ISOLATION OF AVIAN PARAMYXOVIRUSES FROM VILLAGE CHICKENS AND WILD BIRDS IN KENYA

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A Thesis Submitted in Part Fulfillment for a Master of Science Degree in the Department of Veterinary Pathology and Microbiology

University of Nairobi

2000

DECLARATION.

This thesis is my original work and has not been presented for a degree to any other University.

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DEDICATION.

This thesis is dedicated to my husband Dr.Jonathan Orengo, my Children Lenny Japheth Weda and Dora Keller Khakayi, and my parents Mr. and Mrs Francis Lichoti.

ACKNOWLEDGEMENTS

Special thanks go to my Supervisors Prof. P.N. Nyaga and Dr. Lilly C. Bebora who guided and assisted me and were patient to read and correct all the handwritten drafts and the typed manuscript.

My gratitude also goes to my friends for their technical assistance: Messer's Francis Njoroge, Julius Kibe and Justus Nzioka for media preparation and Indeche who used to feed the chicken.

The co-operation of the management and personnel of the slaughterhouses in Nairobi are acknowledged with great thanks.

After all is said and done, I give all glory to God who enabled me to do all that I planned to.

The German Academic Exchange Service (DAAD) provided a scholarship through the University of Nairobi that made this study possible and I am really grateful to them. The research work for the study was supported by funds from the Israel-Kenya joint project financed by the United States Agency for International Development (USAID) through the US-Israel Co-operative Research Programme (CDR) grant number DHR-105, CO8-26 of Professors M.

Lipkind of the Kimron Veterinary Research Institute, Israel, and P. N. Nyaga of the University of Nairobi for which I am very grateful.

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ABSRACT

The potential of village chickens in Kenya has not been fully exploited because most farmers do not practice disease control, supplementation, and there is no proper housing for the birds. Control of viral diseases especially Newcastle disease which is one of the major setbacks for village chicken farmers has not been dealt with. This work was designed to find out which strains of Avian paramyxovirus (APMV) occurred in the rural chickens and wild birds and if these chicken and wild birds could be potential reservoirs of virulent Newcastle disease virus (NDV). Field samples (cloacal and whole blood) were collected from live market village chickens at the time of slaughter. The samples were processed for virus isolation and the isolates recovered characterized using their haemagglutination-elution patterns, receptor specificity for red blood cells from different animal species, haemagglutinin thermostability, virulence in 9-day old embryonated eggs, cross reactivity of the isolates among themselves and control strains tested by the haemagglutination inhibition (HI) test with homologous and heterologous and reference sera for paramyxovirus serotypes APMV-1 to APMV-9. One hundred and sixty two avian paramyxovirus serotype-1 (APMV-1) and one avian paramyxovirus serotype-4 (APMV-4) were isolated from the rural chickens and wild birds composed of one hundred and forty six isolates from chicken, eleven from wild birds, five from Lake Bogoria water and one (APMV-4) from flamingo faeces. Haemagglutination-elution patterns showed that of the isolates recovered, 13 were fast eluters, 10 were moderate eluters, 41 were partial and 23 were non-eluters at 4°C. Elution appeared unrelated to any aspect of the virus history or other virus character. Receptor specificity of the isolates regarding red blood cells from seven species showed that all virus isolates agglutinated chicken red blood cells and in addition, dog red blood cells were agglutinated by most NDV isolates. Thermostability of haemagglutination

activity of the isolates showed that at 56°C all the isolates were inactivated within 8 hours. Of the twenty-one NDV isolates tested for virulence, 10 were velogenic, 7 were mesogenic and four were lentogenic. Flocks with velogenic strains constitute a reservoir of virulent Newcastle disease virus and this could be a potential danger to the chicken industry. The NDV isolates showed antigenic differences; however 8 pairs showed no cross-reactivity, 3 pairs of isolates were slightly related asymmetrically, and 33 pairs showed one-sided moderate relationship. Some vaccine strains were reacting asymmetrically with some of the isolates i.e. antisera against LaSota and F-strain vaccine strains could not neutralize B26, K2 and K11 virus isolates. Serology served as a useful diagnostic function allowing detection of infections in unvaccinated flocks and also an indication of exposure. Three hundred and eighteen samples were screened and 87 serum samples were positive and some showed cross-reactivity with the APMV-2 to APMV-9. After adsorption of the antisera with NDV-L, only APMV-3 showed specific haemagglutination inhibition against B37, W1, W17, KR139 and B6, the other inhibitions against the other APMV isolates were cross-reactions indicating the possibility that PMV-3 occurred in village chickens. NDV is widespread throughout Kenya. It was concluded that velogenic NDV strains are widespread in village chickens sold through live bird markets. Such birds are a reservoir of and can spread velogenic NDV and be a danger to the poultry industry. Wild birds harboured virulent NDV and pose danger to the poultry industry.

1.INTRODUCTION

Live bird markets in Kenya provide an outlet for direct sale of village poultry to consumers. These markets are located in the urban areas where most of the chicken is consumed. The chickens are collected from various parts of the country and sold in these urban and pre-urban markets. Chicken traffic between these rural sources and urban markets can be a vehicle for the transmission of a number of disease agents including Newcastle disease virus. Chickens, which are in the latent or convalescent phases of infection can excrete the Newcastle disease virus and infect the unvaccinated healthy in contact birds for a long time (White and Jordan, 1963 as cited by Awan *et. al.*, 1994). The poultry vendors could also carry the virus on their shoes from farm to farm as they go around collecting the chickens from the farmers. In Kenya most farmers sell their chicken when there is an outbreak of Newcastle disease so as not to incur severe loses (Musiime, 1991).

Although village chickens are the dominant form of poultry kept in the developing world, they are a natural resource whose potential is not fully exploited for the welfare of the rural populations. In Kenya 70% of the 25 million poultry population (M.O.L.D, 1991) are village chickens and they produce slightly more than 50% of all the eggs consumed in the country (Ndegwa and Janseen, 1994). The most devastating disease in village poultry is Newcastle

disease Musiime, 1991). Newcastle disease, (ND) is an epidemic disease in intensive poultry farming and is responsible for high economic losses (Awan *et. al* 1994). Together with Newcastle disease, Gumboro disease, fowl pox and Marek's disease have been identified also as major viral poultry disease contraints in Kenya

(Musiime, 1991).

Avian paramyxovirus serotypes 1-9 have been isolated from wild, captive, caged, and domestic birds in most countries where surveillance programmes have been undertaken (Ostrowski *et al.,* 1995, Alexander, 1980, Alexander, 1979). Newcastle disease virus (NDV), the type species of avian paramyxoviruses (APMV'S) produces the most severe disease while the other species produce a mild respiratory disease or egg production problems in their hosts (Waterson *et.al,* 1967). Besides the fowl, other poultry are susceptible to APMV-1 and about 30 species of wild birds including some migrant sea birds have been shown to be susceptible to the virus. The wide host range makes control of Newcastle disease difficult, however susceptibility does not necessarily imply that the birds are ever naturally infected (Waterson *et.al* 1967).

