06(H    ................................................................................
A/duck/Cd'Ivoire/1787-18/06(H5    ................................................................................
A/chicken/Ng/1047-34/06(H5N1)     ................................................................................
A/chicken/Ng/1047-30/06(H5N1)     ................................................................................
A/chicken/Ng/1047-62/06(H5N1)     ................................................................................
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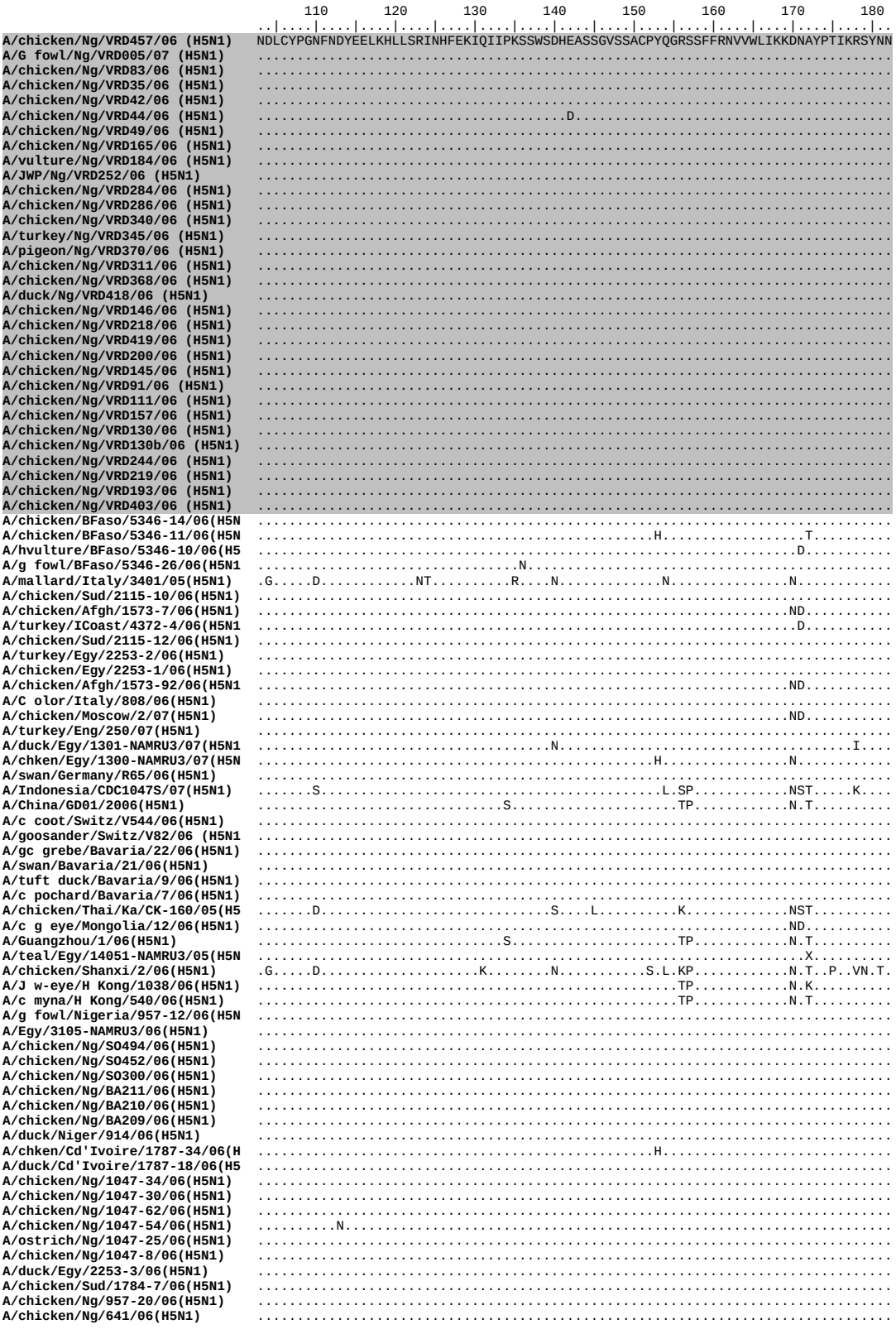
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A/G fowl/Ng/VRD005/07 (H5N1)
A/chicken/Ng/VRD83/06 (H5N1)
A/chicken/Ng/VRD35/06 (H5N1)
A/chicken/Ng/VRD42/06 (H5N1)
A/chicken/Ng/VRD44/06 (H5N1)
A/chicken/Ng/VRD49/06 (H5N1)
A/chicken/Ng/VRD165/06 (H5N1)
A/vulture/Ng/VRD184/06 (H5N1)
A/JWP/Ng/VRD252/06 (H5N1)
A/chicken/Ng/VRD284/06 (H5N1)
A/chicken/Ng/VRD286/06 (H5N1)
A/chicken/Ng/VRD340/06 (H5N1)
A/turkey/Ng/VRD345/06 (H5N1)
A/pigeon/Ng/VRD370/06 (H5N1)
A/chicken/Ng/VRD311/06 (H5N1)
A/chicken/Ng/VRD368/06 (H5N1)
A/duck/Ng/VRD418/06 (H5N1)
A/chicken/Ng/VRD146/06 (H5N1)
A/chicken/Ng/VRD218/06 (H5N1)
A/chicken/Ng/VRD419/06 (H5N1)
A/chicken/Ng/VRD200/06 (H5N1)
A/chicken/Ng/VRD145/06 (H5N1)
A/chicken/Ng/VRD91/06 (H5N1)
A/chicken/Ng/VRD111/06 (H5N1)
A/chicken/Ng/VRD157/06 (H5N1)
A/chicken/Ng/VRD130/06 (H5N1)
A/chicken/Ng/VRD130b/06 (H5N1)
A/chicken/Ng/VRD244/06 (H5N1)
A/chicken/Ng/VRD219/06 (H5N1)
A/chicken/Ng/VRD193/06 (H5N1)
A/chicken/Ng/VRD403/06 (H5N1)
A/chicken/BFaso/5346-14/06(H5N
A/chicken/BFaso/5346-11/06(H5N
A/hvulture/BFaso/5346-10/06(H5
A/g fowl/BFaso/5346-26/06(H5N1
A/mallard/Italy/3401/05(H5N1)
A/chicken/Sud/2115-10/06(H5N1)
A/chicken/Afgh/1573-7/06(H5N1)
A/turkey/ICoast/4372-4/06(H5N1
A/chicken/Sud/2115-12/06(H5N1)
A/turkey/Egy/2253-2/06(H5N1)
A/chicken/Egy/2253-1/06(H5N1)
A/chicken/Afgh/1573-92/06(H5N1
A/C olor/Italy/808/06(H5N1)
A/chicken/Moscow/2/07(H5N1)
A/turkey/Eng/250/07(H5N1)
A/duck/Egy/1301-NAMRU3/07(H5N1
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A/swan/Germany/R65/06(H5N1)
A/Indonesia/CDC1047S/07(H5N1)
A/China/GD01/2006(H5N1)
A/c coot/Switz/V544/06(H5N1)
A/goosander/Switz/V82/06 (H5N1
A/gc grebe/Bavaria/22/06(H5N1)
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A/tuft duck/Bavaria/9/06(H5N1)
A/c pochard/Bavaria/7/06(H5N1)
A/chicken/Thai/Ka/CK-160/05(H5
A/c g eye/Mongolia/12/06(H5N1)
A/Guangzhou/1/06(H5N1)
A/teal/Egy/14051-NAMRU3/05(H5N
A/chicken/Shanxi/2/06(H5N1)
A/J w-eye/H Kong/1038/06(H5N1)
A/c myna/H Kong/540/06(H5N1)
A/g fowl/Nigeria/957-12/06(H5N
A/Egy/3105-NAMRU3/06(H5N1)
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A/chicken/Ng/SO452/06(H5N1)
A/chicken/Ng/SO300/06(H5N1)
A/chicken/Ng/BA211/06(H5N1)
A/chicken/Ng/BA210/06(H5N1)
A/chicken/Ng/BA209/06(H5N1)
A/duck/Niger/914/06(H5N1)
A/chken/Cd'Ivoire/1787-34/06(H
A/duck/Cd'Ivoire/1787-18/06(H5
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A/chicken/Ng/1047-30/06(H5N1)
A/chicken/Ng/1047-62/06(H5N1)
A/chicken/Ng/1047-54/06(H5N1)
A/ostrich/Ng/1047-25/06(H5N1)
A/chicken/Ng/1047-8/06(H5N1)
A/duck/Egy/2253-3/06(H5N1)
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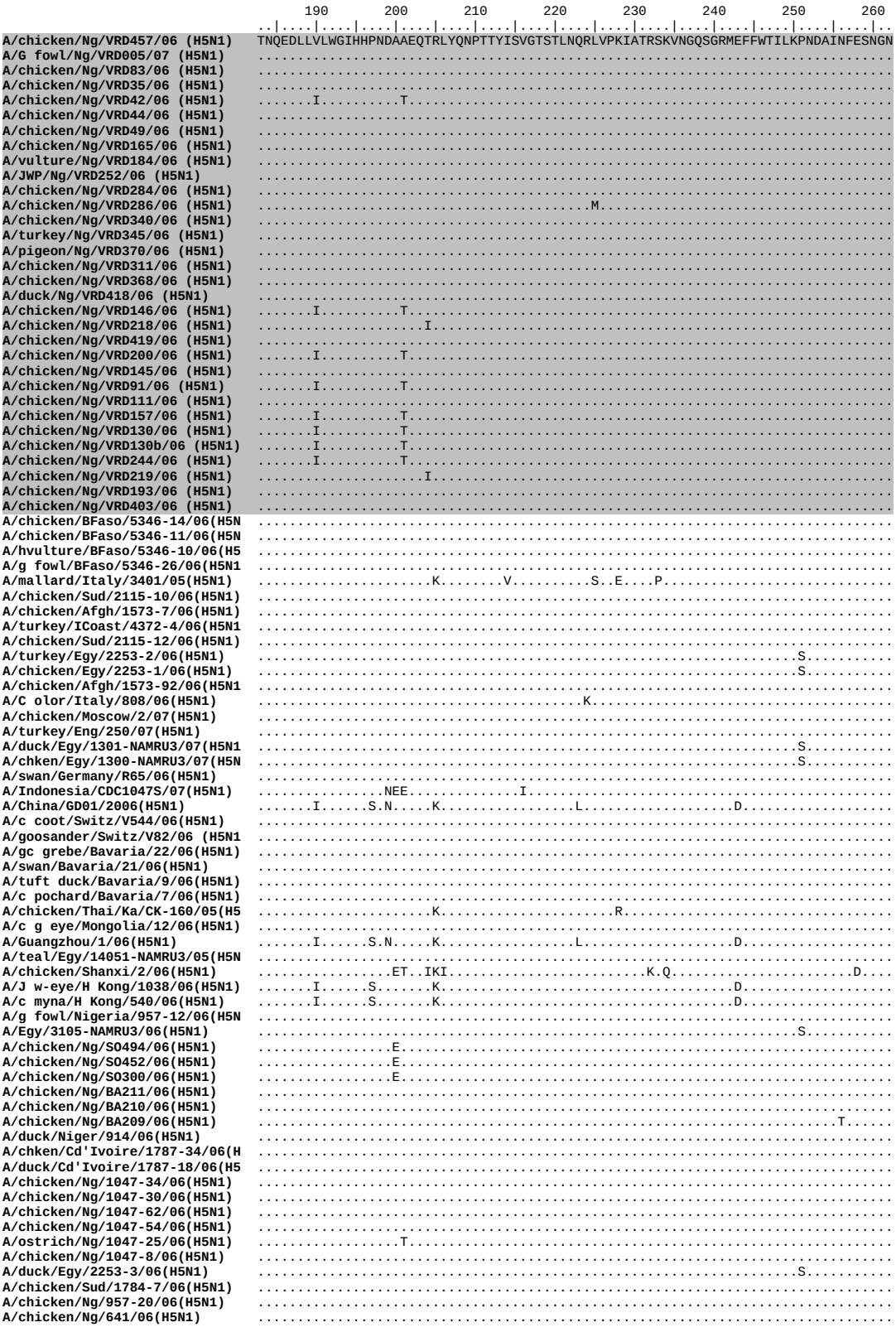
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A/chicken/Ng/VRD42/06 (H5N1)
A/chicken/Ng/VRD44/06 (H5N1)
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A/chicken/Ng/VRD340/06 (H5N1)
A/turkey/Ng/VRD345/06 (H5N1)
A/pigeon/Ng/VRD370/06 (H5N1)
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A/chicken/Ng/VRD146/06 (H5N1)
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A/chicken/BFaso/5346-11/06(H5N
A/hvulture/BFaso/5346-10/06(H5
A/g fowl/BFaso/5346-26/06(H5N1
A/mallard/Italy/3401/05(H5N1)
A/chicken/Sud/2115-10/06(H5N1)
A/chicken/Afgh/1573-7/06(H5N1)
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A/turkey/Egy/2253-2/06(H5N1)
A/chicken/Egy/2253-1/06(H5N1)
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A/C olor/Italy/808/06(H5N1)
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A/turkey/Eng/250/07(H5N1)
A/duck/Egy/1301-NAMRU3/07(H5N1
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A/Indonesia/CDC1047S/07(H5N1)
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A/c coot/Switz/V544/06(H5N1)
A/goosander/Switz/V82/06 (H5N1
A/gc grebe/Bavaria/22/06(H5N1)
A/swan/Bavaria/21/06(H5N1)
A/tuft duck/Bavaria/9/06(H5N1)
A/c pochard/Bavaria/7/06(H5N1)
A/chicken/Thai/Ka/CK-160/05(H5
A/c g eye/Mongolia/12/06(H5N1)
A/Guangzhou/1/06(H5N1)
A/teal/Egy/14051-NAMRU3/05(H5N
A/chicken/Shanxi/2/06(H5N1)
A/J w-eye/H Kong/1038/06(H5N1)
A/c myna/H Kong/540/06(H5N1)
A/g fowl/Nigeria/957-12/06(H5N
A/Egy/3105-NAMRU3/06(H5N1)
A/chicken/Ng/SO494/06(H5N1)
A/chicken/Ng/SO452/06(H5N1)
A/chicken/Ng/SO300/06(H5N1)
A/chicken/Ng/BA211/06(H5N1)
A/chicken/Ng/BA210/06(H5N1)
A/chicken/Ng/BA209/06(H5N1)
A/duck/Niger/914/06(H5N1)
A/chken/Cd'Ivoire/1787-34/06(H
A/duck/Cd'Ivoire/1787-18/06(H5
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A/chicken/Ng/1047-30/06(H5N1)
A/chicken/Ng/1047-62/06(H5N1)
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A/ostrich/Ng/1047-25/06(H5N1)
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A/duck/Egy/2253-3/06(H5N1)
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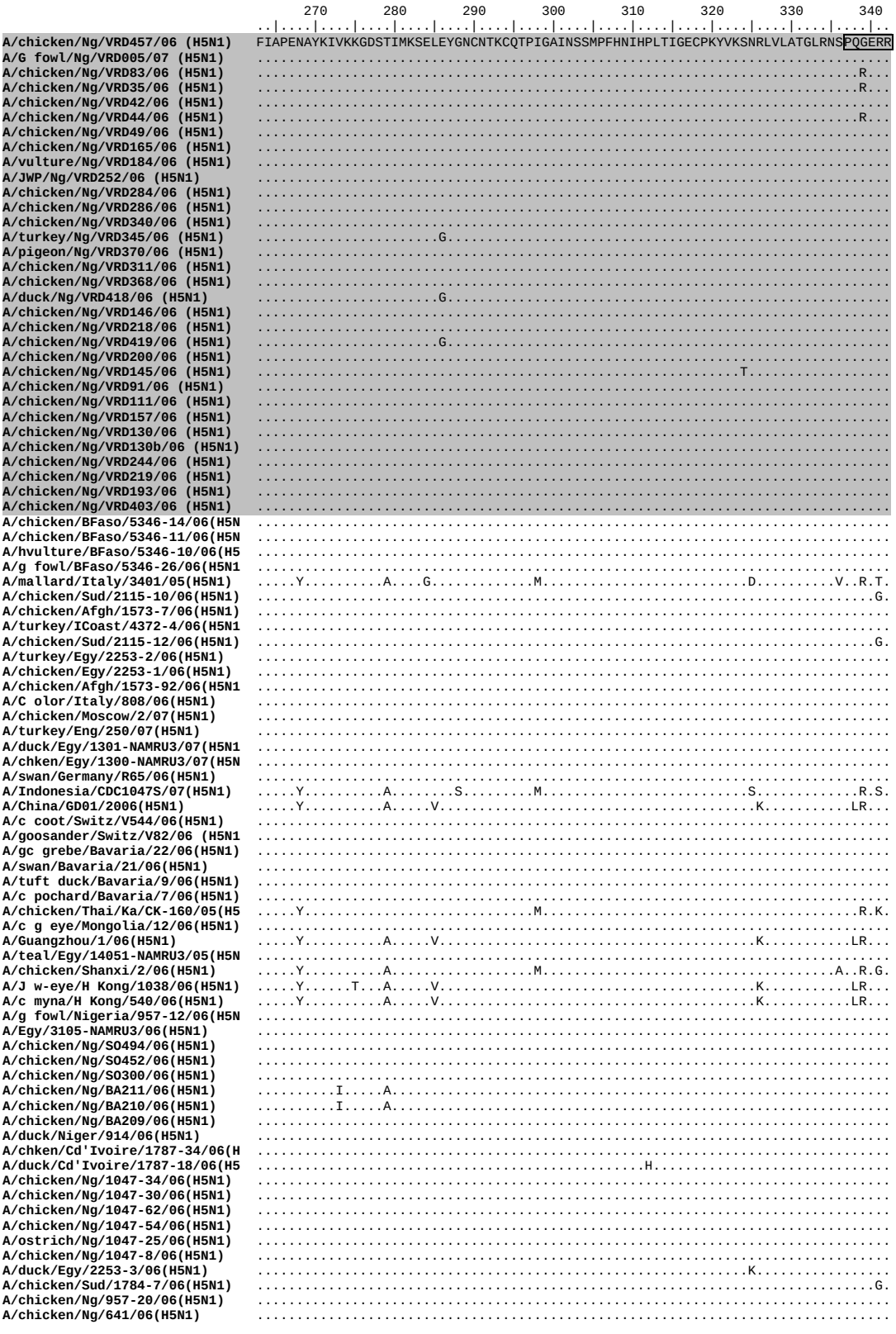
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A/chicken/Ng/VRD83/06 (H5N1)
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A/chicken/Ng/VRD42/06 (H5N1)
A/chicken/Ng/VRD44/06 (H5N1)
A/chicken/Ng/VRD49/06 (H5N1)
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A/vulture/Ng/VRD184/06 (H5N1)
A/JWP/Ng/VRD252/06 (H5N1)
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A/turkey/Ng/VRD345/06 (H5N1)
A/pigeon/Ng/VRD370/06 (H5N1)
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A/g fowl/BFaso/5346-26/06(H5N1
A/mallard/Italy/3401/05(H5N1)
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A/goosander/Switz/V82/06 (H5N1
A/gc grebe/Bavaria/22/06(H5N1)
A/swan/Bavaria/21/06(H5N1)
A/tuft duck/Bavaria/9/06(H5N1)
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A/c g eye/Mongolia/12/06(H5N1)
A/Guangzhou/1/06(H5N1)
A/teal/Egy/14051-NAMRU3/05(H5N
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A/J w-eye/H Kong/1038/06(H5N1)
A/c myna/H Kong/540/06(H5N1)
A/g fowl/Nigeria/957-12/06(H5N
A/Egy/3105-NAMRU3/06(H5N1)
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A/duck/Niger/914/06(H5N1)
A/chken/Cd'Ivoire/1787-34/06(H
A/duck/Cd'Ivoire/1787-18/06(H5
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A/ostrich/Ng/1047-25/06(H5N1)
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A/chicken/Ng/VRD403/06 (H5N1)   ........................................................