Currently there are nine serotypes of avian paramyxoviruses, namely, APMV-1 to APMV-9, with NDV as APMV-1. In Kenya APMV-1 isolates have been

recovered from exotic breeds of chickens (Nyaga, 1982), while APMV-2 and APMV-4 have been reported in wild birds (Mbugua, *et.al*, 1982; Nyaga *et.al.* 1995). Bell and Mouloudi (1988) found village chickens throughout Morocco to be reservoirs of virulent APMV-1. Out of the 100 tracheal swabs collected from village chickens in Morocco, forty-one isolates of NDV were recovered and the 12 isolates that were characterized were found to be velogenic. In Mauritania, tracheal swabs were taken from 80 village chickens from three regions and six isolates of NDV were recovered, of which four formed plaques on chicken fibroblast monolayers without overlay with trypsin, indicating virulence (Bell *et.al*, 1990a). Newcastle disease virus has also been recovered from surveillance of live bird markets in the Northern United States at the U.S. Department of Agriculture, National Veterinary Service Laboratories (King et al., 1997). All the isolates tested were of low virulence to the chicken. Panigrahy *et al.*, (1993), isolated velogenic viscerotropic Newcastle disease (VVND) from domestic psittacine birds in six states: Illinois, Indiana, Michigan, Texas, California, and Nevada. They also isolated VVND from quarantine birds intended for importation into the United States. Virulent Newcastle disease virus in rural chickens has been reported from Ethiopia (Bawke *et al.*, 1991), Nigeria (Olabode *et al.*, 1992), Sudan (Fadol, 1991), Ivory Coast (Couaccy-Hymann *et al.*, 1991), Mozambique (Fringe & Dias, 1991), Uganda (George, 1991), South-East Asia (Copland, 1987), Sri Lanka

(Gunaratne *et al.*, 1992), Thailand (Janviriyasopak *et al.*, 1989a,b), Myanmar (Lwin, 1992), Nepal (Mishra, 1992) and Vietnam (Nguyen, 1992). The isolation of Newcastle disease virus from village chickens indicate that virulent NDV is widespread.

In wild birds, at least 236 species have been reported to be susceptible to natural or artificial NDV infection (Kaleta & Beldauf, 1988). In Nigeria, velogenic, mesogenic and lentogenic strains of NDV have been found in wild birds and these were considered as a reservoir and a source of dissemination of the virus to susceptible rural poultry (Olabode *et al.*, 1992). Newcastle disease virus was isolated by Ostrowski *et al.*, (1995) from a Houbara bustard (*Chlamydotis undulata*) that was showing an acute respiratory disease while Alexander *et al.*, (1979) recovered NDV of low virulence from a wild Mallard duck and it could be distinguished from the vaccinal viruses. This indicated that non-vaccinal NDV of low virulence was present in wild birds in Great Britain. Lipkind *et al.,* (1997), isolated a mixed population of viruses of APMV-1 and APMV-4 and four cases of infection by APMV-1 andAPMV-2 in wild and domestic birds in Israel. In the case of the double infection of the wild mallard by APMV-1 and APMV-4, vaccination was not involved. The isolate derived from the apparently healthy bird turned out to be virulent for chickens (Lipkind *et al.,* 1997). Jörgensen *et al.*, (1998) isolated APMV-1 from dead birds in a population of 77 ostriches and four

emus held in quarantine. Samples from 17 of the dead ostriches examined virologically yielded three APMV-1 isolates and their ICPI was in the range of 1.63-1.69. Characterization of the isolates by means of mouse monoclonal antibodies and by restriction site analysis revealed that the three isolates were indistinguishable and similar to APMV-1 viruses present in a simultaneous epizootic of Newcastle disease in backyard poultry in Denmark. Isolated outbreaks in chickens in Switzerland in 1995 also showed the same binding pattern. It seemed, the Ostriches and the emus were the source of infection for the chickens. Rosenberg *et al.* 1974 obtained four isolates of NDV from free-flying Canadian geese in the Atlantic flyway that were lentogenic. Stallknecht *et al.*, (1991) isolated APMVs 1, 4, 6 and 8 from migrating and resident ducks in coastal Louisiana. These were typical of PMV'S commonly associated with free-flying waterfowl. Wild birds could be a source of infection for the chickens when they are in the same vicinity and sometimes they share food with the village chickens especially leftovers which the owners throw out for the chickens.

Seroconversion to NDV in unvaccinated birds is an indication of exposure to the virus. In Morocco, Bell and Moulodi (1988) collected serum samples from six regions from non-vaccinated chickens and they found antibodies against NDV from each region ranging from 5 to 83%. In Mauritania, serum samples were taken from 80 chickens in rural poultry flocks in three different regions and

antibodies against NDV were detected in 4.6% of the chickens (Bell *et al.*, 1990a). As cited by Awan *et al.*, (1994) Courtecuisse *et al.*, (1990) detected NDV antibodies in 14% of unvaccinated traditionally managed birds from 31 villages in Tessoua, Maradi Department. In Malaysia during field trials (Aini & Ibrahim, 1990) blood samples from 1200 unvaccinated chicken gave less than 10% detectable antibodies to NDV. In another study in Malaysia, 15% of free-range domestic fowl showed antibodies to NDV. Serological tests in small holder, backyard poultry flocks in Germany showed reactors for NDV in 32 out of 37 flocks, but no clinical cases of Newcastle disease were noted (Schobries *et al.*, 1989). A serological survey carried out in Nigeria (Ezeokoli *et al.*, 1984), showed 72% prevalence of antibodies against NDV in free range and 62% in traditionally managed backyard flocks. In Tanzania Minga *et al.*, (1989), screened blood samples from 120 chickens 4 months of age or older from five villages for ND and 13.3% of the chickens tested seropositive. Agbede *et al.*, (1992), in Cameroon collected blood samples from 60 village chickens from each of three regions and seropositivity for NDV antibodies was 52, 48 and 47%, with an overall mean of 49%. In a similar study in Benin Chrysostome *et al.*, (1995) conducted a serological study in three regions and seropositivity of chickens for NDV was 56, 75 and 69%. So far there is no report in Kenya on the prevalence and ecology of avian paramyxovirus serotypes 1 to 9 in live bird markets hence the study.

NDV is maintained in the village poultry by cycling of virus through the rural chickens, other domestic species and wild birds (Martin, 1992). The isolates recovered by Jorgensen *et al.*, (1998) from the ostriches and emus were similar to those recovered from outbreaks on farms in Denmark in the vicinity during the same period, thus indicating that the ostriches might have been the source of infection. A health and productivity study in native village chickens was carried out in Thailand (Thitisak *et.al.* 1989) where monthly blood samples were taken from 920 non-vaccinated wing tagged birds and 448 offspring. The mean haemagglutination inhibition titres for NDV was high in newly hatched chicks and declined as maternal immunity waned at about 90 days. Thereafter, mean titres rose steadily as the birds' age increased, peaking in birds over 3 years of age. This indicated persistence of NDV in the flocks. Samuel and Spradbrow (1989) working on the V4 strain of Newcastle disease virus in a small open range flock of Bantams found out that the virus spread to the uninoculated birds and persisted in the flock for two years, infecting chickens that were introduced by natural brooding and rearing. All new clutches of chicks seroconverted by 80 days of age and the titres of the adult birds showed a concurrent rise, suggesting that the chicks were amplifying the virus. The modes of spread and of persistence of the virus were not determined. These observations could indicate that Newcastle disease virus can be maintained in a flock of birds where there is

constant introduction of chicks and birds that have been once exposed to NDV can still be infected and excrete live virus without showing clinical disease.

The main objective of the project was:

1. To find out whether village chicken and wild birds in Kenya harbored avian paramyxoviruses.
2. If they do harbor the avian paramyxoviruses, are these virulent or apathogenic strains.
3. Are these strains any different from the standard vaccine strains in the market?
4. Can the vaccines in use now protect the birds from the wild strains?