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A/chicken/BFaso/5346-11/06(H5N  ........................................................
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A/g fowl/BFaso/5346-26/06(H5N1  ........................................................
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A/Indonesia/CDC1047S/07(H5N1)   ........................................................
A/China/GD01/2006(H5N1)         ........................................................
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A/goosander/Switz/V82/06 (H5N1  ........................................................
A/gc grebe/Bavaria/22/06(H5N1)  ........................................................
A/swan/Bavaria/21/06(H5N1)      ........................................................
A/tuft duck/Bavaria/9/06(H5N1)  ......................................K.....
A/c pochard/Bavaria/7/06(H5N1)  ........................................................
A/chicken/Thai/Ka/CK-160/05(H5  ........................................................
A/c g eye/Mongolia/12/06(H5N1)  ........................................................
A/Guangzhou/1/06(H5N1)         ........................................................
A/teal/Egy/14051-NAMRU3/05(H5N  ........................................................
A/chicken/Shanxi/2/06(H5N1)     ....................................S.......
A/J w-eye/H Kong/1038/06(H5N1)  ........................................................
A/c myna/H Kong/540/06(H5N1)    ........................................................
A/g fowl/Nigeria/957-12/06(H5N  ........................................................
A/Egy/3105-NAMRU3/06(H5N1)      ........................................................
A/chicken/Ng/SO494/06(H5N1)     ........................................................
A/chicken/Ng/SO452/06(H5N1)     ........................................................
A/chicken/Ng/SO300/06(H5N1)     ........................................................
A/chicken/Ng/BA211/06(H5N1)     ........................................................
A/chicken/Ng/BA210/06(H5N1)     ........................................................
A/chicken/Ng/BA209/06(H5N1)     ........................................................
A/duck/Niger/914/06(H5N1)       ........................................................
A/chken/Cd'Ivoire/1787-34/06(H  ........................................................
A/duck/Cd'Ivoire/1787-18/06(H5  ........................................................
A/chicken/Ng/1047-34/06(H5N1)   ........................................................
A/chicken/Ng/1047-30/06(H5N1)   ........................................................
A/chicken/Ng/1047-62/06(H5N1)   ........................................................
A/chicken/Ng/1047-54/06(H5N1)   ........................................................
A/ostrich/Ng/1047-25/06(H5N1)   ........................................................
A/chicken/Ng/1047-8/06(H5N1)    ........................................................
A/duck/Egy/2253-3/06(H5N1)      ........................................................
A/chicken/Sud/1784-7/06(H5N1)   ........................................................
A/chicken/Ng/957-20/06(H5N1)    ........................................................
A/chicken/Ng/641/06(H5N1)       ........................................................
```

Figure 3.4.b **Continuation of the amino acid alignment of full-length H5 genes. The haemagglutinin peptide cleavage site (H_0) at position 337-347 is identified in box. Highlighted alignments indicate Nigerian viruses contributed by this study**.

```
                                  10        20        30        40        50        60        70        80
                         ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
A/chicken/Nig/VRD457/06(H5N1)    TNKVNSIIDKMNTQFEAVGREFNNLERRIENLNKKMEDGFLDVWTYNAELLVLMENERTLDFHDSNVKNLYDKVRLQLRD
A/G fowl/Ng/VRD005/07(H5N1)      ................................................................................
A/chicken/Nig/VRD83/06 (H5N1)    ................................................................................
A/chicken/Nig/VRD35/06 (H5N1)    ................................................................................
A/chicken/Nig/VRD42/06 (H5N1)    ................................................................................
A/chicken/Nig/VRD44/06 (H5N1)    ...............................................................................N
A/chicken/Nig/VRD49/06 (H5N1)    ................................................................................
A/chicken/Nig/VRD165/06(H5N1)    ................................................................................
A/vulture/Nig/VRD184/06(H5N1)    ................................................................................
A/JWP/Nig/VRD 252/06 (H5N1)     ................................................................................
A/chicken/Nig/VRD284/06(H5N1)    ................................................................................
A/chicken/Nig/VRD286/06(H5N1)    ................................................................................
A/chicken/Nig/VRD340/06(H5N1)    ................................................................................
A/turkey/Nig/VRD345/06 (H5N1)    ................................................................................
A/pigeon/Nig/VRD370/06 (H5N1)    ................................................................................
A/chicken/Nig/VRD311/06(H5N1)    ................................................................................
A/chicken/Nig/VRD368/06(H5N1)    ................................................................................
A/duck/Nig/VRD418/06 (H5N1)     ................................................................................
A/chicken/Nig/VRD146/06(H5N1)    ................................................................................
A/chicken/Nig/VRD218/06(H5N1)    ................................................................................
A/chicken/Nig/VRD419/06(H5N1)    ................................................................................
A/chicken/Nig/VRD200/06(H5N1)    ................................................................................
A/chicken/Nig/VRD145/06(H5N1)    ................................................................................
A/chicken/Nig/VRD91/06 (H5N1)    ................................................................................
A/chicken/Nig/VRD111/06(H5N1)    ................................................................................
A/chicken/Nig/VRD157/06(H5N1)    ................................................................................
A/chicken/Nig/VRD130/06(H5N1)    ................................................................................
A/chicken/Nig/VRD130b/06(H5N1)   ................................................................................
A/chicken/Nig/VRD244/06(H5N1)    ................................................................................
A/chicken/Nig/VRD219/06(H5N1)    ................................................................................
A/chicken/Nig/VRD193/06(H5N1)    ................................................................................
A/chicken/Nig/VRD403/06(H5N1)    ................................................................................
A/chicken/Nig/VRD203/06(H5N1)    ................................................................................
A/chicken/Nig/VRD216a/06(H5N1)   ..................K......K.......KKE.....................................
A/turkey/Nig/VRD262/06 (H5N1)    ................................................................................
A/chicken/Sudan/2115-10/2006(H   ................................................................................
A/turkey/Ivory Coast/4372-4/20    ................................V...............................................
A/chicken/Sudan/2115-12/2006(H   ................................................................................
A/turkey/Egypt/2253-2/2006(H5N   ................................................................................
A/chicken/Egypt/2253-1/2006(H5   ................................................................................
A/cygnus olor/Italy/808/2006(H   ................................................................................
A/chicken/Moscow/2/2007(H5N1)   ................................................................................
A/turkey/England/250/2007(H5N1   ................................................................................
A/duck/Egypt/1301-NAMRU3/2007(  ................................................................................
A/chicken/Egypt/1300-NAMRU3/20  ................................................................................
A/China/GD01/2006(H5N1)         ................................................................................
A/common coot/Switzerland/V544  ................................................................................
A/goosander/Switzerland/V82/06  ................................................................................
A/great crested grebe/Bavaria/   ................................................................................
A/swan/Bavaria/21/2006(H5N1)    ................................................................................
A/tufted duck/Bavaria/9/2006(H   ................................................................................
A/common pochard/Bavaria/7/200  ................................................................................
A/chicken/Thailand/Kanchanabur  ................................................................................
A/common goldeneye/Mongolia/12 ................................................................................
A/Guangzhou/1/2006(H5N1)       ................................................................................
A/teal/Egypt/14051-NAMRU3/2005  ................................................................................
A/chicken/Shanxi/2/2006(H5N1)   ..............G.V..I...............................................................
A/Japanese white-eye/Hong Kong  ................................................................................
A/crested myna/Hong Kong/540/2  ................................................................................
A/guinea fowl/Nigeria/957-12/2   ................................................................................
A/Egypt/3105-NAMRU3/2006(H5N1) ................................................................................
A/chicken/Nigeria/SO494/2006(H  ................................................................................
A/chicken/Nigeria/SO452/2006(H  ................................................................................
A/chicken/Nigeria/SO300/2006(H  ................................................................................
A/chicken/Nigeria/BA211/2006(H  ................................................................................
A/chicken/Nigeria/BA210/2006(H  ................................................................................
A/chicken/Nigeria/BA209/2006(H  ................................................................................
A/duck/Niger/914/2006(H5N1)     ................................................................................
A/chicken/Cote d'Ivoire/1787-3   ................................................................................
A/duck/Cote d'Ivoire/1787-18/2   ................................................................................
A/chicken/Nigeria/1047-34/2006   ................................................................................
A/chicken/Nigeria/1047-30/2006   ................................................................................
A/chicken/Nigeria/1047-62/2006   ................................................................................
A/chicken/Nigeria/1047-54/2006   ................................................................................
A/ostrich/Nigeria/1047-25/2006   ................................................................................
A/chicken/Nigeria/1047-8/2006(  ................................................................................
A/duck/Egypt/2253-3/2006(H5N1)  ................................................................................
A/chicken/Sudan/1784-7/2006(H5  ................................................................................
A/chicken/Nigeria/957-20/2006(  ................................................................................
A/chicken/Nigeria/641/2006(H5N  ................................................................................
```

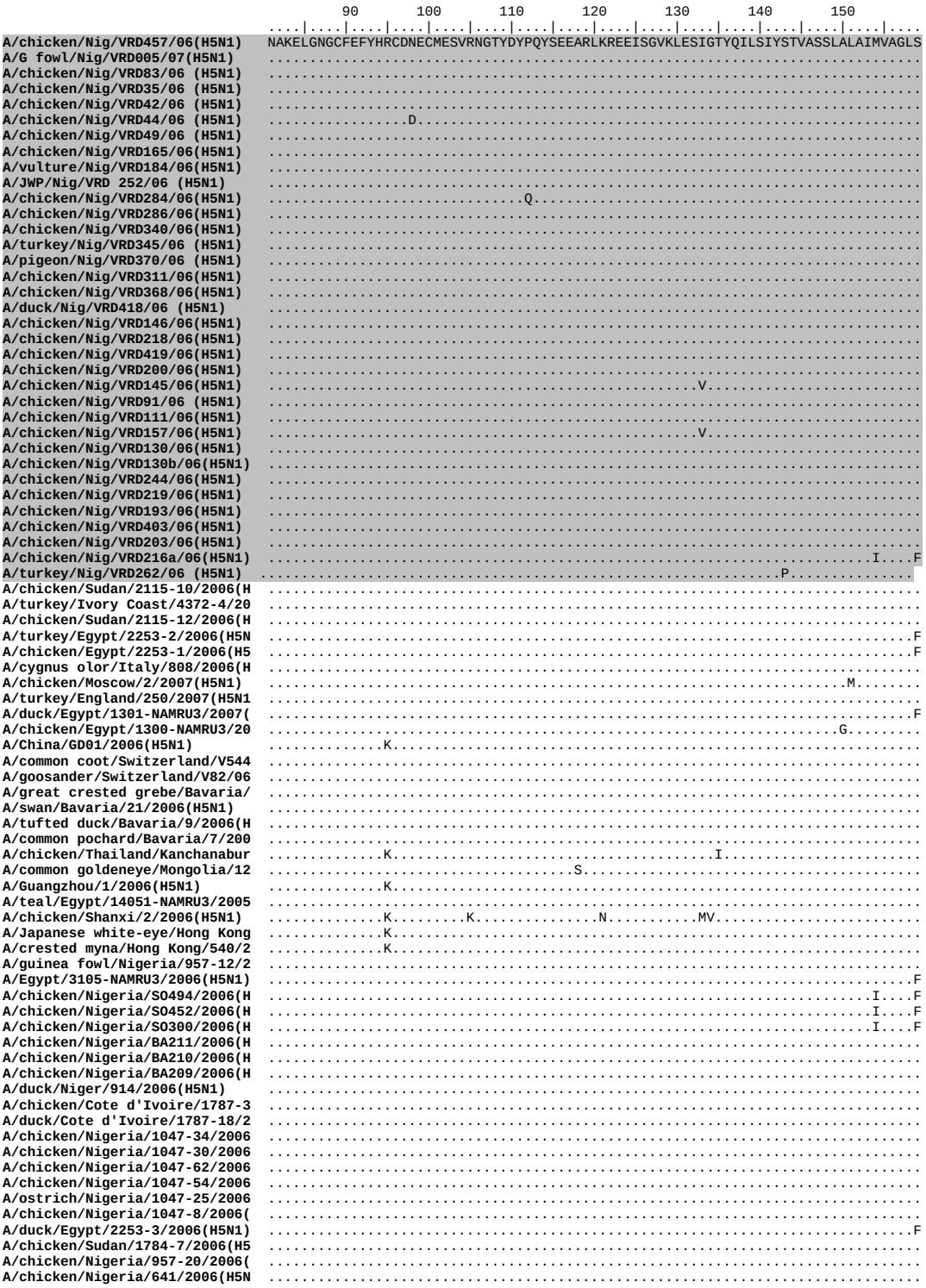


Figure 3.4 b. **Amino acid alignment of full-length H5 genes. The haemagglutinin peptide cleavage site (H_0) at position 337-347 is identified in box. Highlighted alignments indicate Nigerian viruses contributed by this study**. *(The longer sequence ending at haemagglutinin peptide position 389 were analysed to include all of the viruses from Africa).*

3.3 RESULTS OF THE ANALYSES USING GEOGRAPHICAL INFORMATION SYSTEM

DNR did not differ significantly over time (Fig. 3.5a). This indicated that the average DNR was independent of time in the first 24 weeks of the epidemic.

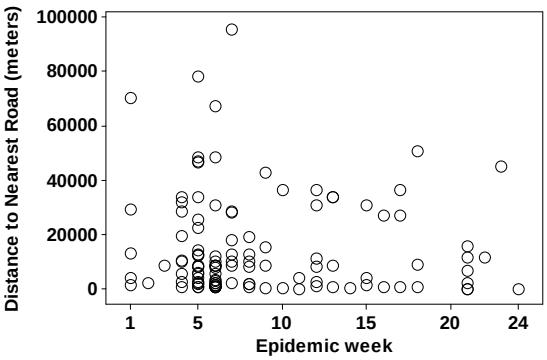


Fig. 3.5a. Plot of time (epidemic week) against distance of infected farm to the nearest major road. Pearson correlation r = – 0.062, P = 0.517 (n=113 farms)

In contrast, the (log) farm distance to the nearest road (DNR) was negatively correlated with the number of infected farms (Fig. 3.5b). This indicated that as the farm DNR decreased, the number of infected farms increased, finding that suggested proximity to the road network facilitated AI dispersal.

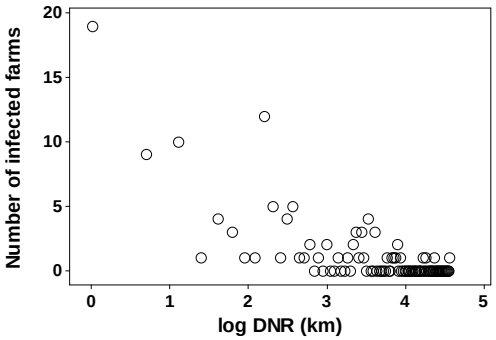


Fig.3.5b. Plot of number of infected farms against (log) DNR. Pearson correlation r = – 0.72, P < 0.0001 (n=113 farms)

Transmissibility, as estimated by Rø, differed across states. Six patterns were observed (Fig. 3.5c). They suggested that AI dispersal differed over space and time. This assessment, if conducted on weekly basis, could monitor the efficacy of control measures and/or lead to different measures (regionalization).

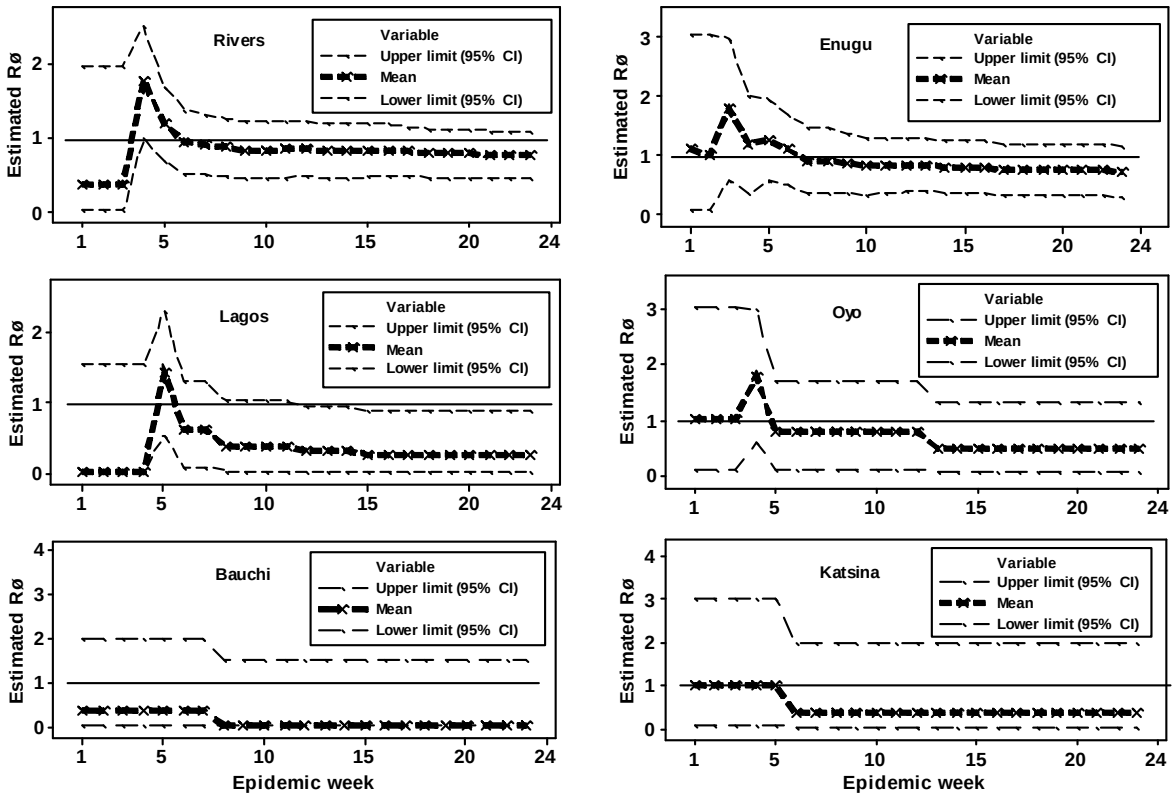


Figure 3.5c Transmissibility, as estimated by Rø, differed across states. Six patterns were observed. They suggested that AI dispersal differed over space and time. This assessment, if conducted on weekly basis, could monitor the efficacy of control measures and/or lead to different measures (regionalization).

AI transmisibility in Nigerian states. Six states, whose profiles are representative of the 15 states where AI cases were reported, are shown. A: an epidemic peak was observed in Rivers state at week four (one week before the national average shown in Fig. 3.5c); the upper limit of the confidence interval for Rø was above 1 in all 24 epidemic weeks. Except for the lack of an epidemic peak (not shown by the upper limit of the confidence interval for Rø), a similar profile was observed in the Enugu state (B). Lagos state displayed a later epidemic peak (week 5), and neither the mean nor the upper limit of the confidence interval for Rø displayed values above 1 (C). After epidemic week 12, a profile resembling that of Enugu state was observed in Oyo state, where the upper limit of the confidence interval for Rø displayed no epidemic peak (D). While neither Bauchi (E) nor Katsina (F) states showed epidemic peaks, both revealed an upper limit of the confidence interval for Rø above 1 throughout the first 24 epidemic weeks.

A future-oriented test, based exclusively on what was learned or available by epidemic day 4, attempted to generate an inference upon which decision-making could be made and implemented before the first infectious period (estimated to be ≤10 days) ended. The goal

was to identify a factor or factors associated with epidemic dispersal in order to be controlled.

As commonly observed in outbreaks of infectious diseases, early cases were few in number and did not always occur (or were reported) daily. In addition, the actual location of susceptible farms was unknown. In spite of these limitations, decisions, to be effective (low cost/high benefit) need to be implemented very early. This data and those presented above (Fig. 3.6a to Fig. 3.9, pages 75-80) can be used to assist in effective planning of an effective control programme.