The objective was achieved by testing the following hypotheses that rural chickens and wild birds in Kenya harbour avian paramyxoviruses and that all the avian paramyxovirus serotype-1 isolates are virulent, antigenically identical and have identical biological chemical, and physical properties.

2.LITERARURE REVIEW

2.1. Avian paramyxoviruses

Avian paramyxoviruses have been classified into nine serotypes (Alexander 1990) with Newcastle disease virus as the type species for avian paramyxovirus serotype 1 (APMV-1). Avian paramyxoviruses have an RNA genome and belong to the family, paramyxoviridae, subfamily paramyxovirinae, and genus rubulavirus. Virions of the family paramyxoviridae are 150nm or more in diameter and pleomorphic. They are usually spherical in shape, although filamentous and other forms are common. The virus nucleic acid is single stranded, non-segmented, non-infectious and negative sense to the mRNA with molecular weight, 5.2-5.7x10^6 daltons. An RNA directed RNA polymerase is also present in the virus. It has a helical symmetry and is 13-18nm in diameter, with a 5.5-7nm pitch. The virions also contain 6-7 transcriptional elements that encode 10-12 proteins. These proteins include three nucleocapsid-associated proteins i.e., an RNA-binding protein (NP), a phosphoprotein(P) and a large putative polymerase protein(L);three membrane associated proteins,i.e., an unglycosylated envelope protein (M) and two glycosylated envelope proteins comprising of a fusion protein (F) and an attachment protein (HN). The lipid envelope surrounds the nucleocapsid and is derived from lipids of the host cell plasma membrane. The F protein is synthesized within the infected cell as a

precursor and is activated following cleavage by cellular proteases to produce the virion disulfide linked F1 and F2 subunits. HN is associated with haemagglutinin and neuraminidase activities. Virions are very sensitive to heat, lipid solvents, non-ionic detergents, formaldehyde and oxidizing agents (Murphy *et al.*, 1995).

During maturation, assembly of paramyxoviruses occurs by a budding process at the cell membrane, and the envelope proteins are incorporated into the plasma membrane. The nucleocapsid aligns beneath an area of the membrane that contains the proteins. The modified membrane then envelops the nucleocapsid during the budding process, which results in the release of the virions (Choppin & Compans, 1975). Since the lipid bilayer is derived from host cell membrane, its lipid composition reflects that of the plasma membrane (Klenk & Choppin, 1969, 1970a, 1970b). This is as a result of the removal of neuraminic acid residues by viral neuraminidase (Klenk *et al.*, 1970a, 1970b).

The APMV-1 virus has been known since the early studies of Dawson and Elford (1949) to be pleomorphic. This irregularity of form makes morphological comparison between strains difficult. Waterson & Cruickshank (1963) compared the appearances of eight strains of NDV of varying virulence. In the electron microscope, the appearances of the internal ribonucleoprotein (RNP) were the same in all these strains; they differed only in the ease with which the coat

fragments were disrupted by ether treatment. There was no definite correlation of any morphological features with virulence.

2.2. Haemagglutination activity

Vaccine and avirulent field viruses may be distinguished by the heat resistance of their haemagglutinin and ability to agglutinate equine red blood cells (Hanson 1975; Hanson and Spalatin, 1978; Alexander *et al.*, 1979d). The haemagglutinin molecule initiates infection by binding to neuraminic acid containing receptors on the cell surface. The receptor-binding site is a pocket located at the distal end of each monomer. The residues forming the pockets are largely conserved between subtypes (Weis *et al.*, 1988). However amino acid exchanges at the pocket convey altered specificity for N-acetyl neuraminic acid linked to galactose either by alpha, 2,6 linkages or alpha 2,3 linkages (Rogers, *et al.,* 1983). Contact between the 5-N-acetyl group of neuraminic acid and Trp-153 and Gly-134 of the pocket play an important role in receptor binding (Weis *et al.*, 1988). Following receptor mediated endocytosis the fusion protein (F) mediates fusion with the cell membrane. Fusion depends on proteolytic cleavage of the fusion protein (F) (Klenk *et al.*, 1975; Lazarowitz and Choppin, 1975) and on a conformational change triggered by the low pH in the endosome. Cleavage is exerted by the host proteases and is an important determinant for the spread of infection and pathogenicity. Several protease inhibitors have been described that block fusion

protein cleavage and therefore interfere with the spread of infection (Hanson *et al.*, 1997).

2.3. Elution-patterns

The rate of elution of NDV strains can be used as a genetic marker as well as a character for identifying the strains, since there is no correlation between the haemagglutination patterns and other properties of the same strain (Spalatin *et.al.* 1970). Out of the 53 strains and 16 clones studied by Spalatin, both slow and rapid eluters were represented. The attachment and dissociation of virus from red blood cells is determined in part by the interaction of environmental temperature, sensitivity of cells, balance of electrolytes, pH of medium, and concentration and relationship of the components, as well as, by the virus (Spalatin *et al.*, 1970). The rapidity of agglutination and elution varies from virus to virus and strain to strain (Spalatin *et al.*, 1970).

2.4. Haemagglutination activity

Avian Paramyxoviruses have been found to possess different abilities to haemagglitinate red blood cells (RBCs) from various animal species (Macpherson and Swain, 1956; Nyaga 1982). According to Nyaga (1982), all the 32 isolates studied haemagglutinated RBCs from fowl, cow, sheep, and dog.

Only 10 of the isolates haemagglutinated RBCs from the horse and only one isolate failed to haemagglutinate rbcs from the goat. Of the 8 isolates studied by Macpherson and Swain (1956) none of them haemagglutinated rbcs from the horse and cat. Winslow *et al.,* (1950) studied 25 strains and 19 haemagglutinated cow RBC S, 10 haemagglutinated sheep, 13 haemagglutinated horse and all of them haemagglutinated dog RBCs. This indicates that dog RBCs are more sensitive to viral haemagglutinin.

2.5. Haemagglutinin thermostability

Studies on the thermal stability of the haemagglutinin of various APMV-1 strains have shown that infectivity and haemagglutinins are inactivated independently (Hanson *et.al*, 1949). Similarly, Nyaga (1982) reported that one APMV-1 isolate whose haemagglutinins became inactivated after 8 hours at $56^{O}C$ lost infectivity after 6 hours while two isolates whose haemagglutinins became inactivated after 20 minutes still retained infectivity after 60 minutes at $56^{O}C$.

2.6. Virulence of Newcastle disease virus

Strains of APMV-1 show marked differences in their ability to cause disease and death in susceptible birds. The pathogenicity has been determined using the mean death time (MDT) in eggs, the intravenous pathogenicity test index (IVPI) in day

old chicks and the intracerebral pathogenicity index (ICPI) test in 6-week-old chicks (Alexander, 1995). Isolates of NDV have been recovered from completely symptomless adult birds in various parts of the world (Waterson *et.al.* 1967). These include a strain described by Bankowski (1960) in the USA, the strain Queensland v_4 in Australia and strain Ulster recovered from Northern Ireland by McFenran. Strain Ulster causes no drop in egg production and is virtually nonpathogenic for young chicks (Waterson *et.al.* 1967).

Virulence of NDV may be based on the ability of the virus to kill chick embryos, and of the speed this occurs. The range of virulence as judged by the mean death time (MDT) in 10 day old embryonated eggs varies from about 40 hours for the most virulent strains to infinity for apathogenic strains (Waterson *et al.,* 1967).