In choosing a very conservative assumption to test whether the (few) farms reporting infections by epidemic day 4 were at the same distance to the nearest road intersection than the remaining (susceptible) farms, it was assumed that susceptible farms were located within 10 km from the major road network.

If infected farms were closer to major road intersections than average susceptible farms then, blocking traffic (in order to prevent bird traffic), if implemented efficaciously and immediately after the decision was chosen, would impede new cases beyond the segments already infected and probably diminish the traffic within segments already infected. Such control policy would have a low implementation cost and would require minimal technology and would not depend on previous training.

Three tests were conducted to address that question. The first test compared the median DNI of infected farms (n=5) to that of 10 000 spatial points assumed to represent the (unknown) population of susceptible farms. The median DNI of the infected farms represented 38% the DNI of the 10 000 points (24.2 and 63.7 km, respectively, P =0.055, Mann-Whitney test).

Second, the null hypothesis was tested that the distribution of the DNI was the same among farms infected early, e.g., within the first 10-day infectious period, as among points randomly located within 10 km of the major road network. The alternative hypothesis was that the DNI was smaller among early infected farms than among points randomly located within 10 km of the major road network. This question was addressed with a randomization test (S4). For each of 20 000 random samples of 5 points within 10 km of a major road, the median DNI was computed. The median DNI of the 5 farms infected by

day 4 of the AI epidemic, 24.23 km, was greater than the medians of only 731 of these 20 000 random samples, giving an observed *p*-value of 0.03655. Hence, the null hypothesis was rejected (the DNI of the 5 infected farms was significantly smaller than that of points located within 10 km from major roads) and, therefore, as early as epidemic day 4, the available evidence indicated that proximity to major road intersections promoted epidemic dispersal.

Third, the probability of obtaining DNI values of 35.029 km or less for at least 4 of the 5 points in the sample (an event observed in farms infected by day 4) was tested. The DNI was computed for each of 10 000 points randomly selected from the area located within 10 km of a major road. Of those 10 000 points, 2 632 points were 35.029 km or less. Hence, the estimated probability that DNI $\leq$ 35.029 km for a randomly chosen point within 10 km of a major road was 0.2632. In contrast, the estimated probability that DNI $\leq$ 35.029 for at least 4 of 5 such points in a random sample was then

$$5\times(0.2632)^4\times(1 - 0.2632) + \times(0.2632)^5 = 0.01894.$$

Thus the event observed for the 5 farms infected by day 4 had estimated *p*-value of 0.01894. Therefore, infection was an event more likely to occur in farms near major road intersections (less than 35 km) than in those randomly distributed, even if located at a relatively close distance (<10 km) from road lines.

The analyses of the epidemiological data using the GIS and computational analyses indicated that large clusters of outbreaks were found in the northern states of Plateau, Kaduna, Kano, Bauchi and Abuja- the federal capital territory, and the southern states of Lagos and Ogun (Fig. 3.6a, page 75). HPNAI H5N1 was spatially diffused in the country for the whole year 2006, irrespective of time of outbreaks and period where no outbreak was recorded (Fig. 3.6b, page 76). A centripetal (outward from a central point) mode of diffusion was noticed from the site of primary infection nationally (Fig. 3.8, page 79).

It was also noticed that based on dates of outbreaks in Nigeria, multiple outbreaks rather that a single epidemic front with declining trends prevailed (Fig. 3.7a&b, pages 77 & 78). Spatial-temporal (case location per time) analyses revealed a continuing spread of the virus southward along the major highways and intersections as the course of the outbreaks progressed (Fig. 3.9, page 80). A comparison of the estimated transmission rate in the country and selected locations indicated a peak transmission around the 3rd-5th

epidemic week. These findings correlated well with the observed cases and field reports (Fig. 3.10, page 81).

The first peak of the HPNAI H5N1 epidemic occurred around the 5th epidemic week (19th-26th February, 2006) with a second peak occurring in the 21st epidemic week (Fig. 3.10, page 81). The analysis using the ecologic niche modeling indicated that there is a potential risk of the spread of the HPNAI H5N1 viruses within West Africa (Fig. 3.11, page 82)

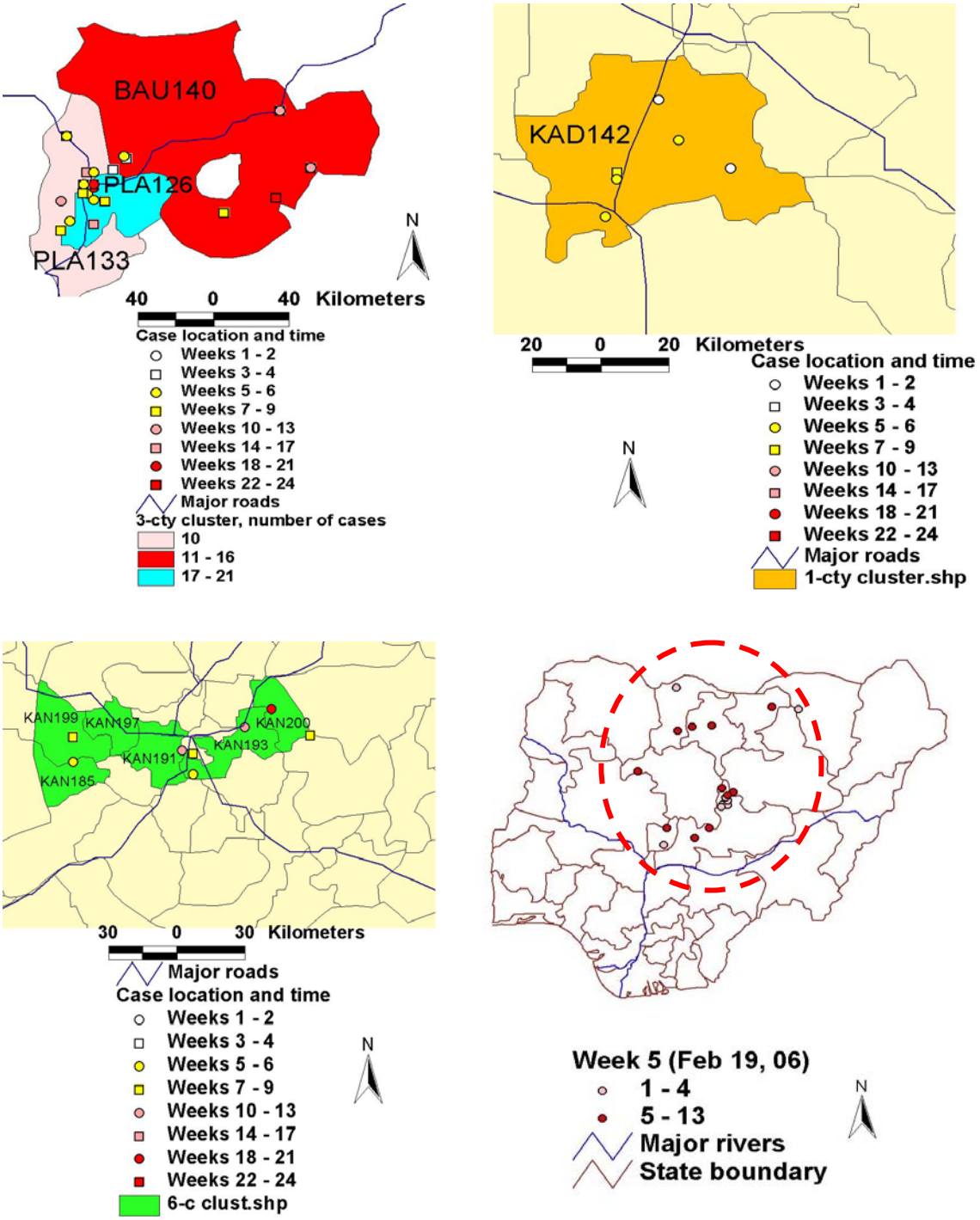
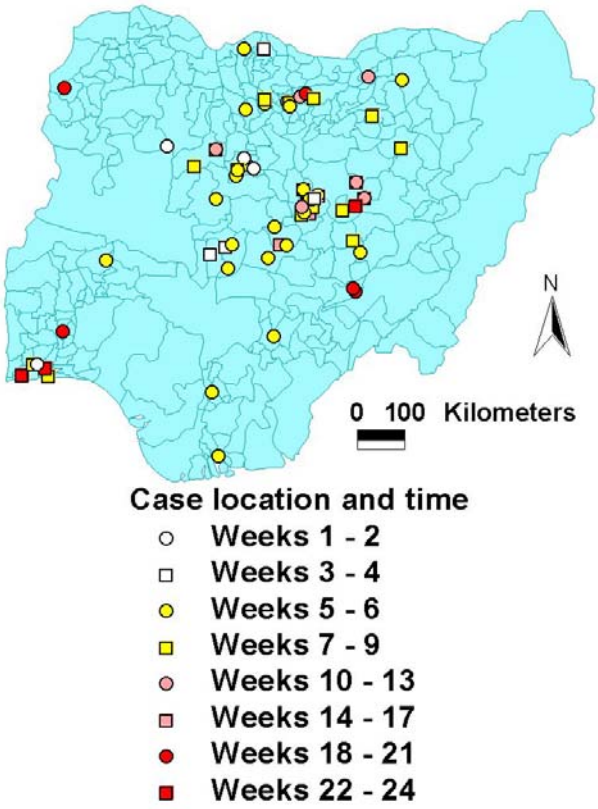


Figure 3.6a. Clusters of selected states (Bauchi, Plateau, Kaduna and Kano) and period of highest numbers of outbreaks in Nigeria. While some spatial clusters were observed (which reported at least twice as many cases per county sq km than average, for at least 10 epidemic weeks), neither spatial autocorrelation nor migratory birds appeared to explain the national diffusion. The week of February 19th represents the week with the highest numbers of infection recorded per week.

A



B

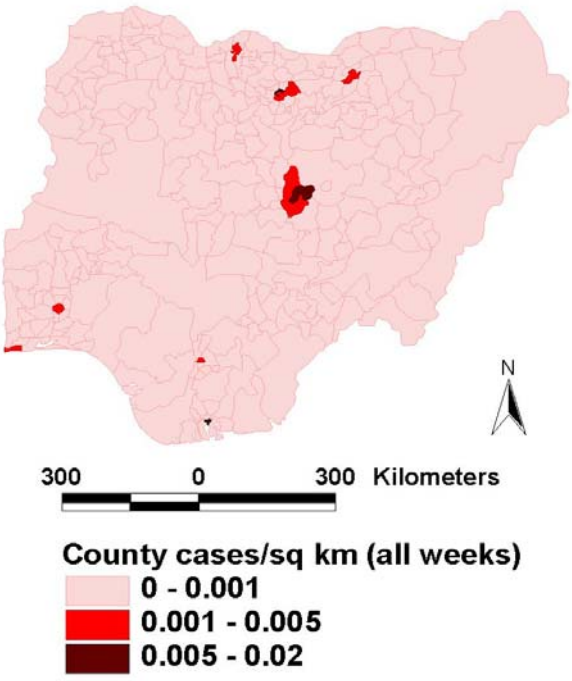


Figure 3.6b. Spatial diffusion of Avian Influenza in Nigeria (January-June, 2006). A: Weekly diffusion (n=113 poultry farms). B: Local government-based number of cases (infected poultry farms) per square kilometer.

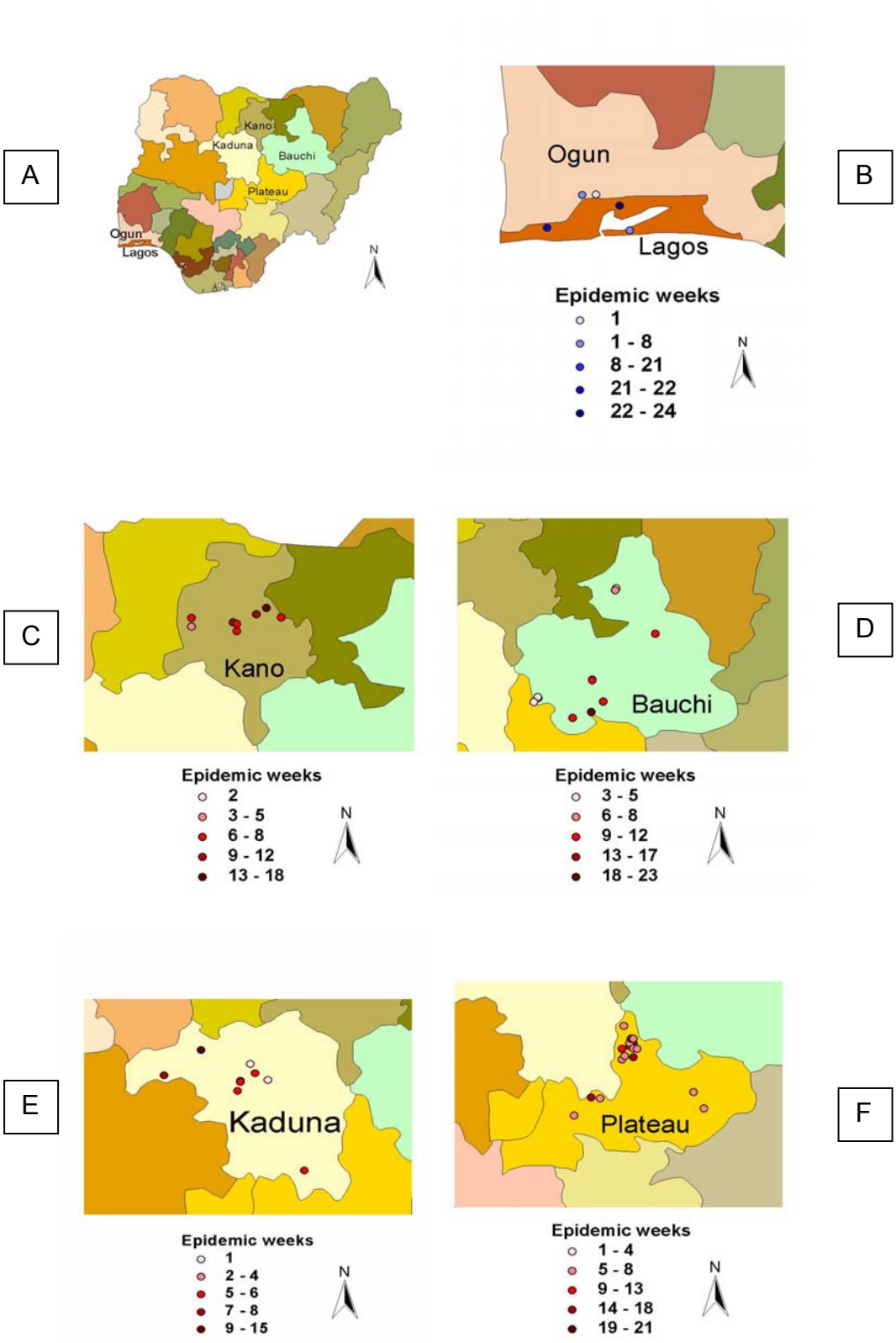


Figure 3.7a. Temporal diffusion patterns at selected areas. A: Nigerian states. B-F: Persistent outbreaks reported over 10 or more weeks in the region/state of Ogun/Lagos, Kano, Bauchi, Kaduna, and Plateau, respectively.

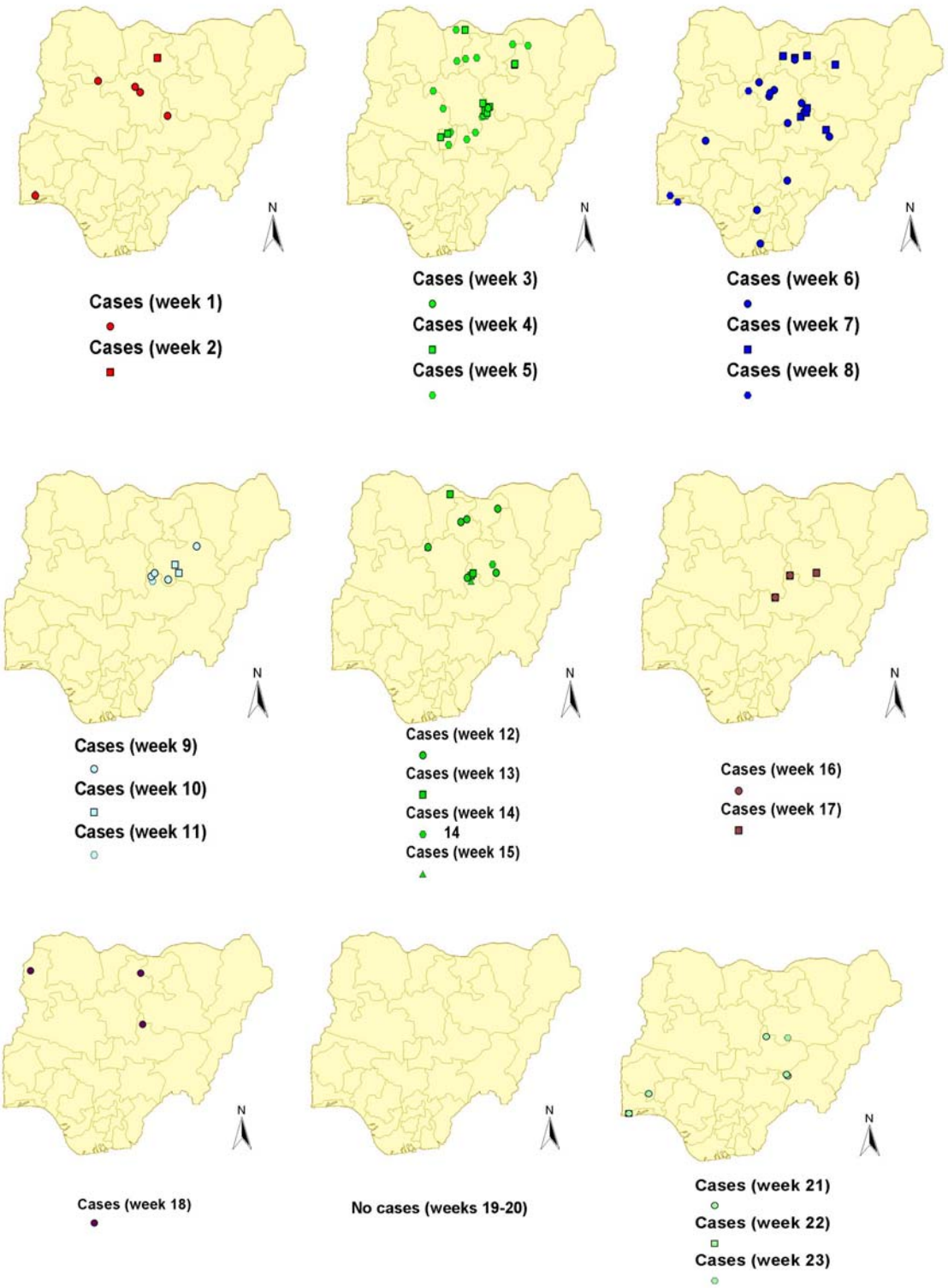


Figure 3.7b. The temporal diffusion indicated neither a "single epidemic front" nor a declining epidemic trend that indicated the end of the epidemic. Instead, multiple outbreaks (even after periods when no new cases were reported) were observed.

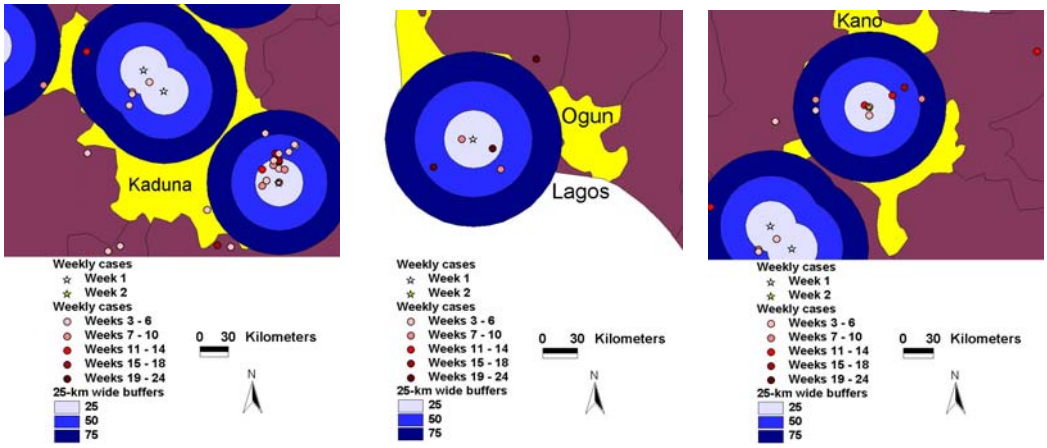


Figure 3.8. National and selected state diffusion pattern indicated outward diffusion to neignhbouring states. 25km buffer zone rings were placed concentrically and an outward spread was noticed.

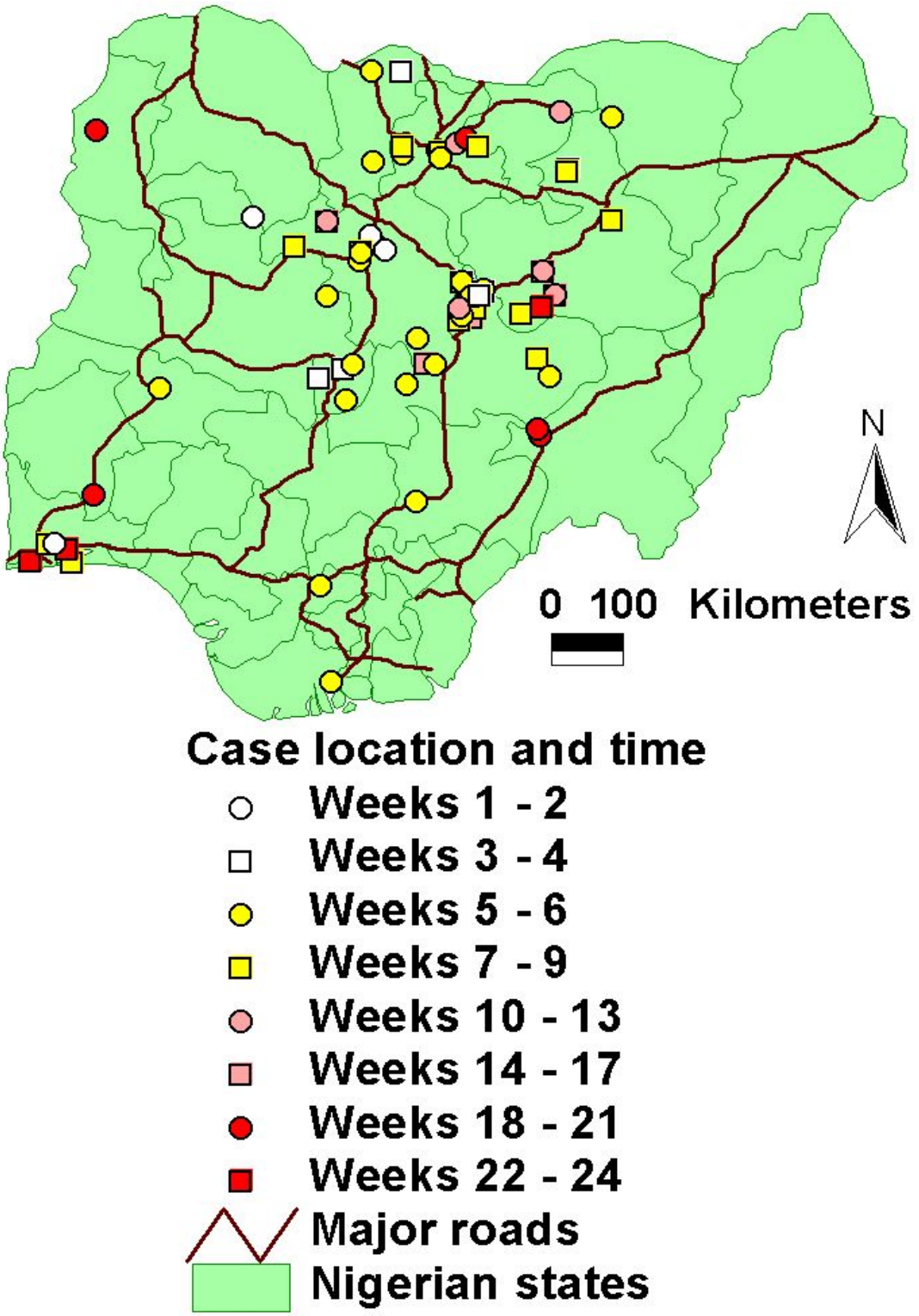


Figure 3.9. **Case location and time for Nigeria, 2006 (including road network). Major intersections of roads were infected. This indicated that roads possibly contributed to the spread of infection. The data suggested a mixed profile (both local and long-distance diffusion was observed over periods of rapid territorial expansion followed by a lack of new reports then followed by new periods of local and national diffusion.**

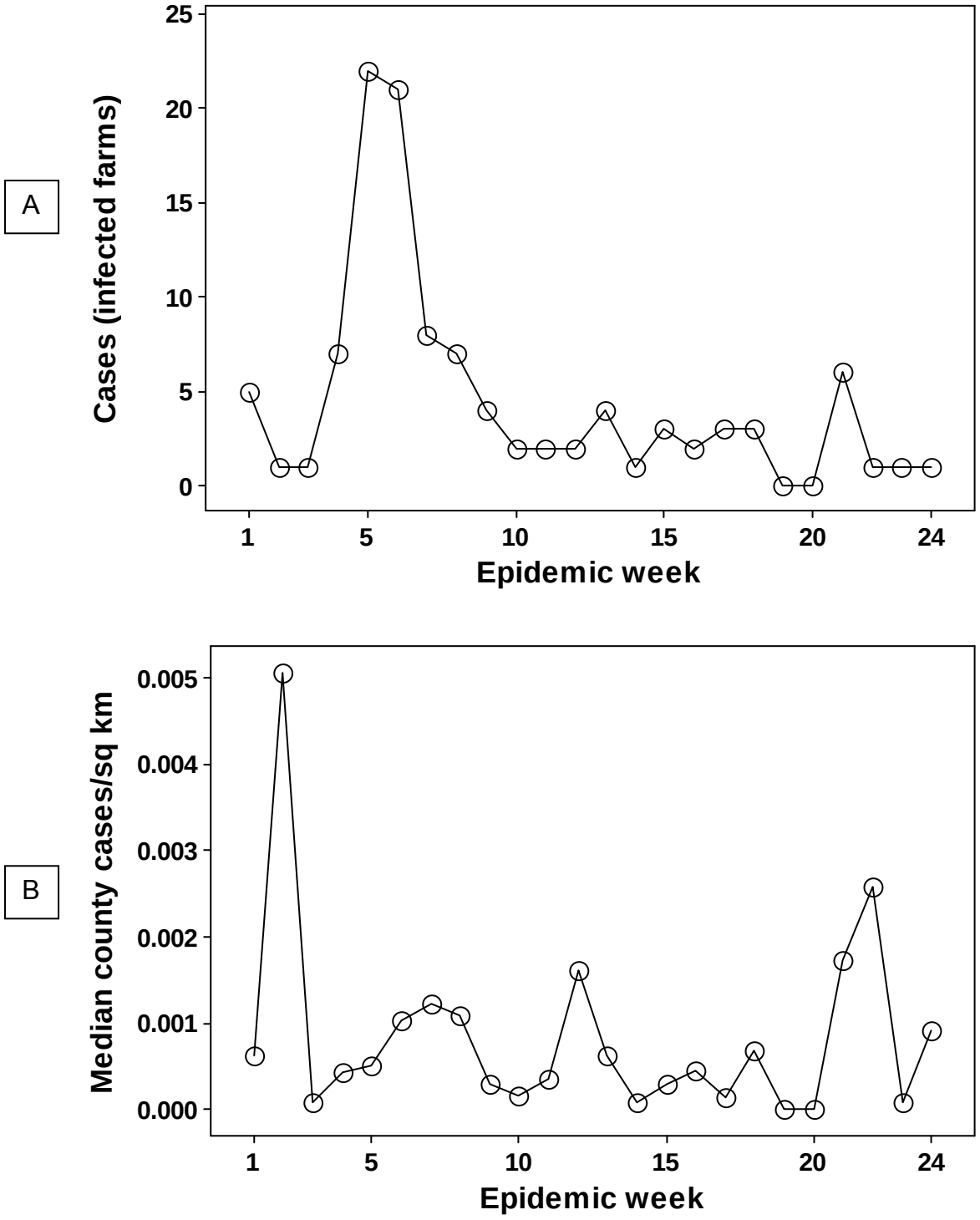
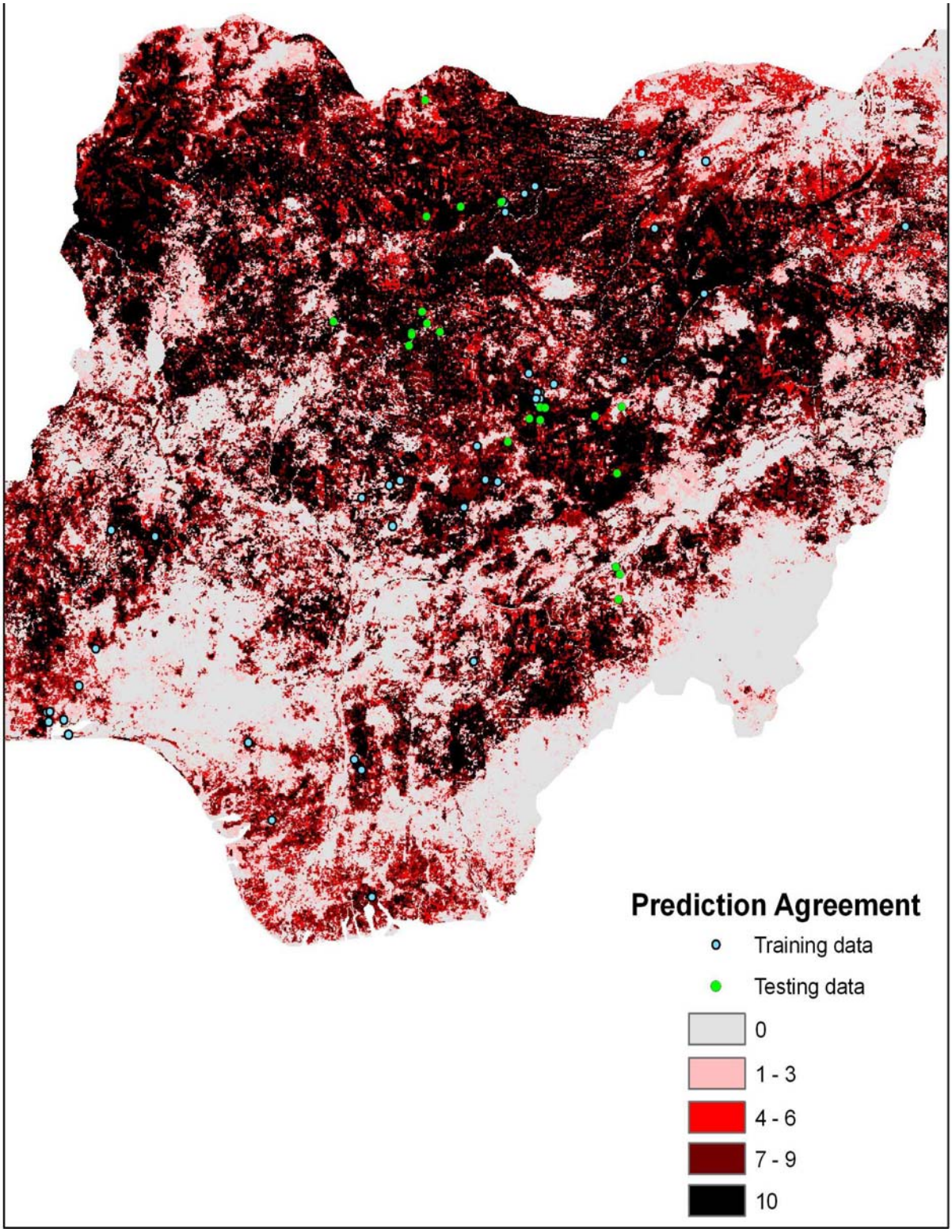


Figure 3.10. Weekly epidemic progression. A: number of cases (infected farms). B: cases expressed as number of Local government areas infected farms/sq km. Both expressions indicated a secondary peak at or around week 21.

Figure 3.11a. Ecologic niche modeling of Nigeria HPNAI (On-diagonal ENM predicts off-diagonal points) The numbers represent the degree of agreement with the prediction e.g. 0 out of 10, 1-3 out of 10, etc.



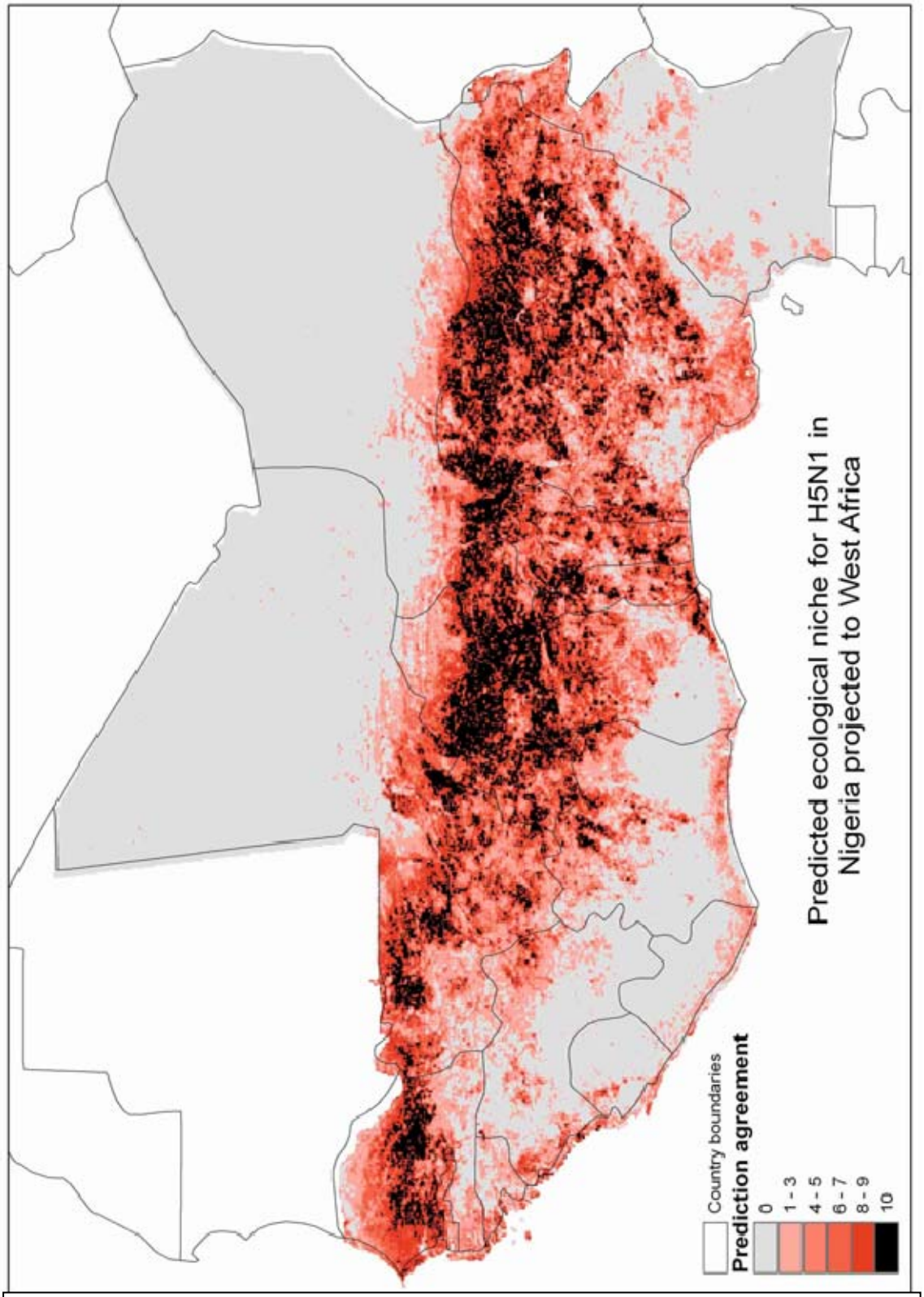


Figure 3.11b. Ecological Niche Modeling for HPAIV H5N1 Nigeria projected into West Africa

Further Notes on the maps and figures listed in Figure 3.5-3.11

Figure 3.5

Each state in Nigeria has animal control posts, it was assumed therefore that the spread of an animal disease could effectively be stopped by blocking all the entry routes (control posts) entering a state or by intensifying surveillance effort at the posts. These aspects of disease control were concluded lacking or ineffective during the course of the 2006 HPNAI H5N1 epidemic as the infection spread from one state to the other e.g. Plateau infection spread to Bauchi (Fig 3.5a); Infections were also noticed to spread along the major intra-state roads e.g. Kaduna and Kano (Fig 3.5b&c).

The week of February 19^{th}-26^{th} represented the week with the highest number of outbreaks. This corresponded to the week when live bird markets (LBMs) and hatcheries reported outbreaks in Northern Nigeria. Poultry diseases like HPNAI H5N1 are likely to be widely disseminated following infection of such facilities (LBMs and hatcheries) as birds, which may be infected are usually distributed from these sources to various locations. Molecular analyses similarly suggested widespread HPNAI H5N1 viral spread following infection of LBMs and hatcheries.

Fig 3.6

A. Represented all the infected locations in Nigeria in 2006. Following the time-wise trend of the map, it revealed initial infections of states of Kaduna, Kano, Plateau and Lagos followed by outward spread of infections from these primary sources.
B. Represented the intensity of infection per local government area. Since local government areas are the smallest administrative areas where major executive decision may be made, such maps are relatively important to enhance such decisions. This map revealed geo-spatial information about the relative number of infections per unit area, an information that may be vital in planning for location-based controls and for definition of quarantine areas as infected, buffer or free zones.

Fig 3.7a

a. Map of Nigeria with annotation of some of the infected states.
b. Lagos-Ogun infection largely occurred along the border. The area represents the axis where the highest poultry density in Nigeria is located. This has major implications for the development of the poultry industry in Nigeria.

c, d, e, f: Spread of HPNAI H5N1 in selected states of Nigeria. Spread along the border towns of each of the affected states presented viable options for infection of neighbouring states. This particular pattern of spread was noticed in Kaduna (Katsina and FCT), Kano (Jigawa and Katsina), Lagos (Ogun), Bauchi (Yobe and Gombe) and Plateau (Bauchi, Nasarawa and Taraba). All of these states were subsequently infected following these uncontrolled primary infections in contiguous states.

Fig 3.7b (1-9)

Weekly analyses of spread of the HPNAI H5N1 virus in Nigeria revealed an intensification of the virus circulation in the weeks three to eight (3-8). The effective monitoring, quarantine and control programme within the first two weeks of the infection may have prevented further outward spread and re-emergence of the virus within the poultry population between weeks nine and twenty-three (9-23).

Fig 3.7c

An effective implementation of the HPNAI H5N1 zones-infected, buffer and free- is expected to have positively contributed to the prevention and control strategies.

Fig 3.11a

Nigeria map showing the likelihood of continuing intensification of the spread of the HPNAI H5N1 virus based on available ecological factors (environmental factors, farming and cultural practices, difference in land covers and virus characteristics). The states of Northern Nigeria and parts of south-west and south-east Nigeria are at higher risks of re-infection, re-emergence and continued spread of the virus.