On the basis of the MDT, Hanson and Brandly (1955) divided strains into velogenic (40-60 hours), mesogenic (60-90 hours), and lentogenic (more than 90 hours) groups. The lower the MDT value the more virulent the strain, for example vaccine strains such as F and B1 have MDT values of 108, while the California CG 179 strain used by Bang (1951) as a typical virulent strain had MDT value of 40 hours.

2.7. Antigenic relationships

Antigenic differentiation in APMV S has been based on the haemagglutination-inhibition (HI) tests and subsequently confirmed by Neuraminidase-inhibition (NI) tests (Alexander, 1974a; Alexander and Chettle, 1979c; Alexander *et al.*, 1979b; Alexander *et al.*, 1979c; Collings *et al.*, 1975; Kessler *et al.*, 1979, Lipkind *et al.*, 1979; Tumova *et al.*, 1979a; Lipkind and Shihmanter, 1985). Analysis of antigenic relationships has revealed that pairs of viruses may be very close when compared by either the HI and NI tests or both but may respectively be strikingly different from each other when compared with the other members of APMV-1 group. Viruses that seem to be identical by mutual cross reactions but different in the spectrum of quantitative pattern of their cross reactions with other APMV-1 serotypes, are currently taken to be antigenically different (Rishe & Lipkind, 1987).

In order to explain these variations in the antigenic relationships, Rishe and Lipkind (1986) proposed that the HA and N-ase antigenic sites are topographically distinct entities on the HN molecule and undergo independent antigenic drift. They also proposed a model of the genomic material coding for the HN glycoprotein to consist of a conserved *"common- to- all"* the APMV S portion and an equally conserved *"serotype-specific"* portion at the end of which is a *"variable"* portion. The variable portion is small for APMV-1 but rather

large in the other APMV'S. Biancifiori (1993) observed a considerable antigenic diversity between pigeon isolates and LaSota strain.

2.8. Neuraminidase activity

APMV's bind to their host cells by attaching to neuraminic acid residues terminally located on glycoproteins. Receptors present on infected cells may interfere with the release of progeny viruses and these viruses have therefore, developed strategies to remove receptors after infection. This is mediated by down regulation of protein receptors or by receptor destroying enzymes that cleave the receptor determinant from the host receptors. Paramyxoviruses using N-acetylneuraminic acid (Neu 5Ac) as a receptor determinant have the receptor-destroying enzyme as neuraminidase (Klenk *et al.*, 1997).

2.9. Assay for antibodies in field sera

Presence of HI antibodies may be an indicator of the presence of infection by strains of NDV in unvaccinated populations (Spradbrow, 1993/94). Variability in seroprevalence is probably due to different stages of infection in the populations at the time of sampling and, to a lesser extent, to the variability in the results of the HI test (Awan *et al.*, 1994). Alexander (1991) stated that poultry sera do not

cause non-specific inhibition of NDV haemagglutinin above dilutions of 1:8 and titres greater than 1:8 are usually regarded as indicative of exposure. Prevalence of low HI antibodies is suggestive of an interepidemic phase or the early phase of infection, while a high prevalence is indicative of the post epidemic period (Awan *et al.*, 1994). Infection within the village poultry population by velogenic Newcastle disease virus (VNCDV) can be maintained in a flock of poultry through a cycle of waning immunity followed by immunity boosting symptomless infection which is then passed on to other birds with sufficiently lowered immunity (Hanson 1976).

2.10. Occurrence of avian paramyxovirus serotype-1

Newcastle disease was first reported from the city of Jakarta in Indonesia in 1926 and subsequently in the following year it was reported in other parts of Asia and also in England (Spradbrow, 1987). Serological surveys in conjunction with isolation studies have shown that velogenic NDV strains are endemic in village poultry populations even in isolated villages and possibly in isolated flocks (Awan *et al.,* 1994). Although NDV is endemic in village poultry, the clinical disease usually follows an epidemic pattern. ND outbreaks often occur once or twice a year at regular intervals affirming the endemicity of the virus, however, 'mini'outbreaks in individual flocks and sporadic cases in individual birds may

occur (Awan *et al.*, 1994). Factors affecting the occurrence of Newcastle disease in village poultry include the weather, exposure to virus, virulence of virus, age of the chicken, immune status and concurrent disease. Mortality rates in village chicken during outbreaks of velogenic Newcastle disease are often comparable to those in none-immune commercial poultry (Martin, 1991). Infection within the village poultry population by velogenic Newcastle disease virus (VNCDV) can be maintained in a flock of poultry through a cycle of waning immunity followed by immunity boosting symptomless infection which is then passed on to other birds with sufficiently lowered immunity (Hanson 1976). Newcastle disease is enzootic in many countries with substantial village poultry population (Spradbrow, 1991). The disease occurs year round often with major epizootics once or twice a year that inflict heavy losses on village flocks (Spradbrow, 1991). Velogenic, mesogenic and lentogenic pathotypes of NDV occur in village poultry and also some have been isolated from numerous species of wild birds elsewhere and these may be actually reservoirs for the domestic birds (Lancaster, 1966; Hanson, 1976; Alexander, 1988).

Newcastle disease virus survives periods of weeks to months in the environment in tropical and subtropical climates although this varies with viral strain. Epizootics occur at times of climatic stress, notably rainy seasons (Nyaga, 1982). The susceptibility of an individual bird depends on the strain, concurrent disease

and parasitism. While the interaction of different strains of virus within the village chicken population are not understood, they do affect the immune status of individual birds and of the flock (Martin, 1992). In recent years, the roaming and scavenging village chickens have received increasing recognition as an issue deserving internationally funded attention (Heath, Lindsey, Macmanus, 1991). Computer modelling using field data indicates that vaccination programmes that reduce mortality due to NDV will result in significant economic benefit to people in the developing world (Heath, Lindsey, Mcmanus, 1991).

2.11. Control of Newcastle disease by vaccination

Traditional vaccines (for example, F Strain, Hitchner B1, LaSota) rely on a continuous cold chain for viability until applied to the target bird. Vaccination is mainly carried out on commercial farms and in some foci of outbreaks among rural chickens near urban centers. The owners of village chickens are not keen to have these birds vaccinated when there is no disease outbreak. However in the period of an outbreak they sell off the birds or slaughter for consumption and later on restock. The constraints that hinder village chicken owners from vaccinating their birds are:

(i) Large dose packaged expensive vaccines (most vaccines produced are mainly formulated for large-scale use).

(ii) Cold chain requirements of currently available vaccines. In order to

circumvent these problems, a heat resistant vaccine V_4HR was developed specifically by Webster for use in hot climates so as to reduce the dependence on cold chains for transport and to improve virus stability (Heath and Macmanus, 1991). When reconstituted and mixed with feed it can be applied as an oral vaccine to village poultry. The efficacy and economic benefits of Webster's V_4HR vaccine when applied in this manner has been assessed in a number of countries particularly those in South East Asia, by the Australian Centre for International Agricultural Research (ACIAR). Extensive field trials in village chicken using Webster's NDV V_4HR vaccine have demonstrated satisfactory protection against velogenic NCD outbreaks in these environments (Spradbrow, 1991). In an economic study of the impact of vaccinating village chickens against Newcastle disease in the Philippines, Johnstone *et.al.* (1992) concluded that the ratio of benefits to cost of a vaccination project would be 13.8, with an internal rate of return of 65% (Chrysostome *et.al.* 1995).