Fig 3.11b

Projecting the Nigerian HPAI ecological data into the West African sub-region revealed that the likelihood of infection in the northern zones of the sub-region is high. The infection is likely to spread from the extreme west part of the sub-region (Nigeria) to the far north Senegal and Gambia. Since the time of the production of this risk map, Burkina Faso, Cote d'Ivoire, Ghana, Niger and Togo have all reported outbreaks. Other countries that are yet to record outbreaks are only claiming freedom from infection based on lack of reports of outbreak. It will be necessary for all countries within the sub-region to carry out active surveillance to actually determine their statuses.

3.4 FINANCIAL COST IMPLICATIONS OF THE HIGHLY PATHOGENIC AVIAN INFLUENZA H5N1 IN NIGERIA IN 2006

Using the values generated in section 2.4 (pages 67-74), the total cost implication was calculated as follows:

Ci = PSʊ + PSβ + PSδ + PSγ

Actual Cost Implication

Ci= {$6,806,483+ $42,068,373+ $3,516,156 + $3,303,031}

Ci = $55,694,043

Scenario A (Mild generalized outbreaks 10% Commercial flock)

Ci = {$67,342,342 + $154,612,296 + $18,798,906 + $3,303,031}

Ci A = $244,056,575

Scenario B (Severe generalized outbreaks 70% Commercial flock)

Ci = {$471,396,397 + $83,748,327 + $131,592,341 + $3,303,031}

Ci B = $690,040,096

Note that these costs did not include the price of medical supplies donated.

Although our analyses did not consider the broiler industry, we are aware that there was a reported monthly regional export market losses of 12,000 tonnes of poultry meat (Personal communication, Poultry Association of Nigeria, 2006). These losses translated into 144 000 tonnes/annum and at an average cost of N350/kg ($2.7/kg) of meat, the broiler industry in Nigeria will have recorded annual direct losses of $392,217,899.

3.5 OPTION OF VACCINATION AS AN ADDITIONAL CONTROL MEASURE AGAINST AVIAN INFLUENZA H5N1

The final cost associated with the vaccination and eradication of infected populations over a three year period is estimated at $91,868,561 (Table 3.1.); and the final net benefit was estimated to be $5,347,325,735 (Table 3.2.). Based on the prevailing discount factor in Nigeria, discounted cost and benefits were calculated for a three- year period and the values were presented in table 3.3. The adjusted figures arrived at were used to calculate the Benefit Cost Ratio (BCR) of the project.

Calculated BCR = $\frac{4,267,315,737}{82,751,364}$

BCR = **51.57**

Table 3.1. Costs associated with a vaccination and test and slaughter policy

Year	Poultry Estimates (headcount)	Vaccinated population (70%)	Cost of Vaccination (2 doses given 4 weeks apart) ($)	Labour, distribution and administration costs ($)	Compensation and eradication ($)	Associated laboratory cost ($)
0 (2006)	156,800,000	109,760,000	13,171,200	8,936,928	11,076,044	155,642
1 (2007)	163,934,400	114,754,080	13,770,490	9,336,454	5,538,022	116,732
2 (2008)	171,393,415	119,975,390	14,397,047	9,754,159	5,538,022	77,821
3 (2009)	179,191,815	-	-	-	-	-

Cost of vaccine per bird per 2 doses is $0.12; cost of vaccine administration is $0.04 per bird; cost of distribution and other administration is $156,128 per annum; compensation was paid at $ 11.71 per chicken; total birds lost as at January 2007was 945,862; associated laboratory cost is $156,128 which would decrease by 25% per annum

Table 3.2. Associated benefits of a vaccination with test and slaughter policy

Year	Reduced Compensation ($)	Prevention of egg losses ($)	Regaining of external trade ($)	Normality in egg prices ($)	Value of salvaged birds ($)	Prevention of facilities redundancy ($)
0 (2006)	-	-	-	-	-	-
1 (2007)	5,538,022	9,785,968	393,120,000	1,327,794,875	6,649,410	738,378
2 (2008)	2,769,011	4,892,984	393,120,000	1,388,431,539	3,324,705	369,189
3 (2009)	2,769,011	4,892,984	393,120,000	1,456,315,773	3,324,705	369,189

Calculations were based on 284 eggs per bird per annum; Chicken eggs sell for $2.19per tray of 30 eggs; Nigerian Poultry Association claimed export of 12,000 tonnes of poultry meat per month to regional markets at $2.73 per Kg; Mean observed reduction in egg price during peak infection of HPAI was $1.01/ tray of 30 eggs; value of spent chicken is $7.03; average cost of facility for 1000 chicken per annum is $780.64

Table 3.3. Costs and Benefits associated with the control programme over a three year period.

Year	Discount Factor ??(0.12)	Cost ($)	Benefit ($)	Net cash flow ($)	Cumulative cash flow ($)	Discounted cash flow ($)	Discounted cost ($)	Discounted benefit ($)
0 (2006)	1.00	33,339,814	0	-33,339,814	-33,339,814	-33,339,814	33,339,814	0
1 (2007)	0.89	28,761,698	1,743,626,653	1,714,864,955		1,526,229,810	25,597,911	1,551,827,721
2 (2008)	0.80	29,767,049	1,742,907,420	1,713,140,371	3,424,671,345	1,370,512,297	23,813,639	1,394,325,936
3 (2009)	0.71	0	1,860,791,662	1,860,791,662	5,285,463,007	1,321,162,080	0	1,321,162,080
Totals		91,868,561	5,347,325,735	5,255,457,174	10,388,325,512	4,184,564,373	82,751,364	4,267,315,737

CHAPTER FOUR

DISCUSSION

Prior to this study, only 15 Nigerian isolates of avian influenza H5N1 were present in the public sequence depositories (GenBank and EMBL). This study has enriched the pool of the HPAI H5N1 viruses deposited from Nigeria. A total of 35 newly isolated viruses were subjected to molecular characterization and the haemagglutinin sequences of these viruses (identical sequences excluded) were deposited in the GenBank. This dataset currently represents the widest spatio-temporal and species related diversities from Nigeria publicly available.

The current phylogenetic analyses concurred with previous reports (Ducatez *et al.*, 2006) that Nigeria was infected by multiple sources of the virus H5N1. The phylogenetic relationships and time of occurrence of viruses in sub-lineage A indicates a high probability of shared epizootics with Egypt. These viruses were limited to South West (SW) Nigeria (Lagos) and did not spread further. These present classification using the six (6) sub-lineages were based on finite branching orders, current molecular markers and bootstrap values. The use of more Nigerian viruses in this study and the more numbers of sub-division of the viruses indicated that more changes had occurred in the viruses circulating in Nigeria since the work of Ducatez and co-workers in early 2006.

The West African sub-region operates as a free trade zone with poor quarantine and border controls. It is highly likely that trans-border movements of humans along with trade of poultry and poultry products and weak biosecurity played a significant role in the spread of viruses in sub-lineage D, that which contained a large cluster of geographically-diverse viruses from infected African nations. The Sudanese outbreaks possibly occurred as a result of the initial shared infection between Nigeria and Egypt, which Sudan later contacted through long distance poultry or poultry products movement, probably from Nigeria. Although the Cameroonian virus sequences were not included in this study due to unavailability, the outbreaks in that country roughly coincided with the time period of early infection in Nigeria (21st February).

Sub-lineages A and D were some of the earliest HPAI H5N1 viruses to affect the continent and outbreaks caused by these viruses were restricted to the commercial poultry in Nigeria and were similarly found early in some other West African countries and Sudan (between 1st March and 1st April). This strongly suggests that imported infected commercial stock may have been the source of outbreak.

The importance of the live bird markets (LBM) in the spread of the virus in West Africa is particularly evident in this study. The viruses in sub-lineage H (infecting vultures, pigeon, guinea fowl, free-range chicken and other birds from a wildlife park) were geographically and chronologically dispersed in Nigeria following infection of the LBMs, inappropriate disposal methods and isolation of the virus in the LBMs and hatcheries. Field data which are supported by these phylogenetic analyses indicated that outbreaks caused by this group of viruses were found in locations as distant as 954 km from an infected LBM infection. Previous studies have similarly confirmed the role of wet markets, LBMs and movement of poultry and poultry products without recourse to biosecurity in the viral ecology and spread of avian influenza and other viruses (Guan *et al*., 2000, Henzler *et al.*, 2003, Webster, 2004).

Poultry movement in Nigeria follows a particular trend: local guinea fowl (*Numida meleagris*), ducks, turkeys, local free range chickens and spent hens are usually moved towards the south of the country especially around the festive period (Christmas, Easter, Eid-el-Fitri and Eid-el-Maulud). Day-old-chicks and input supplies are the major poultry related products moved up-north. These movements and trend-lines of dates of occurrences of outbreaks could explain the wide geographical diversities in outbreaks caused by the sub-lineages originating from northern Nigeria and highly restricted spread of those from south-west Nigeria (sub-lineages A and E). Furthermore, it was reported that Nigeria continued to import poultry and poultry products from contaminated zones in 2005 despite the ban on such importation and these are distributed within the country (Gauthier-Clerc *et al*., 2007).

Vultures probably contracted infection from disposed carcasses and viscera. Infection of pigeons may have occurred due to their co-habitation with infected free-range chickens, turkeys and ducks, as suggested by the time-line of infections and the phylogram. The infection of Wildlife park birds occurred in early April, a period that roughly coincides with Easter and Eid-el-Kabir and increasing human movement to the park. The park is situated

in the epicenter of Northern Nigeria outbreaks, with no outbreak reported before this time. Inadvertent human introduction or an infected source of feed for the meat-eating animals within the park may be responsible for such introduction.

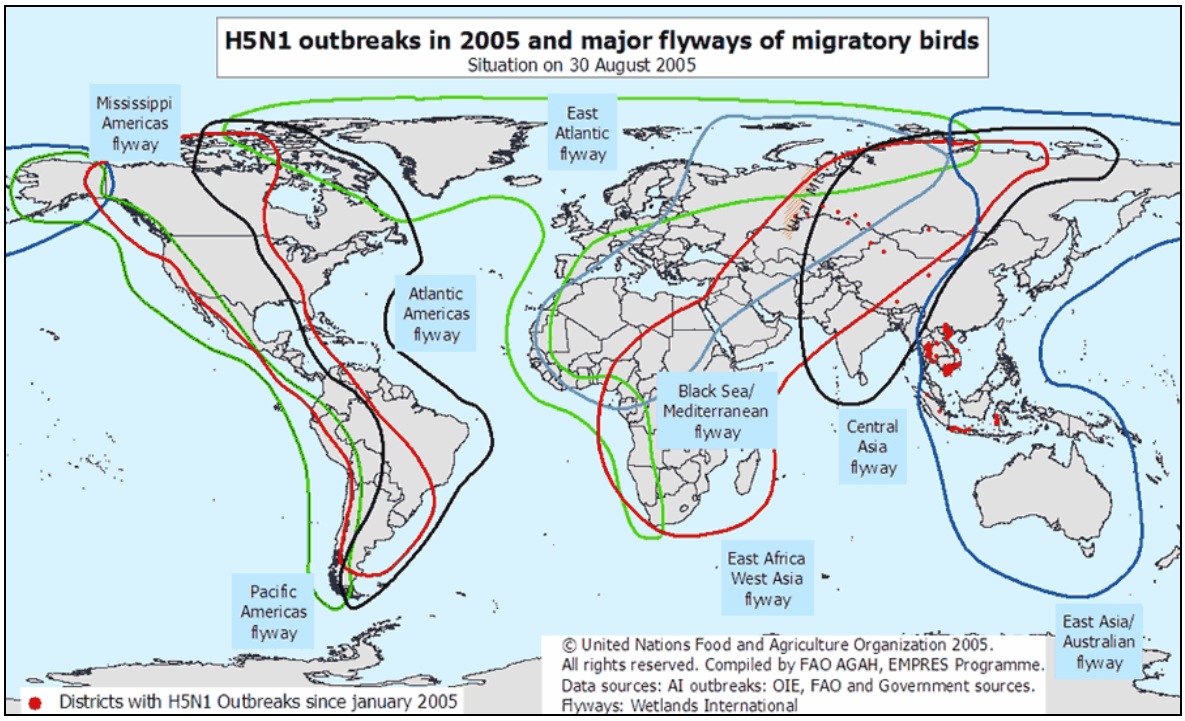


Figure 4.1. ***Migratory Bird movement across the world. Source: FAO, 2005***

Other works had similarly refuted the fact that wild water-birds or migratory fowls were primarily responsible for the outbreaks or spread of HPAI H5N1 in Nigeria and other parts of Africa (Gaidet, Dodman, Caron, Balança, Desvaux, Goutard, Cattoli, Hagemeijer and Monicat, 2007, Feare, 2007, Gauthier-Clerc *et al*., 2007) (Fig. 4.1). The infection of wild life, parks and free range birds have an ominous implication for other continents as many migratory bird species over-winter in Africa, and West Africa trades wild birds in the international market (Bird Life International, 2006a). Other regions of the world have been infected through migratory birds, legal and illegal importations (Sims *et al.*, 2005, van Borm *et al*., 2005).

Although, the FAO postulated that backyard farms and free range village birds are at higher risk of infection (FAO, 2004), we found Sectors 2 and 3 of the poultry industry (as described by the FAO, 2004)-which are closely associated with the large scale importers and hatcheries/distributors of day old chicks and operate with minimum to no biosecurity-are more widely affected. A more coordinated surveillance system is thus encouraged in the West African sub-region in particular and Africa in general to determine the situation of

HPAI H5N1 in other countries which currently claim freedom from outbreaks, since all African countries are at high risk of infection.

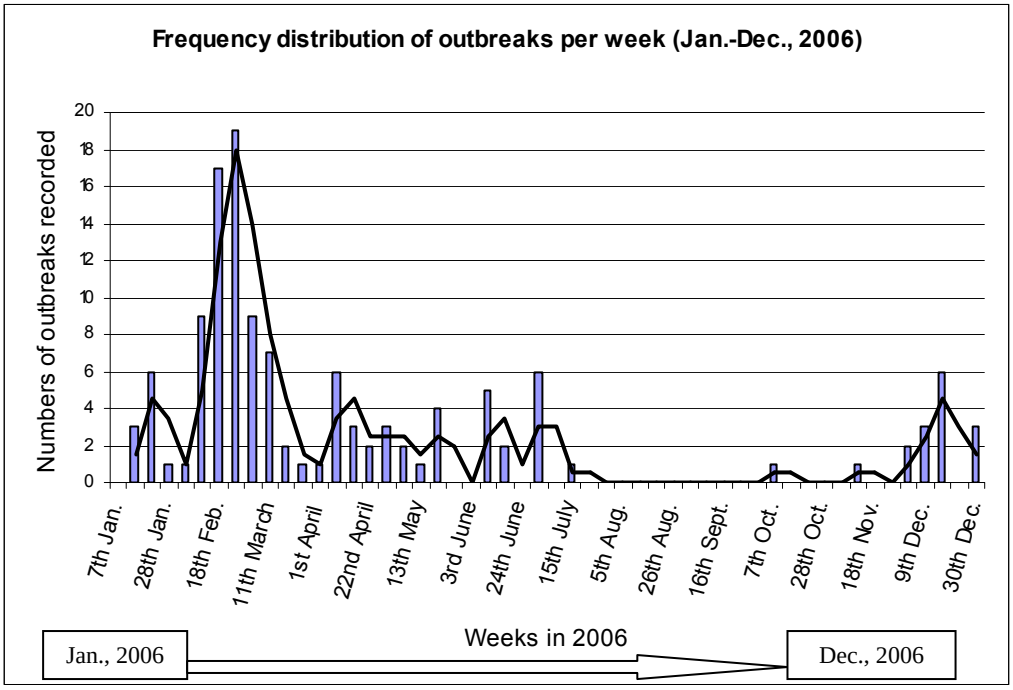


Figure 4. 2. **Distribution of numbers of outbreaks per week (Jan.-Dec., 2006)**

The role of the different control measures introduced and their contribution to the epidemiology of HPAI H5N1 in Nigeria will also require further evaluation. Control measures were introduced at about the seventh week of the outbreaks in Nigeria (20th February, 2006), with consequent reduced reports of new infection (Fig. 4.2). However, the effectiveness of the movement restriction was highly questionable as unaffected individual farmers in affected locations sometimes sneak their stock into HPAI free areas or dispose of such.

The viruses from Nigeria included in this study were collected over a year (January 2006 to January 2007) and showed varied drift in their genetic constituents over time. Salzberg, Kingsford, Cattoli, Spiro, Janies, Aly, Brown, Couacy-Hymann, de Mia, Dung, Guercio, Joannis, Ali, Osmani, Padalino, Saad, Savic, Sengamalay, Yingst, Zabrosky, Zorman-Rojs, Ghedin and Capua, (2007) indicated that a particular sub-lineage for example A/chicken/Nigeria/1047-62/2006 were re-assortants generated in Africa. Four similar strains (according to homology with the haemagglutinin proteins) were identified during this study.

High mutation rates represent opportunities for evolution of avian influenza viruses particularly in the African continent where primary health care facilities are not easily accessible, human disease monitoring and veterinary infrastructures are doubtful; and hygiene measures in poultry handling/slaughtering are poor. Since the present study analyzed the current isolates using the haemagglutinin genes, there is a need to fully characterize all of the Nigerian isolates, conduct more detailed epidemiological research in other parts of Africa, particularly in countries that have not reported outbreaks and use outcomes to develop better control strategies in view of the fact that the current control policies seem ineffective for the African continent.

Thus, it can be concluded that the Nigerian epidemic was caused by multiple strains of HPAI H5N1 in 2006 and the spread was linked to commercial poultry and not wild birds. The infections were human and trade mediated; and the pattern of poultry movement in Nigeria similarly played some roles.

Investigation using the GIS analytical tools (Fig. 3.5 to Fig. 3.9, page 93 to 101) fully supported the molecular analyses. Spatial and temporal based clusters correlated well with the sub-lineages inferred from the phylogenetic trees. This GIS study revealed that the northern states of Plateau, Kaduna, Kano, Bauchi and FCT are at higher risk and more prone to widespread infection and this fact was supported by the genetic markers detected by molecular analyses. The temporal study indicated that multiple outbreaks occurred in Nigeria rather than a single sustained infection, a finding similarly suggested by the molecular studies. The national and state-specific reproduction numbers *Rø* indicated the epidemic peaked at around February 19th, coinciding with the observed number of weekly cases. Hence, the estimated 10-day mean infectious period fitted well with the observed data. Given the additional outbreaks noticed after the epidemic peak, *Rø* ~ 1, and the fact that subsistence agriculture is practiced in Nigeria (system associated with live bird markets and unregulated animal movement), findings are consistent with the hypothesis of human-driven diffusion (for instance, early re-population of infected premises with new, susceptible animals), behavior potentially leading to endemicity. The GIS and molecular studies similarly concurred that north-central Nigeria was the epicenter of HPNAI H5N1 infection in Nigeria. Road-mediated spread was highlighted in the spatial study and indications from the molecular analyses pointed to the fact that genetically related viruses were isolated from locations as distant as approximately 1,000km.

Although the ecological niche model predictions used for Nigeria and West Africa are exploratory (Fig. 3.11a, b & c, page 102-104), they tested the basic hypothesis that environmental correlates existed in the HPAI H5N1 situations in the sub-region.

The following limitations of the current study are recognised:

1. Imprecision inherent in geo-referencing infection sites which sets a base level of error, and guarantees some predictive failures. Indeed, given that poultry is frequently traded and moved to markets, transmission may frequently occur at sites not coincident with detection sites—a number of Nigerian HPAI H5N1 cases were detected in poultry markets, to which infected birds were presumably transported over unknown distances from the actual transmission sites.