2.12. Avian paramyxovirus disease in Kenya

In Kenya the poultry population is about 25 million, 70% of which comprises

village chickens and the rest are exotic breeds. With the ever-increasing prices of red meat, village chickens have become the main source of animal protein in the form of meat and eggs for the rural human population. The latter comprise about 80% of the country's total population (Musiime, 1991). Urban centers also seem to be consuming large quantities of village chickens brought in from rural markets. Available information shows that Newcastle disease is widely distributed throughout the country and occurs mostly during the cold and dry periods in the year peaking June-July and November-December (Nyaga 1982). ND was first encountered in Kenya on the Mombasa Island (Daubney 1936) and later spread throughout the country. Rural village chickens are concentrated in the Eastern, Nyanza, and Western provinces. Intensive poultry production is concentrated in the central province (Musiime, 1991). Records in the annual reports of the Veterinary Department show that large numbers of Newcastle disease outbreaks have been reported from Central and Rift Valley Provinces, and have occurred during the cold and dry periods in the year with peaks in June-July period (Musiime, 1991; Nyaga, 1982; Nyaga *et.al.* 1985). What is recorded is normally small, as the majority of the outbreaks that occur in the country especially among village chickens are not reported.

Due to continuous increase in the human population, more land is used to grow food for human consumption. In Kenya, this land was previously used for meat

production; hence supply of red meats has been drastically reduced. However, women and unemployed youths have found entrepreneurial avenues in trade with small-scale chicken enterprises and the sale of village chickens. The village chickens are therefore filling the red meat gap and the protein requirements rather rapidly and so becoming important in terms of food security. Studies on the constraints to their production are therefore opportune in order to increase their productivity. Newcastle disease has been cited as one of the major constraints in village poultry production. Studies on it's spread in the village poultry is important in order to map out strategies of control, thus the aim of this project was to study the epidemiology of Newcastle disease and other avian paramyxoviruses in live bird market village chickens and wild birds. Wild birds were considered in this project because they interact with the village poultry and if they harbor Newcastle disease virus, they could also be a source of infection to the rural poultry.

3.MATERIALS AND METHODS

3.1 Experimental Design.

The field samples for this project were collected from slaughterhouses within the city of Nairobi. The live bird market in Kenya, offers an outlet for the village chicken. Chicken vendors move from village to village buying birds from farmers and transport them to the city, where the chicken fetch a reasonable income. Due to time factor and financial constraints samples were collected from these markets within a period of 3 years. It was assumed that within this period, the samples collected would be a representative of the village chicken in the country. Wild bird samples were collected from Nairobi area and Nakuru district (Lake Bogoria) that harbors different species of wild birds.

3.2. Reagents

Reagents used i.e. normal saline; Phosphate Buffered saline; Alsever`s solution; Fetuin; Periodate reagent; Arsenite reagent; Thiobarbituric acid reagent; and Butanol reagent were prepared as in appendix 1

3.3. Chickens

Day old chicks were purchased from a local hatchery and raised in the isolation

pens at the Department of Veterinary Pathology and Microbiology, University of Nairobi. Before use these chicks were screened to ascertain they were free of Newcastle disease virus antibodies. Seven-week-old chickens were used for antisera production, and three chickens were used for each isolate. Three chickens were spared and used as a source of erythrocytes for use in haemagglutination and haemagglutination inhibition tests.

3.4. Embryonated eggs

Specific pathogen free embryonated eggs were obtained from Kenya Veterinary Vaccines Production Institute (KEVEVAPI) and incubated at 37^{O}C. These were candled on the tenth day after incubation. Any infertile eggs and those with dead embryos were discarded, while those with live embryos were used for sample inoculation.

3.5. Reference strains of avian paramyxovirus serotypes1-9

Reference avian paramyxovirus serotypes 1-9 (except for type-5) and corresponding antisera were kindly provided by Prof. M. Lipkind of the Kimron Veterinary Institute, Israel. These were used to type the local avian paramyxovirus isolates.

3.6. Field samples

Local virus isolates were recovered from field samples obtained by using cloacal or faecal swabs taken from slaughter chickens and wild birds. In addition, for wild birds, water samples were collected. Cotton swabs were used to collect cloacal samples from market chickens at slaughterhouses within Nairobi between April 1994 and May 1997. These samples were from indigenous chickens that were apparently healthy. The respective slaughterhouses were at Westlands, Kariokor, and Burma markets. The chickens came from various parts of Eastern, Western, and Rift Valley provinces. Samples were also collected from wild birds and from sick chickens that were brought to the poultry clinic. A total of 607 cloacal swabs and 317 serum samples were collected.

3.7. Collection and processing of samples

3.7.1. Cloacal swabs: -

Sterile cotton swabs were twirled in the cloaca of the chickens or wild birds and rubbed on the cloacal mucosa so as to collect enough faecal material. The swabs were then put into transport media, taken to the laboratory in a cool box and kept frozen at -20^{o}C until when processed further. Innocula were prepared by squeezing each swab to the sides of the Bijoux bottle and extracting its contents

into the transport media, followed by centrifugation at 2000xg. The supernatant was decanted and stored at -20°C until inoculated into eggs for virus isolation.

3.7.2. Serum samples

Blood was collected from the jugular vein of each chicken into separate universal bottles at the time of slaughter. The bottles were then kept slanted to allow the clot to form in a slanted manner. The samples were taken to the laboratory, incubated at 37°C for an hour and stored overnight at 4°C. The separated serum was centrifuged at 500xg for 15 minutes, decanted, aliquoted into screw capped vials and kept frozen at -20°C until when needed.

3.8. Virus isolation: Egg Inoculation and harvesting of allantoic fluid

All eggs to be used for virus isolation were sprayed with ethyl alcohol and allowed to dry prior to virus innoculation. A hole was punched above the airspace and a volume of 0.1ml per sample of the innoculum processed from the swab samples was inoculated each into four eleven-day-old embryonated eggs through the allantoic route using a 36xgauge needle inserted 4 millimeters. The hole was then sealed with paraffin wax and incubated at 37°C with the pointed end down for a maximum of 6 days. The eggs were candled each day, the dead

ones removed and chilled together with those surviving up to day 6.

Allantoic fluids were harvested individually from the chilled eggs and tested for haemagglutinating activity. Samples giving positive haemagglutination activity were retained while the negatives were passaged twice to confirm absence of virus. The positive samples were stored at -20^{0}C in aliquots.

3.9. Production of polyclonal antisera

Three seven-week-old layer chickens were each respectively inoculated intramuscularly into the thigh muscle with 0.5ml of heat inactivated (56^{0}C) selected virus isolates. The inoculated birds were kept in separate cages and boosted after every 2 weeks for a period of 4 months. Two weeks after the last booster, the birds were bled, serum prepared and kept at –20^{O}C until when used.

3.10. Haemagglutination test

3.10.1. Preparation of red blood cells

Five milliliters blood was collected from chicken in Alsevers solution and centrifuged at 500xg for 5 minutes. The supernatant and buffy coats were removed and the red blood cells washed three times in phosphate buffered saline

(PBS) pH 7.2. The red blood cells were then resuspended in PBS to a final concentration of 0.5% v\v, (Hsiung, 1973) stored at 4°C and used within 3-4days.