2. Another important challenge for these analyses is that of distinguishing true spatial and ecological biases in case distributions (i.e., the ecological niche!) from the spatial and ecological biases in distributions of the major known HPNAI H5N1 host in Nigeria (the chicken). The total Nigerian chicken population is 140 million, including “backyard chickens,” raised without biosecurity measures (~60%); commercially farmed chickens under high biosecurity (~25%); and semi-commercial chickens, raised with some biosecurity measures (~15%) (Adene and Oguntade 2006). Most commercial birds (65%) are raised in the southwestern part of the country, in and around Lagos (Adene and Oguntade 2006). Free mingling of backyard poultry and wild birds has been identified a risk factor for HPNAI H5N1 transmission (de Benedictis et al., 2007).

In this study, HPNAI H5N1 outbreak localities do not necessarily coincide with areas of high backyard chicken populations for example, the state with the highest backyard chicken populations (Imo, southeast Nigeria) has had no cases of HPNAI H5N1, despite having a roughly tenfold higher density of backyard chickens than Plateau, the state with the highest number of HPAI H5N1 outbreak sites.

Similarly, little coincidence was observed between HPAI H5N1 outbreaks and areas of high density of commercially farmed birds in the southeast (Adene and Oguntade 2006).

Recent studies in Southeast Asia (Gilbert, Xiao, Chaitaweesub, Kalpravidh, Premashthira, Boles and Slingenbergh, 2007) identified predictable foci of HPAI H5N1 activity based on free-range duck farming and rice-paddy cultivation. Although that association has clearly and easily interpretable foundations, these results suggest that predictable ecology may

be more pervasive in HPAI H5N1 geography than might have been expected. Several elements in the HPAI H5N1 transmission cycle could be responsible for this predictivity: ecological biases associated with initial arrival of virus propagules in a poultry population via migratory birds (Chen, Li, Li, Shi, Shinga, Deng, Qi, Tian, Fan, Zhao, Sun and Kawaoka, 2006; Olsen, Munster, Wallensten, Waldenstrom, Osterhaus and Fouchier, 2006), transmission among Nigerian poultry flocks (Gilbert, *et al.*, 2006; Yasue, Feare, Bennun and Fiedler, 2006), or even with transportation routes within Nigeria that might be responsible for communicating infections—most likely, the truth lies in a combination of such factors (Kilpatrick *et al*., 2006). The precise basis for this predictivity has yet to be identified, but the existence of an environmental signal in HPAI H5N1 transmission may offer valuable clues as to its nature.

Perhaps most importantly, projecting the Nigerian ecologic niche models across the entire region yielded a prediction of West African HPNAI-H5N1 distributions that was highly predictive of what independent test data could be assembled. Such validated model predictions offer the possibility of public health applications, providing information that may be used to prioritize surveillance and remediation activities. Similarly, such predictions may be helpful to policy makers planning expansions to and investment in the Nigerian chicken industry, particularly as regards investment in biosecurity measures. The spatial limits of the predictivity this analysis has documented remains an open question—initial demonstrations of predictable HP-H5N1 geography across West Africa awaits further testing and comparison with HP-H5N1 occurrence information from other regions.

Since the policy makers will best understand the HPNAI H5N1 situation in Nigeria in financial terms, the effect of using vaccination as a control strategy in Nigeria was examined, in addition to enhanced biosecurity, stamping out, movement restrictions and effective surveillance (Capua and Marangon, 2003). This decision was based on the fact that Nigeria has poor veterinary infrastructure, poor biosecurity and disorganized farming systems. Based on available estimates, it was concluded that the benefit cost ratio (BCR) of vaccination as an additional choice of control measure was approximately 52 times better than a no-control policy from an economic point of view. If implemented in a strict sense, the control measures could begin to yield a quantifiable results within a year of its operation and within the same year, it could have fully justified the financial cost associated with the implementation of the three year programme. Though cheaper alternatives were identified in the decision tree analysis (DTA), the nature of the poultry

industry in Nigeria as well as several other developing and transiting countries makes vaccination the most suitable option.

The use of vaccination as a control option may restrict external trade in poultry and poultry products, but it will help in protecting the industry from eventual collapse since it reduce virus shedding (Cauthen, Swayne, Schultz-Cherry, Purdue and Suarez, 2000; Capua and Marangon, 2006) that may be associated with a no control policy and considering the economic benefit of control through vaccination combined with test and slaughter policy, it will be a worthwhile venture. Other disadvantages of vaccination may include problems and costs associated with monitoring and traceability of vaccinated poultry and poultry products.

Further benefits associated with good control measures may include environmental, ecological and medical, and these have been evaluated and quantified by previous workers (Mullooly, Bennett, Hornbrook, Barker, Williams, Patriaca and Rhodes, 1994; Bridges, 2000; Bloom, Canning, and Weston, 2005). However, a careful assessment of the benefits and demerits of vaccination must be considered before a final decision can be taken.

It is acknowledged that this analysis has certain limitations and economic assumptions, which are partly explained by the weak surveillance and reporting structures in Nigeria, as well as lack of readily available data, but it is expected that this model will serve as baseline data for future economic analyses and it will be a first step towards improving HPNAI control in Nigeria. Costs associated with the intangible factors in these estimates and assumptions may vary widely with changes in disease outbreak patterns, spreads and infectiousness. Other workers had similarly identified uncertainties associated with disease situations (Ramsay *et al.*, 1999; Horst *et al.*, 1999). While vaccination may hold potentially immense benefit for a country like Nigeria, problems associated with it should be carefully considered and thoroughly reviewed before a final decision on vaccination can be taken by policy makers.

The option of a community initiative in the prevention of the spread of epizootics like HPNAI H5N1 was evaluated with the following findings: Vom is a community in Plateau state with a high probability of infection with HPAI since it lies in the epicenter and major spread route of disease in the country. It also receives large numbers of individuals who

deal in poultry, feed, and poultry products. Furthermore, it serves as a repository for carcass submission (oftentimes transported inappropriately) in the wake of the outbreaks; a dead migratory bird was also diagnosed to be HPAI H5N1 positive within the vicinity of one of the largest farms in the area (NVRI, unpublished data).

The community took some pre-emptive measures which included the following:

• Stopping visits by egg buyers. The community agreed to transport eggs to buyers and leave egg trays behind rather than allow buyers to visit for collection.

• Biosecurity was improved by regular decontamination (spraying with disinfectant) of vehicles after every such egg transportation.

• Farm workers had to decontaminate regularly and almost all of the free-range poultry in the community was culled by owners or restricted indoors.

• Visitors were restricted from visiting farms in the community.

• Extra care was implemented by decontaminating feed bags and feed mill premises by tow millers or by buying of finished feed.

• The Veterinary Institute also organized awareness programs for farmers at the peak of the outbreaks to stop panic, correct error in sample submission, and engage more in on-farm assessment/sample collection rather than sample transport to the laboratory.

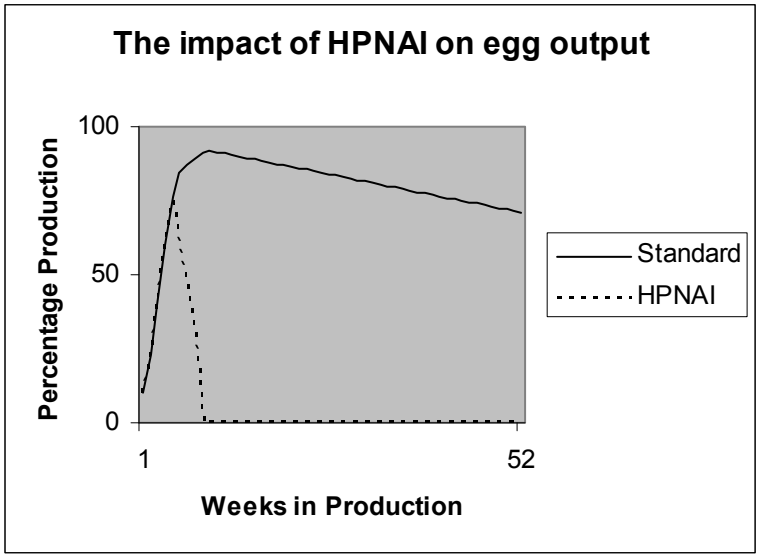
Although all of the above strategies implemented had been previously advocated (Law and Payne, 1996; farmers did not adhere to them, as is the situation in the poultry industry in many third world nations. However, the estimated 50-60 farming families that participated raised an estimated 100,000 poultry (mostly layers) did not suffer a single outbreak during the crisis period.

Assessment of these community initiatives identified the following: 1. The measure of intensive awareness programs engaged in by the Veterinary Institute contributed tremendously to controlling the disease spread as drastic reduction in incidence rate was noticed from the period of this community awareness. 2. The emphases on the application of biosecurity in tackling disease entities are non-negotiable in successful poultry production enterprises in tropical Africa. 3. The national governments of developing economies must develop functional regional laboratories and improve sample submission systems, if combating disease emergencies will be successful endeavors.

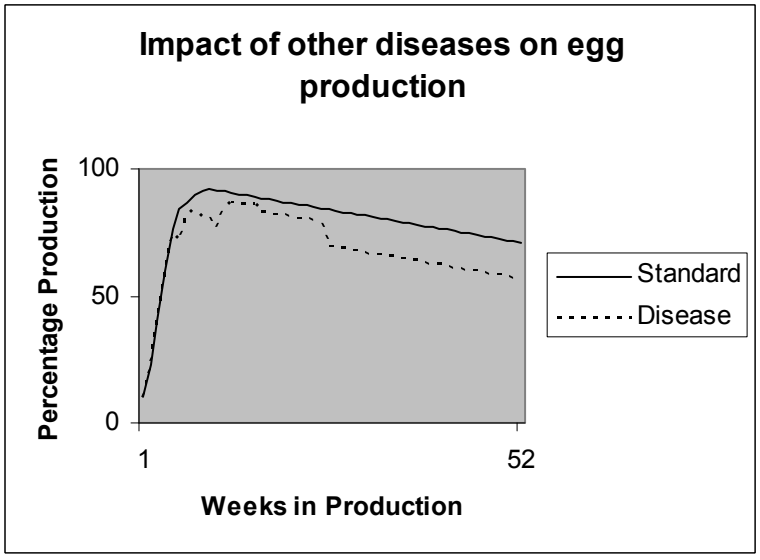
Finally, since poultry farms are usually grouped together due to convenience of assessing inputs, efforts should be directed at a community approach to combat disease entities rather than an individual approach, which currently operates.

The cost associated with the infection of avian influenza H5N1 in Nigeria in the year 2006 was huge considering the financial involvement, although economics suggests that such costing should not be assigned monetary values alone (Hanson and Hanson 1983; Howe 1985; McInerney 1988). The cost implication of the disease is, however, important as a starting point to assessing the true effect of the outbreak. Previous estimates of the cost of avian influenza outbreaks using direct costs grossly undervalue costs associated with HPNAI. A mild scenario of infection affecting 10% of the commercial laying bird population will cost the country in the region of $245 million and a worsening situation may lead to losses of around $700 million in the layer industry alone. From the results, any severe outbreaks of HPNAI in a country like Nigeria will mean an extremely huge economic loss and will negatively affect the agricultural industry in the sub-region.

Graphical representation of the effect of highly pathogenic avian influenza (Fig. 4.3) proved that these previous methods may not be sufficient to estimate the cost of this disease. This study considered that the productive lifespan and the potential value of the animals involved should be taken into consideration if a comprehensive evaluation of the cost of animal disease is to be done. A point-of-lay commercial bird or a breeder chicken, although may cost less than a rooster/broiler or a turkey respectively at any point, is more valuable than the latter in term of economic benefit since the laying hen or the breeder will bring economic benefit for at least a year. ‘Economic value is not simply prices’ (McInerney 1988)



*Note: Area between standard and disease graph is the cost. HPNAI cause complete loss in poultry production either through cessation of production, death or culling. Most of the Nigerian farmers reported above 90% reduction in production and whole flock was finally culled (100% loss) (Analysis of the Nigerian situation)



*Note: Area between standard and disease graph is the cost. Several other avian diseases cause partial loss of poultry production. However, not all will disease attract culling of the affected flock

Figure 4.3. Graphs showing Evaluation of Cost of Animal Diseases. *Hypothetical values were used to construct the figures only for the purposes of illustration. It indicated that while the losses associated with HPNAI H5N1 will lead to complete termination of production (death or culling), many (but not all) other diseases affecting poultry can either be managed, treated or may not result in complete eradication of the flock or sources of income*.

Apart from financial losses, the HPNAI H5N1 outbreak also had severe impacts on trade and tourism, created scarcity/unavailability of animal protein due to public health

misconceptions, led to higher prices for alternative and often lesser quality products, and increased the costs of livestock farming. There are concerns that HPNAI may become enzootic in the sub-region or in the African continent, which may then become a source of infection or re-infection to other parts of the world. Efforts to step up controls at the borders, surveillance and effective analysis systems are considered justified by the huge resources that will be lost due to such outbreak, if calculated over the productive life. There is a need for restructuring of the poultry industry which aims for higher levels of biosecurity. A scientifically based contingency plan and fair compensation schemes also needs to be developed by all governments. This must be established and tested periodically before the outbreak of any disease, since time lost to decision-making during disease outbreaks has huge economic impacts.

Furthermore, a separate analysis of the socio-economic changes forced on the affected farmers, and the costs of different control efforts is also necessary to assist the decision makers in prioritizing all efforts aimed at controlling HPNAI in Nigeria. An assessment of the effect of the HPNAI on other categories of services providers including day-old-chick suppliers, feed millers and other input suppliers, the hospitality industry, and sole traders in poultry products as well as animal pharmaceutical industries will also be essential to comprehensively assess the overall effect of HPNAI.

CHAPTER FIVE

CONCLUSION

Avian influenza H5N1 is now present in the African continent. It has continued to spread while efforts to contain it do not seem to match the rate of spread or potential effect of the virus in the poultry industry. At the time of writing, Ghana and Togo had confirmed outbreaks and Algeria has suspected the infection, therefore the risk that more African countries will be infected is high (see Fig.4.3.7). Apart from financial losses in Nigeria, Egypt claimed to have lost in the region of US$882.4 million (Ministry of Agriculture, Egypt, 2006). While the epizootic continued in the poultry population, it is saddening that some individual farmers do not believe that HPAI H5N1 exists at all and some are deliberately twisting the facts surrounding the outbreaks (The Guardian, 2007, Fasina *et al.*, 2007 unpublished reports). This situation has ominous implications and can entrench the continued spread of the virus in the poultry population and a high potential for human infections since such farmers are responsible for the presentation of poultry and poultry products-which at times are not inspected.

This work is therefore in agreement with other papers that although wild birds (migratory birds included) may have contributed to the introduction of the virus in other regions of the world, there is currently no evidence that they are involved in the spread of avian influenza in Africa; rather, human activities, poultry trade and movement, and poultry related industries are responsible for the vast majority of outbreaks recorded in Nigeria (Birdlife International, 2006c; Salzberg *et al.*, 2007; Gauthier-Clerc *et al.*, 2007). Although the spread of the outbreaks was not poultry population or density correlated, much interplay of factors were detected to be involved and different sectors were affected as described in Fig. 5.1 below.

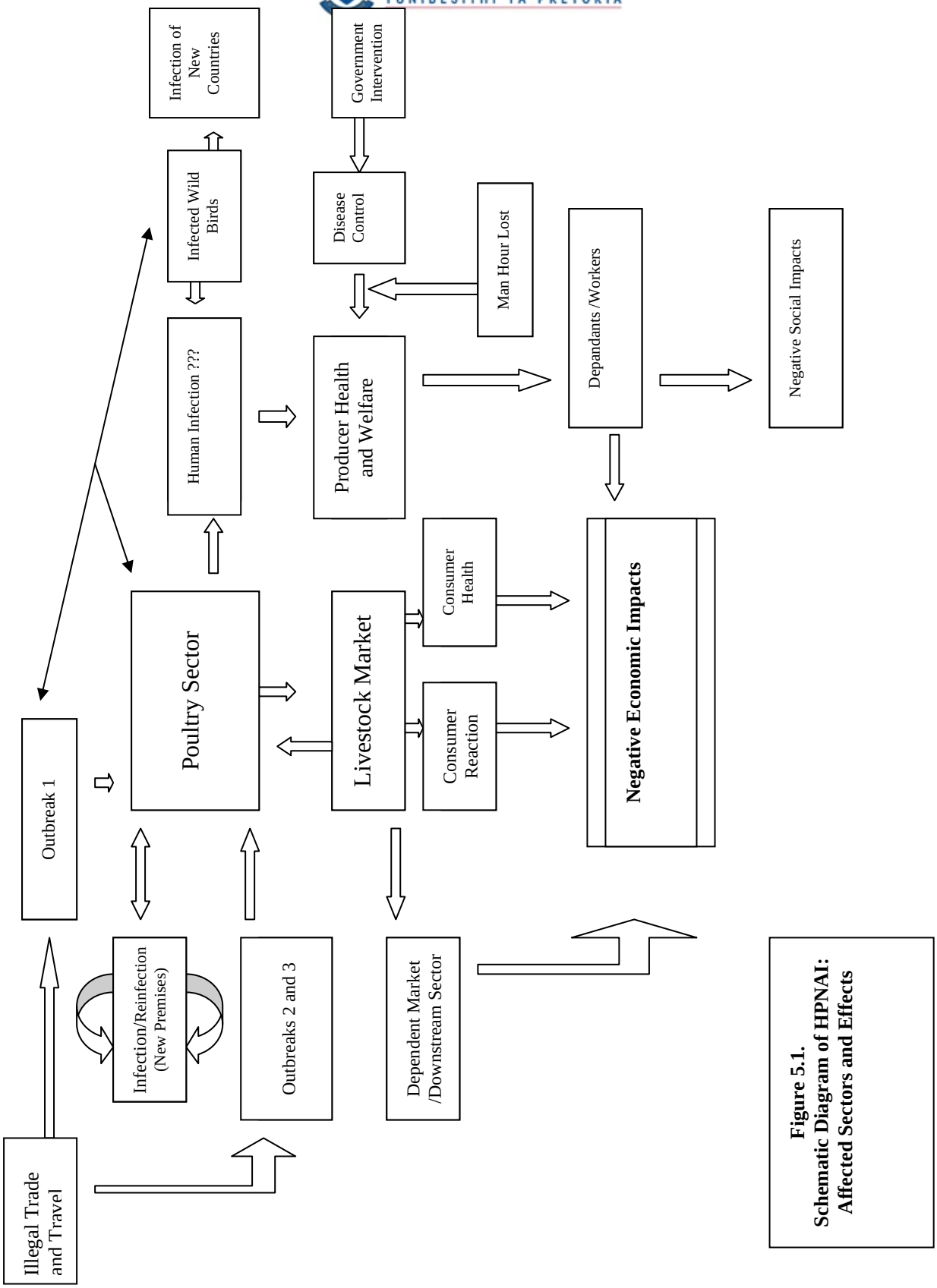


Figure 5.1.
Schematic Diagram of HPNAI: Affected Sectors and Effects

Finally, it is evident that both animal and human diseases and emergencies like HPNAI should not be viewed from narrow perspectives of animal infection considering their diverse impacts and socio-economic importance, a multi-disciplinary approach to

managing disease situation is strongly encouraged and advocated for by this work. Animal disease such as avian influenza apart from its poverty elevation impact can also become a major human pandemic. The African population will keep expanding, especially in the urban areas with more need for food of animal origin particularly poultry and this will require a huge expansion of poultry resources and closer intimacy between human and poultry that share limited land resources (Enserink, 2006). This presents opportunities for a shared infection between man and his poultry and any such infection with zoonotic potential like HPAI could present with serious human health problems. The politics and policies that shape the control of livestock diseases like HPNAI are undoubtedly complex, but the need to free Africans from poverty, make them food secure and save the continent from emerging diseases like pandemic influenza should justify overriding all complexities in favour of effective animal disease control.

5.1. FUTURE PERSPECTIVES

In conclusion, the situation of avian influenza (H5N1 and all other Influenza A) viruses in animals (most importantly poultry) and humans is not over yet especially in Nigeria, Africa or even in East Asia. Most of the countries that claim freedom from infection are basing such declarations on absence of HPAI reporting, however epidemiological data collected are often inaccurate, inadequate or fragmented and can not be relied upon. The situations therefore call for a need to place the issue of avian influenza in Africa in perspective.

There is a need to conduct more intense empirical studies into the following:

1. HPAI H5N1 viral ecology in the Nigerian farms and LBM
2. Identification of risk factors and the contribution of individual risk factor to the overall HPAI situation in Africa
3. Comprehensive viral surveillance at farm level and border towns to determine the presence or otherwise of avian influenza in each country
4. State of co-interaction between animal species (especially free range birds, dogs and cats) and the impact on the spread of HPAI spread in the affected African countries
5. Threat of continued avian influenza H5N1 spread and with particular reference to the animal-human interface

6. Comprehensive socio-psychological and socio-economical implications of avian influenza especially for the poor rural population which form the majority in Africa
7. Completion of the sequences of internal genes of viruses previously partially characterized in this study and for a continuing genetic characterization of HPAI H5N1 viruses from new outbreak locations.

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URL OF FIGURES ACCESSED ON LINE

1. Fig. 1.9: http://www.wakingupcosts.net/399
2. Fig. 1.11: http://www.poultrymed.com/files/index.html
3. Fig. 1.12: http://www.scientificpsychic.com/health/virus.html
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6. Fig. 1.15: http://avianflu.realage.com/imagescontent/
7. Fig. 1.16: http://www.rhodes.edu/biology/glindquester/viruses/pagespass/flu/flu.html
8. Fig. 4.1: http://www.fao.org/ag/againfo/subjects/en/health/diseases-cards/migrationmap.html

PHOTO CREDITS

Figure 1.4 and 1.5: Dr. T. M. Joannis.

Figure 1.6 and 1.7: Food and Agricultural Organization of the United Nations.

Appendix A

National Department of Agriculture

DEPARTMENT OF AGRICULTURE
DEPARTEMENT VAN LANDBOU
REPUBLIC OF SOUTH AFRICA
DIREKTORAAT DIEREGESONDHEID
PRIVAATSAK/PRIVATE BAG X138
2006 -06- 02
PRETORIA 0001
DIRECTORATE OF ANIMAL HEALTH
DEPARTMENT OF AGRICULTURE

Directorate of Animal Health
Import-Export Policy Unit
Private Bag X138
Pretoria, 0001
Republic of South Africa

Tel: (27)-012-3197514
Fax: (27)-012-3298292

IMPORTER:
ARC-ONDERSTEPOORT VETERINARY INSTITUTE
OLD SOUTPAN ROAD
ONDERSTEPOORT
PRETORIA
0110

PERMIT NO: 13/1/1/30/1-**94**
Valid from: 2006-06-02
Expiry date: 2006-10-02

VETERINARY IMPORT PERMIT
(Issued in terms of the Animal Diseases Act, 1984

Authority is hereby granted for you to import into Republic of South Africa:

60 ISOLATES X CHEMICALLY-INACTIVATED HPAI H5N1 VIRUSES FROM NIGERIA

from: **DR.DAVID SHAMAKI, ASSISTANT DIRECTOR OF RESEARCH NATIONAL VETERINARY RESEARCH INSTITUTE, VOM, PLATEAU STATE, NIGERIA PMB01**
subject to the following conditions:

1. the consignment must be accompanied by this original permit;
2. the INACTIVATED VIRUSES to be securely packed and transported in leakproof containers, sealed by an authorised official of the Veterinary Administration of the exporting country;
3. the consignment to be airfreighted through port of entry **JOHANNESBURG INTERNATIONAL AIRPORT**
4. the **INACTIVATED VIRUSES** to be kept and used for purposes of testing/research at the laboratories of ARC-BIOTECHNOLOGY DIVISION,ONDERSTEPOORT VETERINARY INSTITUTE, ONDERSTEPOORT under the personal supervision of CELIA ABOLNIK
5. on completion of tests/research the **INACTIVATED VIRUSES** must be destroyed by incineration;
6. The State Veterinarian: **KEMPTON PARK** Tel: **(011)973 2827** must be advised timeously of the arrival of the consignment.
7. This permit is subject to amendment or cancellation by the Senior Manager Animal Health at any time and without prior notice being given.

This permit is valid for three (3) months from date of issue and FOR ONE CONSIGNMENT ONLY.

for **SENIOR MANAGER: ANIMAL HEALTH**

NOTE:
From 1st January 2005 any consignment imported into South Africa packed with either wood packing material or dunnage, will require treatment to remove any pests present (by heat or methyl bromide fumigation). Treatment must be indicated on packing material. [Enquiries: Directorate Plant Health & Quality Fax: 012 319 6350 or www.nda.agric.za]

30-1-path specimens

Appendix B

Federal Government of Nigeria

University of Pretoria

Survey on Impacts and Epidemiology of Avian Influenza in Nigeria (2006)

This questionnaire is collated and being conducted as part of an on-going Master of Science Project. It is a non-profit/non-commercial research meant for the public good. The privacy of all participants will be strictly ensured and any information provided will be used only for the purpose of this research.

SECTION A: GENERAL

s/no.		
1	State	
2	Local Government	
3	Rural/Urban	
4	Name (optional)	
5	Marital Status	
6	Age	Below 20……. 20-30……. 31-40……. 41-50……. Above 50……..
7	Children	Yes/No. If yes, how many?…….
8	Occupation (Farmer)	
9	Occupation (Partner)	
10	Other adult (Income earners)	
11	Education level	Number of years of school……

SECTION B: FARM OPERATION

Is poultry farming your main occupation? Yes……..No……

If yes, do you have a secondary occupation? Yes……No……

If no, what is your main occupation? ……

What % of your time is dedicated to poultry farming? …….

Where did you raise money to start poultry farming? ……

Do you still have loans to pay back? ……

How do you dispose/sell your poultry products? ……..

Do egg/poultry buyers collect eggs from your farm? Yes….. No….

Where do they take it to? ……....

What types of bird do you keep?

Types of Birds	Number	Age	Source of bird

Did avian influenza affect your farm in any way? Yes……. No……

If yes, how?

Lose birds	Lose income source	Reduction in income	Spend more on disease prevention	Cannot sell products	Others(state)

Did avian influenza infect your farm? Yes…….No…….

(This section can be skipped for uninfected farms)

When was your farm infected? .....

To whom did you report? ……..

How long does it take you between disease infection and reporting? …….

How easily can you report avian influenza outbreak?

Easy	Not easy	Very difficult

Are you doing any other job now if you lose all your birds? Yes…….. No……

……

……..

Have you gone back to poultry farming? Yes…. No……Later…. Never…...

Were you paid any compensation? Yes……. No……..

Are you happy with the compensation paid? Yes…… No……

Rate the following in order of their importance to you ?

More compensation	Information/counselling	Re stocking	Re financing	Others (state)

What do you think is responsible for infection in your farm (infected farms only)?

……

……

Did you visit any infected farm just before the outbreak? Yes…..No……Not sure…….

If yes, how long? …….

Did you receive visitors from infected farms just before the outbreak? Yes….No….Not sure…..

If yes, how long? …….

How did you sell/dispose off your product during the outbreak?

Sell in open market	Destroy and bury/burn	Dispose off in the refuse dump	Slaughter and eat/sell	Government officials handle it

Did you purchase live poultry? Yes…… No……. Poultry products ? Yes…. No…..before your farm was infected? What quantity? ……

(This section apply to all farms)

SECTION C: EPIDEMIOLOGY

Do you visit other people's farm? Yes…… No……

Do you have infected farms in your immediate neighbourhood? Yes…… No……Not sure…..…

What biosecurity plan do you have for your farm?

Foot bath	Gates and fence	Changing room	Farm cloth and boot	Shower/ bath	Tyre dip	Perimeter fencing	Log Book	Others (state)

Do you actually use the biosecurity measure? Yes…… No…….

Do you keep other categories of animals? Yes…… No……. If yes, list

Are other poultry farms close to your farm? Yes…. No…., If yes, how close…….

How do you dispose your farm litter and other waste materials from the farm?

Burn/bury	Sell as fertilizer	Dump in refuse site	Spread in farm site	Other (state)

Do you compound your animal feed or you buy finished (ready-made) feed? ……

Do you borrow farm equipment? Yes……. No……., If yes, what? .....

What is the water source for your farm? ……

Do you have lake/pool/swamp etc around your farm? Yes……. No……..

Do water birds visit your farm environment? Yes……. No……

Do you have problem with rat in your farm? Yes…… No……

Who is responsible for vaccination and drug administration of your flock?

Self/family	In-House Vet	Shared Vet	Para Vet	Others(state)

Is your poultry building(s) bird-proof? Yes…… No……

What do you do with your dead chickens? ……

SECTION D: IMPACTS **(infected farms)**

Can you estimate your financial loss? ……

Do you think you need assistance? ……

Who do you think should provide the assistance? ……

How much do your family earn per month? ……

What % of this income comes from poultry? ……..

Do you have other sources of income? Yes……. No……..

If yes, list

……

……..

	Yes	No	How many?
Do you have other people living with you?			
Do you have dependants you send money to regularly?			
Do you have people you lend money to?			
Do you pay money to government?			

Money paid to government is for what? .....

Do they get money elsewhere? Yes……No……

Do you have other workers in your farm? Yes……No……How many?.....

Do you have accommodation for them? Yes…….. No……

How far is their place from work? …….

Do they keep poultry of their own? Yes……No……

Do you know if they have got other job if they lose their job in your farm? Yes… No…

(All farms)

Commodity Prices

What is the price of	**January '06**	**April '06**	**October '06**
"Old Layer"			
Tray of eggs			
Broiler			

SECTION E: HUMAN RISK AND RISK COMMUNICATION

Is avian influenza of any concern to you? Yes…… No……Not sure……..

Does avian influenza have food safety problem? Yes…… No……Not sure……..

Will you eat chicken that has avian influenza? Yes….. No…….Not sure……..

Will you eat chicken/eggs from infected flock? Yes….. No……Not sure……..

How will you process it? .....

What can increase the risk of avian influenza?

.....

.....

Did you hear of it before the outbreak in Nigeria? Yes…….. No…….Not sure……..

Do you know how it affects birds? Yes…… No……Not sure……...

What are the symptoms? .....

……..

What other disease is similar to it? .....

……..

Is the infection fatal to (a) birds? Yes…… No…… (b) man? Yes…. No….

List people at risk of infection in order of importance ?

1	
2	
3	
4	
5	

Do you know risky activities that can bring human infection? Yes…. No….

If yes, what? .....

How can you minimize the risks?

……

……..

Do you receive information about avian influenza? Yes…. No…….

From what source ?

Government	Television	Radio	Community	Newspaper	Others(state)

Did you test your birds for avian influenza? Yes…… No……

Did you test yourself for avian influenza? Yes……. No……..

Will you be willing to do the test for yourself? Yes…….. No……..Not sure…….

Will you be willing to do the test for your chicken? Yes…….. No……..Not sure…….

Is there any other thing you want to say about?

- Avian Influenza

  ……

  ……
- Poultry……

  ……..
- Government……

  ……..
- Control Strategy

  ……

  ……
- General……

  ……..

Thank you for your time.

Commodity Prices

What is the price of	January '06	April '06	October '06
"Old Layer"			
Tray of eggs			
Broiler			
Rice			
Corn			
Yam			
Cassava (Gaari)			
Beans			
Beef			
Mutton			
Pork			
Fish			
Goat meat			

Commodity Prices

What is the price of	January '06	April '06	October '06
"Old Layer"			
Tray of eggs			
Broiler			
Rice			
Corn			
Yam			
Cassava (Gaari)			
Beans			
Beef			
Mutton			
Pork			
Fish			
Goat meat			

Appendix C

Financial cost implications of the highly pathogenic notifiable avian influenza H5N1 in Nigeria in 2006

For the financial cost evaluation of HPNAI in Nigeria, the actual situation and scenarios of mild (10%) and severe (70%) generalized outbreaks in the commercial flocks were selected.

In Nigeria, the commercial layer is very important, accounting for almost 90% of all egg production (Adene and Oguntade, 2007). Similarly, ~99% of all infected poultry populations were commercial layers and layer breeders (Data retrieved from National Veterinary Research Institute, Nigeria). Our estimates deal only with this segment which often operates with little to no biosecurity.

A number of assumptions were made:

1. HPNAI caused 100% mortality in affected flocks either through pathologic death or control measures by destruction.
2. 100% cessation in egg production was assumed based on published reports (Capua and Marangon, 2000).
3. HPNAI caused a loss of 6 months in layer/layer breeder systems (downtime and raising new stock to point of lay).
4. Laying birds were in full production and would lay 284 eggs (80% production) for 1 laying cycle, and layer breeders would lay 265 eggs (75% production). 50% of the breeders' offspring would have market value (pullet) and 50% would be cockerels with zero value. 200 chicks per breeder hen are expected and approximately 100 of these chicks would be valued stock (average production standards).
5. All deaths in poultry population in Nigeria occurring during the period of study (January – August, 2006) arose from HPNAI or factors associated with it.

Other baseline data were obtained from Resource Inventory Management, Nigeria National Livestock Resource Survey and FAOSTAT-GLIPHA (FAO 2006a, b & c)

It is difficult to place an economic value on human beings affected by HPNAI. The affected human population was not economically assessed. Prevention of the spread of the disease in livestock would prevent its introduction in the human population.

Table 2.3. Types and number of birds affected between 10 January and 31 August, 2006

SPECIES AFFECTED	NUMBER	PERCENTAGE
Chicken: Layer/Pullet §	770,826	98.12
Chicken: Broiler/Cockerel	2,755	0.004
Chicken: Breeder	11,501	0.015
Guinea Fowl/quail	19	0.000024
Duck/Goose	148	0.000188
Ostrich*	218	0.000278
Turkey	101	0.000129
Wild Bird(multi species)	2	0.0000025
TOTAL	**785, 570**	**~100**

- § Include local , backyard and free range laying hens
- * Ostriches numbers were estimated based on available data

Mathematical Models

$$Ci = PS \{\upsilon + \beta + \delta + \gamma\}$$

$$Or\ Ci = PS\upsilon + PS\beta + PS\delta + PS\gamma$$

Where Ci = cost implications

P = Population of poultry

S = Susceptibility rate of population

υ = Direct Losses: Losses from mortalities (Cost due to mortality of poultry and values of chicks lost from breeders)

β = Indirect Losses: Egg and meat loss (value of direct loss of eggs due to yield reduction, cost of rejection of poultry meat and eggs, and cost associated with glut)

δ = Intangible Losses: Opportunity Cost (Cost of rearing replacement stock to production or sale point, cost of feeding to point of production, cost of retaining facilities and staff during downtime and rearing stage and cost of destroying remaining population of animals)

γ = Miscellaneous costs (cost of intense campaign to win back consumer confidence, cost of control and administrative/governmental policies, and external inputs)

*All calculations were done in Naira (N) (Nigerian currency) and converted to US Dollars at an exchange rate of USD 1 = N128.50

Total Chicken Population in Nigeria = 140,000,000
Commercial Chickens = 40% of 140,000,000 = 56,000,000
Commercial layers and layer breeders = 75% of 56,000,000 = 42,000,000
Commercial Layers = 90% of 42,000,000 = 37,800,000

Layers in Production = 75% of 37,800,000 = 28,350,000
At 80% hen-day production:
Number of eggs per day = 22,680,000
Number of eggs per annum = 8,278,200,000 {eggs in 12 months (365 days)}
Total number of trays (30 eggs per tray) = 275,940,000 trays per annum
At $2.18 per tray: Total annual value of eggs from all commercial layers will be = $601,549,200

Layer Breeders = 10% of 42,000,000 = 4,200,000
Layer Breeders in Production = 75% of 4,200,000 = 3,150,000
At 75% production:
Total expected chicks per breeder per annum = ~200 chicks (100 are saleable pullets)
Total expected number of valued chicks (pullets) per annum = 100 x 3,150,000

If chicks price range between $0.70 and $1.13 with average of $0.93,
Total Value of chicks expected would be= 100 x 3,150,000 x $0.93 = $294,163,424

Total Value of chicks and eggs expected from Layer Breeders and Commercial Layers = $294,163,424 + $601,549,200 = **$895,712,624**

Table 2.4. Parameters used in assessing the economic impacts

s/no.	Description	Symbol	Basic Data	Actual Scenario	Mild Scenario	Severe Scenario
1	Population size at Risk (layers and breeders)	P	42,000,000	0.0056% (758,570)	10 % (4,200,000)	70% (29,400,000)

2	Susceptible population	S	100%	100%	100%	100%
3	Mortality/disposal		100%	100%	100%	100%
4	Commercial Layer population affected		37,800,000	774,069	3,780,000	26,460,000
5	Layer Breeder population affected		4,200,000	11,501	420,000	2,940,000
6	Total market value of adult birds {commercial layer at ~$7 and Layer Breeders at ~$27.30}	Layer	$264,600,000	$5,418,483	$26,460,000	$185,220,000
		Breeder	$114,660,000	$313,977	$11,466,000	$80,262,000
		Total	$379,260,000	**$5,732,460**	**$37,926,000**	**$265,482,000**
7	Value of eggs at ~$2.18 {layers only} & meat {old lay value-at ~$4.36/bird}	Eggs	$601,549,200	$15,974,720	$60,154,920	$421,084,440
		Meat	$164,808,000	$3,374,941	$16,480,800	$115,365,600
		Total	$944,899,200	**$19,349,661**	**$76,635,720**	**$536,450,040**
8	per annum		$294,163,424	$1,074,023	$29,416,342	$205,914,397
9	Value of chicks expected		75%	75%	75%	75%
10	Proportion in production		N280 ($2.18)	N260 ($2.02)	N200 ($1.56)	≤N150
11	Mean Egg price per tray*		Pre outbreak period	6 months	6 months	($1.16) 6 months
	Delay in next production					

* Average egg price derived from field data collected before, during and after the crises period of outbreak. Note that egg price per tray of 30 eggs was progressively dropping as outbreak situation worsened. Layer represents commercial layers, Breeders represents Layer breeders. Other data were derived from UNDP, 2006.

Calculating for ʊ (Direct Costs)

PSʊ1= Actual determined direct value based on the outbreak situation (January-August)

PSʊ2= Estimated direct value based on mild scenario of HPNAI outbreak (10% losses in commercial poultry population).

PSʊ3= Estimated direct value based on severe scenario of HPNAI outbreak (70% losses in commercial poultry population).

PSʊ = Market Value of birds + value of chicks lost

PSʊ1= $5,732,460 + $1,074,023 = $6,806,483

PSʊ2= $37,926,000 + $29,416,342 = $67,342,342

PSʊ3= $265,482,000 + $205,914,397 = $471,396,397

Calculating for β (Indirect Costs)

PSβ= Cost (glut)

Costs associated with glut: Reduction in price observed x (Total annual national production (trays/annum) - Trays lost to mortality in HPNAI)

PSβ1 Cost (glut) 1= ($2.28-$2.02) x (275,940,000-5,650,704) = $42,068,373

PSβ2 Cost (glut) 2= ($2.28-$1.56) x (275,940,000-27,594,000) = $154,612,296

PSβ3Cost (glut) 3= ($2.28-$1.16) x (275,940,000-193,158,000) = $83,748,327

Calculating for δ (Intangible costs)

Since Intangible costs = Costs of rearing replacement stock, facilities retention, staff retention, downtime cost and destruction/disposal of remaining of affected flocks,
Therefore
PSδ = Replacement cost + Downtime cost + Destruction/disposal cost

Replacement cost = (99.985% cost for raising pullets to POL* + 0.015% cost for layer breeders pullets to POL) x total number lost
* *POL: Point of lay bird*
Downtime cost for facilities = Facility cost/bird/annum x Downtime period/annum x number of birds
N100/bird/annum† x 3months/12 months‡ x number of birds

† *$778.21/1000 birds/annum for retaining poultry pen (Field investigations and data from poultry producers, 2006)*
‡ *Average downtime period is 2-4 months (~3 months)*

Destruction/Disposal costs are borne by Government as well as part of the cost of control.