3.10.2. Haemagglutination test

The method of Hsiung (1973) was used. Briefly, two fold serial dilutions of the respective allantoic fluid harvests were made using PBS in microtitre plates (Nunc-Immuno™ plates, MaxiSorp surface made by Nalge Nunc International, Denmark). A volume of 0.025ml of 0.5% suspension of chicken red blood cells was added into each well and the plates were shaken gently. Two sets of plates were prepared and one was incubated at +4°C while the other was incubated at 37°C. A red blood cell control comprising of 0.025ml PBS and an equal volume of 0.5% suspension of chicken red blood cells was set up at each temperature. The test was read after 45 minutes when the red blood cells in the control well had formed a button. In a positive case, the red blood cells formed a mart covering the entire bottom of the well while in negative cases they formed a button at the centre of the well. The end point was taken as the highest dilution of virus showing complete haemaglutination while the titre was the reciprocal of the dilution.

3.11. Haemagglutination inhibition test

The method described by Hsiung (1973), was used. Briefly, two fold serial dilutions of the type specific antisera were made in PBS. Eight haemagglutination units were prepared from respective positive allantoic fluid harvests and a volume of 25 microlitres of the preparation containing 8HAU was added to each of the serum dilutions. The plates were shaken gently and the virus mixture allowed to remain for an hour at room temperature. Serum control was prepared using serum and PBS and virus control set as virus and PBS. A volume of 0.025ml of 5% suspension of chicken red blood cells was added to all the wells and the plates were incubated for 30 minutes. In positive wells, the red blood cells formed a button at the centre, while in negative wells the red blood cells formed a mart at the bottom. The end point of the test was taken as the highest dilution of serum showing complete inhibition.

3.11.1. Typing of the recovered isolates

All the virus isolates recovered were typed using the reference antisera against APMV1-9 and 8HA units of the respective virus isolates in the haemagglutination-inhibition test. The test was read as described earlier

3.12. Neuraminidase assay

The neuraminidase test was carried out using the method of Aminoff (1961) as

described by Palmer, D.F. *et al.,* (1975). Briefly, a set of 3 test tubes (12 x 75mm) were respectively labeled with dilutions and test virus. Fifty microlitres of each of virus dilutions were transferred from the 12 x 75ml master dilution tubes to the bottom of correspondingly labeled 16 x 100mm tubes in the assay series and the virus control series. Two fetuin control tubes were prepared and fetuin added to all the tubes except the virus control tubes. The tubes were covered and incubated at 37^0C for 18 hours. The tubes were removed from the incubator and placed in a 20^0C water bath and allowed to cool to the set temperature. A volume of 0.1ml periodate reagent was added to all the tubes and the reactants mixed thoroughly by shaking the rack. The rack was then placed in the 20^0C water bath and allowed to stand for exactly 20 minutes. The tubes were then removed and 1.0ml arsenite reagent added to all the tubes. The tubes were then shaken vigorously until the brown colour that had developed disappeared. A 2.5ml volume of freshly prepared thiobarbituric acid reagent was added and the reactants mixed thoroughly by shaking the rack vigorously. The tubes were immediately placed in a boiling water bath and the boiling maintained for 15 minutes. The tubes were then rapidly cooled to room temperature in an ice bath. A volume of 4.0ml butanol reagent was added to all tubes and mixed by shaking each tube thoroughly using gloved hands. Shaking the tubes extracted the colour, which passed into the upper butanol phase. The tubes were centrifuged at 500xg for 2 minutes. A sufficient volume of the butanol phase was taken from the fetuin

control tubes to nearly fill a cuvette.

The spectrophotometer was equilibrated at 549 wavelengths and the transmittance (Optical Density (OD) read off. The ODs of all the tubes were read. The results were plotted on a graph paper with the vertical axis labeled "virus dilution factor" and the horizontal axis "optical density". The graph for each isolate was drawn and the line of best fit obtained. From the graph the virus dilution that would give an OD. of 0.85 was obtained. This is the dilution that was used for the neuraminidase inhibition test.

3.13. Haemagglutination-elution assay

The haemagglutination-elution assay was carried out using the method described by Spalatin *et.al.* (1970) with a slight modification. Briefly, the haemagglutination test was set up in three pairs in microtitre plates as described earlier. The plates were incubated at 25^{0}C, 4^{o}C and 37^{o}C and observed for haemagglutination and elution patterns. The test was read at 15mins, 30mins, 1hour, 2hours, 3hours, 4hours, 5hours, 6hours, 12hours, 24hours and then daily up to a maximum of 7 days. Known rapid (F-strain) and slow (LaSota) eluting NDV reference strains were included in the experiment. Complete elution was taken to be when the red blood cells formed a button in all the wells.

3.14. Haemagglutination activity with chicken and mammalian red blood cells

Red blood cells were collected in Alsevers solution from chicken, buffalo, dog, donkey, goat, horse, pig, sheep, waterbuck, and prepared as described earlier. The haemagglutination test was carried out as previously described using chicken red blood cells.

3.15. Haemagglutinin thermostability

A modified method of that described by Nyaga, (1982) was used.
Briefly 1ml of each isolate was put in each of the three Bijoux bottles, and placed at 56^0C. Volumes of 25 microlitres were collected at 10, 30, 60, 120, 180, 240, 300, 360, 480, and 540 and 600 minutes and haemagglutination test carried out.

3.16. Pathogenicity test.

The mean death time (MDT) test was used to assess the virulence of the isolates using a modified method of Waterson *et.al.* (1967). Tenfold serial dilutions of the virus isolates were made separately starting with a dilution of 1:10 and a volume of 0.1ml inoculated into the allantoic cavity of each of the five 9-day old embryonated eggs. The eggs were candled daily and the time each embryo died recorded. The embryos that were not dead by day 7 were chilled. The allantoic

fluid from each egg was harvested and checked for haemagglutinating activity. The MDT was determined as the mean time in hours for the virus to kill the embryos. Hanson and Brandly (1955) grouped NDV isolates into three groups on the basis of MDTs: velogenic (40-60 hours); mesogenic (60-90 hours) and lentogenic (>90 hours).

3.17 Antigenic relationship between the isolates

3.17.1 Cross-haemagglutination inhibition test

The haemagglutination-inhibition test was done as earlier described and all the isolates were run against the homologous and heterologous antisera prepared against the selected virus isolates. The results were read and subjected to analysis by the method of Archetti and Horsfall (1950) and that of Lipkind and Shihmanter (1995). Using the Archetti and Horsfall (1950) method, the calculation of the r-values was done, where r is the squareroot of r_1 x r_2; and the r_1 and r_2 are defined as follows:

r_1 is obtained by dividing the heterologous titre obtained with virus 2 by the homologous titre obtained with virus 1.

r_2 is obtained by dividing the heterologous titre obtained with virus 1 by the homologous titre obtained with virus 2.

The r-values give the extent of antigenic relatedness between the two viruses. Values of r were expressed as reciprocals; r-values of 2 or more were considered significant.

The results were then analysed using the method of Lipkind and Shihmanter (1995) whereby cross reactivity was expressed as the difference d in $\log_2$ units between homologous and heterologous titres. The interrelationships were subdivided into 4 groups: -

a) No antigenic difference i.e. identical, (d values close to zero).

b) Closely related (d values between 1 and 2 $\log_2$).

c) Moderately related (d values between 2 and 4 $\log_2$).

d) Slightly related (d values between 4 and 7 $\log_2$)

Results were analyzed using the t-test. The probability for the difference d in a given number of experiments n was calculated. Differences were regarded as significant when p0.05: When there was no cross reactivity d was expressed as infinity. The same principles of statistical treatment were used in the cases of asymmetric cross reactivity. Thus cross reactivity between viruses X and Y was considered to be asymmetric if the difference d_1 (when anti-X serum against both the viruses was used) was statistically different from d_2 (when anti-Y serum against both the viruses was used.