PSδ1 = {(0.99985 x $4.28 + 0.015 x $13.23) x 785,570) + ($0.78 x 3/12 x 785,570)} = $3,516,156
PSδ2 = {(0.99985 x $4.28 + 0.015 x $13.23) x 4,200,000) + ($0.78 x 3/12 x 4,200,000)} = $18,798,906
PSδ3 = {(0.99985 x $4.28 + 0.015 x $13.23) x 29,400,000) + ($0.78 x 3/12 x 29,400,000)} = $131,592,341

Calculating for γ (Miscellaneous Costs)

Using Nigerian Government budget allocation for 2005 as a guide for 2006

Table 2.5. Budgets and allocations for 2005 fiscal year

Department	Classification number	Expenditure items	2005 allocation	% estimated to be spent on HPNAI
FMA&RD	06200002501004	Publicity &advertisement	$22,757	50%
FMA&RD	02500002000240	(a)	$77,821	50%
FMA&RD	02500002000241	Animal disease control (b)	$77,821	50%
NVRI	02500002000202	National veterinary quarantine services (c)	$38,911	50%
NVRI	02500002000205	Strengthening of Central & outstation laboratories (d)	$155,642	100%
		Research and studies (avian influenza) (e)		
Total:			**$372,952**	**$264,297**

FMA&RD: Federal ministry of Agriculture and Rural Development, NVRI: National Veterinary Research Institute

Source: Nigerian Government (2006a & b)

Compensation reported till date = $182,640 (www.nigeria.gov.ng/avian%20flu%20center)

Other funds and materials acknowledged by the government include:

Monetary Income

1. World Bank Special Emergency Fund = $ 50,000,000 (of which $ 7,000,000 has been released (WHO, 2006)
2. 3 Banks = $171,206

Non-monetary Income

3. DFID = 15,000 protective personnel equipment (PPE)
4. FAO = 7,500 protective personnel equipment and 750 Liters (Diskol)
5. WHO = 10,000 doses of Tamiflu
6. USAID = 1,425 protective personnel equipment
7. Israel Government = 1 ½ Tonnes of Medical equipment

Expenditure

Items 3-7 were assessed in monetary value as below:

1. DFID = 15,000 protective personnel equipment = $1,781,250 (at $118.75/PPE*)
2. FAO = 7,500 protective personnel equipment and 750 Litres (Diskol) (~$20/Litre[†]) = $905,625
3. WHO = 10,000 doses of Tamiflu = $800,000 (at $80/dose of ten tablets[§])
4. USAID = 1,425 protective personnel equipment = $169,219

5. Israel Government = 1 ½ Tonnes of Medical equipment = $?? (details not available to do actual costing)

Total = $2,856,094

* *http://www.gallawaysafety.com/disposableprotectiveclothing-c-76.html*

§ *http://www.coreynahman.com/tamiflu.html*

† *Price of comparable virucidal (disinfectant) (Onderstepoort Veterinary Animal Hospital)*

Other organizations including EU and UNICEF were also acknowledged by the government.

Assuming that all other donations is included in the government spending,

PSγ = 50% (Expenditure items a, b, c, d) + 100% (Expenditure item e) + Reported Compensation + Non monetary expenditure

* *Note that items a-e are listed in Table 3*

PSγ = $108,655 + $155,642 + $182,640 + $2,856,094

PSγ = **$3,303,031**

PSγ 1 =PS γ 2 = PSγ 3

It is impossible to correlate government spending to the scale of the outbreak; this amount was left unchanged for all scenarios. It seems reasonable to assume that this spending would in fact increase in the event of more severe outbreak.

PS γ = PSγ 1 = PSγ 2 = PSγ 3 = **$3,303,031**

Appendix D

Option of vaccination as an additional control measure against avian influenza H5N1 in Nigeria

Nobilis® Influenza H5 Vaccine (H5N2) A/chicken/Mexico/232/94/CPA (Intervet International, Boxmeer, the Netherlands) was chosen as a vaccine of choice based on the reported success associated with its use in other countries (http://www.cidrap.umn.edu/cidrap/content/influenza/avianflu/news/oct2506/vietsuccess/html). Estimated poultry population figures (2006-2009) were arrived at using data available on the FAO statistical website (GLIPHA). It was assumed that 70% of the total poultry population will be covered by the vaccinators in each year of the operation.

Cost associated with vaccination, test and slaughter policy

Cost Year 1 (2006):

1. Cost of vaccine = Number of chicken vaccinated X cost per dose of vaccine X 2 times vaccination
   Cost of vaccine = 109,760,000 x $0.06 x 2 = $13,171,200
2. Labour, administration and distribution (LAD) costs = Number of chicken vaccinated X cost per vaccination X 2 times vaccination + Distribution and administration costs
   LAD costs = (109,760,000 x $0.04 x 2) + $156,128 = $8,936,928
3. Compensation and eradication costs = Dead/culled birds X average price per bird
   Compensation and eradication costs = 945,862 x $11.71 = $11,076,044
4. Associated laboratory costs = $155,642 (http://www.nigeria.gov.ng/dbudget2005.pdf)

**Note: Since the effective implementation of the programme is expected to reduce the rates of infection, spread and death in the poultry populations, it was assumed that:*

- *The mortality rate will reduce by 50% in the second year and the third year*
- *Associated compensation and eradication will consequently reduce by 50%*
- *Government spending on research and laboratory costs will gradually reduce by about 25% in each year of the operation*

These assumptions were noted in calculating the associated costs of the programme for the second year (2007) and the third year (2008)

All iterations above were repeated for the cost year two (2007) and three (2008).

Costs associated with a vaccination, test and slaughter policy

Year	Poultry Estimates (headcount)	Vaccinated population (70%)	Cost of Vaccination (2 doses given 4 weeks apart) ($)	Labour, distribution and administration costs ($)	Compensation and eradication ($)	Associated laboratory cost ($)
0 (2006)	156,800,000	109,760,000	13,171,200	8,936,928	11,076,044	155,642
1 (2007)	163,934,400	114,754,080	13,770,490	9,336,454	5,538,022	116,732
2 (2008)	171,393,415	119,975,390	14,397,047	9,754,159	5,538,022	77,821
3 (2009)	179,191,815	-	-	-	-	-

Cost of vaccine per bird per 2 doses is $0.12; cost of vaccine administration is $0.04 per bird; cost of distribution and other administration is $156,128 per annum; compensation was paid at $ 11.71 per chicken; total birds lost as at January 2007was 945,862; associated laboratory cost is $156,128 which would decrease by 25% per annum

Associated benefits of a vaccination, test and slaughter policy

Benefits Year 1 (2007):

(a) Reduced compensation = Reduced mortality as a result of vaccination X average compensation cost per bird

Reduced compensation = (945,862 ÷ 2) x $11.71 = $5,538,022

(b) Prevention of egg losses = Reduced mortality as a result of vaccination X number of tray of eggs per chicken X price per tray of 30 eggs X % of laying birds

Prevention of egg losses = 472,931 x 284/30 x $2.186 x 85% = $9,785,968

(c) Regain of sale in the regional market = Quantity of poultry meat exported to the regional market per annum X price per kilogram

Regain of sale in the regional market = 144,000,000kg x $2.73 = $393,120,000

(d) Normalization of egg price = (Total population – loss due to mortality) X number of tray of eggs per chicken X average reduction in value of eggs per tray

Normalization of egg price = (163,934,400-472,931) x 284/30 x $1.01 = $1,327,794,875

(e) Value of salvaged birds = Total mortality in previous year X average value of chicken

Value of salvaged birds = 945,862 x $7.03 = $6,649,410

(f) Prevention of redundancy of facilities = Number of bird lost in previous year X cost of facility per bird per year

Prevention of redundancy of facilities = 945,862 x $780.64/1000

All iterations above were repeated for the benefit year two (2007) and three (2008).

Associated benefits of a vaccination with test and slaughter policy

Year	Reduced Compensation ($)	Prevention of egg losses ($)	Regaining of external trade ($)	Normality in egg prices ($)	Value of salvaged birds ($)	Prevention of facilities redundancy ($)
0 (2006)	-	-	-	-	-	-
1 (2007)	5,538,022	9,785,968	393,120,000	1,327,794,875	6,649,410	738,378

2 (2008)	2,769,011	4,892,984	393,120,000	1,388,431,539	3,324,705	369,189
3 (2009)	2,769,011	4,892,984	393,120,000	1,456,315,773	3,324,705	369,189

Calculations were based on 284 eggs per bird per annum; Chicken eggs sell for $2.19per tray of 30 eggs; Nigerian Poultry Association claimed export of 12,000 tonnes of poultry meat per month to regional markets at $2.73 per Kg; Mean observed reduction in egg price during peak infection of HPAI was $1.01/ tray of 30 eggs; value of spent chicken is $7.03; average cost of facility for 1000 chicken per annum is $780.64

Adjusted costs and benefits of a vaccination, test and slaughter policy

Costs and benefits associated with the control programme over a three year period were calculated using established economic analytic tools previously described (Horst *et al*., 1999; Ramsay *et al*., 1999). A 12% discounting factor was used for all calculations as this is obtainable in Nigeria. Final figures were used to arrive at the benefit cost ratio of the project

Costs and Benefits associated with the control programme over a three year period.

Year	Discount Factor ??(0.12)	Cost ($)	Benefit ($)	Net cash flow ($)	Cumulative cash flow ($)	Discounted cash flow ($)	Discounted cost ($)	Discounted benefit ($)
0 (2006)	1.00	33,339,814	0	-33,339,814	-33,339,814	-33,339,814	33,339,814	0
1 (2007)	0.89	28,761,698	1,743,626,653	1,714,864,955		1,526,229,810	25,597,911	1,551,827,721
2 (2008)	0.80	29,767,049	1,742,907,420	1,713,140,371	3,424,671,345	1,370,512,297	23,813,639	1,394,325,936
3 (2009)	0.71	0	1,860,791,662	1,860,791,662	5,285,463,007	1,321,162,080	0	1,321,162,080
Totals		91,868,561	5,347,325,735	5,255,457,174	10,388,325,512	4,184,564,373	82,751,364	4,267,315,737

Appendix E

SPECIFIC LOCATIONS AND SPECIES OF AFFECTED BIRDS

LOCATION	DATE	LATITUDE	LONGITUDE	SPECIES
Jaji	116	10D 49'N	7D 34'E	domestic birds
Igabi				domestic birds
Bukuru	116	9D 42'N	8D 54'E	domestic birds
Onibudo	118	6D 41' 48"N	3D 21' 35"E	domestic birds
Danbari	119			domestic birds
Kano	126	11D 50' 45"N	8D 30' 21"E	domestic birds
W/Mine	202			domestic birds
L-Usuma	207	8D 54'N	6D 53'E	domestic birds
Toro	208	10D 03' 27"N	9D 04' 03"E	domestic birds
Azare	208	11D 40' 42"N	10D 11'31"E	domestic birds
Rantya	209	9D 55' N	8D 51'E	domestic birds
Katako	209	10D 11'N	8D 46'E	domestic birds
Rikkos	209	9D 52'N	8D57'E	domestic birds
Azare	211	11D 40' 42"N	10D 11'31"E	domestic birds
Guako	211	9D 01' 51"N	7D 11' 38"E	domestic birds
Rafukawa	212			domestic birds
Dutsen-safe	213			domestic birds
Katsina	213	12D 59'52"N	7D 35' 58"E	domestic birds
D-Giring	213	9D 34'N	8D 44'E	domestic birds
Bukuru	215	9D 42'N	8D 54'E	domestic birds
K-Vom	215	9D 40'N	8D 44'E	free flying bird
Rayfield	215	9D 50'N	8D 54'E	domestic birds
Rantya	215	9D 55' N	8D 51'E	domestic birds
Potiskum/Nangere/Jakusko	216	12D 22' 04"N	10D46' 16"E	domestic birds
Kokona	217	8D 48' 02"N	8D 02' 07"E	domestic birds
Toro	217	10D 03' 27"N	9D 04' 03"E	domestic birds
Toro	217	10D 03' 27"N	9D 04' 03"E	domestic birds
Hadejia	217	12D 26' 53"N	10D 02' 37"E	domestic birds
Z-Dawanau	217	11D 54'N	8D E	domestic birds
Kano	217	11D 50' 45"N	8D 30' 21"E	domestic birds
Malumfashi	217	11D 48' N	7D 37'E	domestic birds
Kanta	217	9D 04'N	8D 25'E	domestic birds
S-Tasha	217			domestic birds
WTC-GRDP	217			domestic birds
Mando	217	10D 43'N	6D 34'E	domestic birds
Katako	217	10D 11'N	8D 46'E	domestic birds
Naraguta	217	9D 59'N	8D 54'E	domestic birds
Kuje	218	8D 36' 32"N	7D 13' 40"E	domestic birds
Univ Qtrs	220			domestic birds
Katako	220	10D 11'N	8D 46'E	domestic birds
B-Ring road	220	9D 52'N	8D 52'E	domestic birds
Rikkos	220	9D 52'N	8D 57'E	domestic birds
D-Kowa	220			domestic birds
Kawo	221	10D 34' 44"N	7D 26' 56"E	domestic birds
Dankande	221	11D 03'N	8D 09'E	domestic birds
U-Dosa	221			domestic birds
Kakuri	221	10D 28'N	7D 25'E	domestic birds
Oturkpo	222	7D 13'N	8D 09'E	domestic birds
Madubawa/Kano	222	11D 50' 45"N	8D 30' 21"E	domestic birds
Kano	222	11D 50' 45"N	8D 30' 21"E	domestic birds

Katako	222	10D 11'N	8D 46'E	domestic birds
S-Gari	223	9D 26'N	8D 11'E	domestic birds
Apollo Cresent	224			domestic birds
Katako	224	10D 11'N	8D 46'E	domestic birds
Ogidi-Ani	225	6D 05' 55"N	6D 52' 41"E	domestic birds
Apo	225			domestic birds
Port-Harcourt	226	4D 47' 21"N	6D 59' 55"E	domestic birds
S-Barki	226			domestic birds
Katako	226	10D 11'N	8D 46'E	domestic birds
W-Bogga	227			domestic birds
Sarauniya	227			domestic birds
Chwelnyap	228			domestic birds
Azare	301	11D 40' 42"N	10D 11'31"E	domestic birds
Rikkos	302	9D 52'N	8D 57'E	domestic birds
Langtang	302	8D 30' 31"N	9D 51' 56"E	domestic birds
Kano	303	11D 50' 45"N	8D 30' 21"E	domestic birds
K-Vom	304	9D 40'N	8D 44'E	Vulture
Jos	307	9D 55' N	8D 54'E	domestic birds
Kaduna	308	10D 36'N	7D 27'E	domestic birds
Mando	308	10D 43'N	6D 34'E	domestic birds
Kaduna	309	10D 36'N	7D 27'E	domestic birds
Isoko/Ajah	310	6D 28'N	3D 34'E	domestic birds
Sabirale/Agege	310	6D 3' 19"N	3D 19' 33"E	domestic birds
Rikkos	311	9D 52'N	8D 57'E	domestic birds
Old-Airport Jxn	313	9D 48'N	8D 52'E	domestic birds
T-Balewa	315	9D 45' 30"N	9D 33' 26"E	domestic birds
Malali	316			domestic birds
Tina Jxn	319			domestic birds
Bauchi	323	10D 18' 57"N	9D 50' 39"E	domestic birds
K-Ibrahim	324			domestic birds
Bukuru	330	9D 42'N	8D 54'E	domestic birds
GRA/Bauchi	401	10D 18' 53"N	9D 50' 42"E	domestic birds
Gunduwawa/Gezawa	403	12D 02' 02"N	8D 43' 28"E	domestic birds
Gwazaye	403	11D 56' 34"N	8D 27' 35"E	domestic birds
Wildlife Park	403			domestic birds
Hadejia	404	12D 26' 53"N	10D 02' 37"E	domestic birds
U-Dosa	405			domestic birds
K-Ibrahim	406			domestic birds
Apata	413			domestic birds
L-Jubril	414			domestic birds
I-Coomasie	416			domestic birds
Jos	416	9D 55' N	8D 54'E	domestic birds
Bauchi	419	10D 18' 57"N	9D 50' 39"E	domestic birds
Checheniya	426			domestic birds
Gamajiko	426			domestic birds
ECWA Bukuru	426	9D 42'N	8D 54'E	domestic birds
Anjida	502	9D 05'N	8D 17'E	domestic birds
Jos	505	9D 55' N	8D 54'E	domestic birds
Jos	509	9D 55' N	8D 54'E	domestic birds
J-Nyame	512			domestic birds
Anjida	514	9D 05'N	8D 17'E	domestic birds
Maternity/Jos	516	9D 55' N	8D 54'E	domestic birds
Gabasawa	519	12D 06' 24"N	8D 50' 14"E	domestic birds
DogonDutse	520			domestic birds
Molete	606	7D 21'N	3D 52' 40"E	domestic birds
Ojo	606	6D 27'N	3D 13'E	domestic birds
Ibi	607			domestic birds

Gindin Waya	607	8D 05'N	9D 47'E	domestic birds
Dogon-Karfe	608			domestic birds
Molete	611	7D 21'N	3D 52' 40"E	domestic birds
Ojo	612	6D 27'N	3D 13'E	domestic birds
Wukari	625	7D 51'N	9D 47'E	domestic birds
Owutu	626	6D 37'N	3D 31'E	domestic birds
Fagbile	628			domestic birds
Ikeja	711	6D 35' 48"N	3D 20' 35"E	domestic birds
Agbara-Otor (Ho)	905	5D 35'N	5D 52'E	domestic birds
Awka	1007	6D 13'N	7D 05'E	domestic birds
Awka	1009	6D 13'N	7D 05'E	domestic birds
Maiduguri	1110	11D 51'N	13D 05'E	domestic birds
Ehor (Egor)	1128	6D 23'N	5D 36'E	domestic birds
Ilorin	1202	8D 30'N	4D 33'E	domestic birds
Kano	1204	11D 50' 45"N	8D 30' 21"E	domestic birds
Ode Remo	1208			domestic birds
Gezawa	1208	12D 02' 02"N	8D 43' 28"E	domestic birds
Checheniya	1213			domestic birds
S-Tasha	1213			domestic birds
Kawo	1213	10D 34' 44"N	7D 26' 56"E	domestic birds
Kakuri	1213	10D 28'N	7D 25'E	domestic birds
Gezawa	1213	12D 02' 02"N	8D 43' 28"E	domestic birds
Gezawa	1213	12D 02' 02"N	8D 43' 28"E	domestic birds
Fadama-Bauchi	1227	10D 18' 57"N	9D 50' 39"E	domestic birds
Kano	1231	11D 50' 45"N	8D 30' 21"E	domestic birds
Gwale	1231	11D 57' 31"N	8D 28' 35"E	domestic birds
Kebbe, Sokoto	20070105			domestic birds

*Note that individual locations totaled 113. There are two or more outbreaks in some locations. Domestic birds were used because several farmers keep multiple species. Under the date column, 116 will represent 16th January, 2006 and 1231 will indicate 31st December, 2006.