3.17.2 Cross-neuraminidase Inhibition test (NI)

The volume of optimal antigen dilution required was prepared each time the test was carried out. A 0.5 logarithm$_{10}$ dilution steps of the test sera was prepared by adding 0.9ml 0.85% NaCl solution to tube one and 0.44ml 0.85% NaCl solution to other tubes. A volume of 0.1ml of the first serum was added to tube number 1, the contents mixed and with a clean 1ml pipette, 0.2ml transferred to tube number two. This was done for each sera. With a clean 0.05ml microtitration dropper, 0.05ml of each serum dilution was transferred from the master serum dilutions to the bottom of the corresponding tubes in each antigen series. With a clean 0.05ml dropper, 0.05ml of each optimal antigen dilution was added to the serum dilutions of its series. A volume of 0.1ml of 0.85% NaCl solution was added to each of the two-fetuin control tubes. The racks were gently shaken to mix the contents of the tubes, and the tubes were covered and incubated for 1 hour at 37°C. Phosphate buffer-fetuin (0.1ml) was added to each tube in the test and mixed thoroughly by shaking the racks. From this step onwards, the procedure was done as that described for neuraminidase test upto reading of the optical density. The neuraminidase activity was calculated as a percentage of that detected with the same dilution of the normal serum using the formular:

$$\frac{\text{OD. Of immune sera/ virus mixture}}{\text{OD. Of 1:10 normal serum/ virus mixture}} \times 100\%$$

= % neuraminidase activity.

Using a graph paper, the vertical axis was labeled " serum dilution factor" and the horizontal, " neuraminidase activity". The points were plotted and the line of best fit drawn. From the graph, the serum dilution that neutralized the virus but allowed it to retain 50% neuraminidase activity was obtained. This was done for all the isolates. The results were read and subjected to analysis by the method of Archetti and Horsfall (1950) as described earlier.

3.18. Assay of antibodies in field sera

All the serum samples were inactivated at 56^0C for 45 minutes. Serial 2 fold dilutions of the sera were made using PBS in 0.025ml amounts in microtitre plates. A volume of 0.025ml virus suspension (APMV-1, APMV-2, APMV-3, APMV-4, APMV-6 APMV-7, APMV-8 and PMV-9) containing 8HAU was added to each serum dilution well and mixed by gently shaking the plate. The plates were allowed to stand at room temperature for 1 hour and 0.025ml of 0.5% chicken red blood cells were added to each well. After 30 minutes the test was read and the end point was taken as the highest serum dilution showing complete inhibition.

3.19. Absorption of field sera with NDV

The field serum that inhibited the APMVs with titres from 1:16 and above

were selected and absorbedwith APMV-1. This mixture was incubated for one hour at $37^{O}C$ and centrifuged to remove the antigen-antibody complexes. 0.025microlitres were taken and HI carried out as described before with the serum and virus controls set up for comparison purposes. The serum that was still causing inhibition after absorption was taken to be specific for the antigen it inhibited.

4.RESULTS

4.1. Virus isolation

From the 607 samples tested from the live bird market village chickens and wild birds, 162 virus isolates were recovered. This gives a prevalence of 26.7% of APMV-1 in village chickens and wild birds. After haemagglutination-inhibition test of the isolates with specific antisera against APMV1-9, one isolate Bnf6 turned out to be an APMV-4 serotype.This was then further studied and the results are separately reported later in the work.

4.2. Haemagglutination-elution patterns

Using the classification used by Lipkind *et al.* (1995) viruses were placed into 4 groups as shown in table1; Fast eluters (13 isolates) which showed full elution within 4 hours; moderate eluters (10 isolates) showing incomplete elution at 4 hours but full elution at 24 hours; partial eluters (41 isolates) which showed only incomplete elution at 24 hours and non-eluters (23 isolates) which showed no elution after 24 hours.

Table 2 shows the haemagglutination-elution patterns of the NDV isolates according to their source. Of the 1994 NDV isolates from Kariokor, 15 were

partial eluters, 7 were non-eluters, 2 were fast eluters and 2 were moderate eluters. Of the isolates of 1997, 8 were fast eluters and 8 were partial eluters. For the NDV isolates collected from Burma, 3 were non-eluters while 2 were fast eluters and in Westlands, 3 NDV isolates were partial eluters while 4 were non-eluters. For the wild bird isolates from Nairobi, 2 were moderate eluters, 5 were partial and 4 were non-eluters. Lake Bogoria isolates were from flamingo faeces and showed one as a moderate eluter, 3 were partial eluters while 1 was a non-eluter. The other isolates were from Kiambu 1 isolate was a moderate eluter while 2 were partial eluters. All the samples from show birds were partial eluters. Elution commenced from wells of high virus dilution and later from wells of low virus dilution. The wells in the middle eluted last. The presence of rapid or slow eluters was not affected by date of virus isolation or geographic origin of the isolates.

4.3. Haemagglutination activity of various isolates with mammalian and chicken red blood cells

Blood was collected from the chicken and from seven different mammalian species and the haemagglutinating activity was determined.

Table 3 shows the haemagglutination activity on individual basis. It was observed that, donkey; waterbuck and buffalo red blood cells were not as

sensitive as other mammalian red blood cells to virus haemagglutinin. Dog red blood cells were agglutinated by 53 of the 79 isolates. Very slight differences were observed in the agglutinability of cells from the same species (Table 3). Fourteen of the isolates could only agglutinate chicken red blood cells. 28 isolates agglutinated chicken, canine and pig red blood cells. Only one isolate KRC136 agglutinated red blood cells from all the mammals screened. Nine isolates agglutinated red blood cells from 2 mammals while only 4 isolates agglutinated red blood cells from 8 mammals.

It was observed that 58 isolates gave titres above 1:64 isolates with equine red blood cells, 1 with caprine, 8 with canine, and 2 with swine red blood cells.

Very slight differences in virus agglutination were observed between caprine and ovine red blood cells.

Horse and canine red blood cells showed the best haemagglutination patterns with canine red blood cells showing the best pattern of agglutination (Table 4).

Equine, caprine, canine and swine red blood cells gave haemagglutination titres from 1:128 and above with a few of the isolates while fowl red blood cells gave the highest titres with most of the isolates.

4.4. Haemagglutinin thermostability

Heat inactivaton of viral haemagglutinin was done at 56^0C. The heat inactivation

experiments showed marked variation in the stability of the virus haemagglutinins (Table 5).

Isolates were considered to have heat labile haemagglutinins if the haemagglutination titre decreased by 2.0 logs (base 2) or more when heated for 15 minutes. It was observed that (Table 5) 12 isolates possessed heat labile haemagglutinins and 76 isolates possessed heat stable haemagglutinins. From table 5, it was observed that within 10 minutes, isolate B39 had lost haemagglutination activity; 4 isolates, NDV-L, KRC139, KRC290 and KRI55 lost their haemagglutination activity at 1 hour of heating; Bf4, K1, K14, KRC136, KRC238, KTC1, WC21, KRI39 and WC24 lost their haemagglutination activity after 9 hours; 3 isolates namely, TC2, TC3 and WC2 lost their haemagglutination activity after 10 hours. Most isolates lost their haemagglutinin activity between 60 minutes and 180 minutes of heating. Isolate KRC108 retained its titre upto 180 minutes of heating. Almost two thirds of the isolates had lost their haemagglutinin activity after 240 minutes. Isolates B16 and WC2 retained their initial titres even after 240 minutes of heating, while that of KRC150 remained unchanged even after 360 minutes of heating. From table 6 it was observed that the initial titres of the isolates played an important role in the rate of inactivation. Table 7 shows the distribution of the isolates by the place of origin. Most of the NDV isolates from Kariokor were inactivated by 300 minutes and 8 were inactivated by 540 minutes. Four of the Burma NDV isolates were

inactivated by 300 minutes while 2 were inactivated by 480 minutes. The NDV isolates from Westlands, 4 were inactivated by 300 minutes while 3 were inactivated by 600 minutes. Four of the isolates from Lake Bogoria were inactivated by 300 minutes while 1 was inactivated by 540 minutes. Five of the NDV isolates from Nairobi were inactivated by 300 minutes while 6 were inactivated by 540 minutes. Of the isolates from show birds, 2 were inactivated by 300 minutes while 3 were inactivated by 600 minutes. For the Kiambu isolates, 3 were inactivated by 300 minutes while 1 was inactivated by 540 minutes. For the Kawangware isolates, 2 were inactivated by 300 minutes while 1 was inactivated by 540 minutes. Overall of the NDV isolates were inactivated by 300 minutes.

4.5. Virulence of the Newcastle disease virus (NDV) isolates

The MDT was used as a measure of virulence for the isolates. Hanson and Brandly (1955) placed isolates into velogenic (MDT: 40-60 hours), mesogenic (MDT: 60-90hours), and lentogenic (MDT: >90 hours) strains. Accordingly the selected isolates were typed as follows: 10 were velogenic, 7 were mesogenic and 4 were lentogenic (Table 8).

4.6. Serological relationships between isolates as displayed by HI tests.

Cross HI tests were carried out using 19 randomly selected isolates and 2 standard vaccine virus strains. The results show that (Table 9) there was cross reactivity among the isolates studied. Antisera against LaSota and F-strain vaccine strains could not neutralize B26, K2 and K11 virus isolates. Table 9 shows the results of the pooled data. This shows cross-reactions between the isolates, especially the asymmetric cross-reactions.

Table 10 shows the calculations of the relationship between the isolates when different antisera were used (Lipkinds method).

The interrelationships were subdivided into 4 groups: -

e) No antigenic difference i.e. identical, (d values close to zero).

f) Closely related (d values between 1 and 2 $\log_2$).

g) Moderately related (d values between 2 and 4 $\log_2$).

h) Slightly related (d values between 4 and 7 $\log_2$)

37 pairs if isolates showed close symmetric relationship, 38 pairs showed close asymmetrical relationship, 21 pairs showed moderate symmetrical relationship, 21 pairs showed moderate asymmetrical relationship, 3 pairs showed slight asymmetrical relationship and 8 pairs showed no relationship at all. The remaining pairs of isolates showed asymmetrical relationships whereby they could not be grouped. There was marked antigenic difference when B16 antisera was used against KRC108; KRC123 against K13; KRC244 against B16; TC1

against K11; B16 against KRC150; K2 against KRC144; K11 against WC28; K13 against WC28; KRC108 against WC28; KRC123 against WC28; KRC139 against WC28; KRC244 against WC28 and TC1 against F-strain. Antisera against LaSota and F-strain could not neutralize isolates B26, K2 and K11.

Table 11 shows the antigenic relationship between the isolates calculated using the Archetti and Horsfall method According to Archetti and Horsfall (1950) method the r-values were expressed as reciprocals. Values of 2 or more of r were considered significant. All the isolates showed antigenic differences that ranged from 4 to 256 except for 5 pairs i.e. K3 & WC28, K3 & NDV-L, KRC244 & NDV-L, NDV-L & K13, NDV-L and KRC242 that showed no antigenic differences

Table 12 shows the relationships between the isolates after the method of Lipkind in comparison with the Archetti and Horsfall method r-values. According to the Archetti and Horsfall method, only 5 pairs of isolates i.e. K3 & WC28, K3 & NDV-L, KRC244 & NDV-L, NDV-L & K13 and NDV-L & KRC242 were closely related and this agrees with the Lipkinds calculations of the d_1 and d_2 values which showed close symmetrical relationship. For all the isolates that showed close asymmetric, moderate asymmetrical and marked asymmetrical relationships according to the d_1 and d_2 values, the r-values were

zero indicating no relationship between them. The 8 pairs of isolates that showed no relationship according to the d values had zero r-values. The Lipkind method only gives values of zero when antisera were used against their own. The Archetti and Horsfall method gives zero values when the antisera of two pairs of isolates does not neutralise in either one or both directions.

Table 13a shows the cross reaction between Bnf6 and APMV-4 antisera with the other isolates. There was no cross reaction between Bnf6 and APMV-4 with B16, K11, KRC139, KRC244, KWC30, and TCI. Bnf6 haemagglutinated red blood cells from all mammalian species used except for waterbuck and buffalo. The mean death time for Bnf6 was 168 hours while there was no elution at 24 hours. The isolate was inactivated after 180 minutes at 56^{o}C.

Table 13b shows the calculations of the relationship between the NDV isolates and Bnf6. There was asymmetric one sided cross reactivity with Bnf6, B16, K11, KRC139, KRC242, KRC244, KWC30 and TC1 and two-sided asymmetric cross reactivity with K3, K12, K13, KRC108, KRC150 and Lasota. The results of the Archetti and Horsfall method show that Bnf6 is not related to all of the isolates except LaSota with which they are markedly different.

4.7. Antigenic relationship between the isolates as given by NI tests

NI tests were carried out with 10 isolates and 1 vaccine strain using their

corresponding antisera. Results of a typical experiment are presented in Table 14. There is no cross-reaction between LaSota and Bnf6; LaSota and B16; Bnf6 and K3; K12 and B16; KRC123 and K13.There was one sided cross reactivity between K2 and B16; K11 and Bnf6; and between KRC108 with B16, K11 and K12.

When the method of Archetti and Horsfall (1950) was used i.e. table 15 there were significant differences between all the isolates. The r-values calculated from the NI titres showed that except for K13 and K3, K12 and K2 that had r-values of 1.27 and 1.117 respectively which were not significantly different from one another, (agrees with the Lipkinds calculation of the d values) all the other isolates were significantly different from one another. From table 10, the d values of these isolates show moderate symmetrical relationship.

4.8. Prevalence of antibodies to avian paramyxoviruses in field sera

A total of 318 sera were screened against LaSota, NDV-L APMV-1 to APMV-9 except for APMV-5 that was not available. Table 16 shows that B39 gave high HI titres with APMV-4; B37, W17, KR139, W1 and B6 reacted with APMV-3; KR52 reacted with APMV-7; while B17 and B31 reacted with APMV-8. Absorption of these antisera by APMV-1 was done and haemagglutination

inhibition test repeated. The results showed specific reactions of B37, W1, W17, KR139 and B6 with APMV-3, (Table 17). From these observations it can be deduced that APMV-3, is in the field but it has just not been isolated.

Table 18 shows the seroprevalence of NDV in market rural chickens sampled in Nairobi by areas sampled namely Westlands having 12.5%, Kariokor 31.9% and Burma 25.5%. The overall seroprevalence was 27.04%.

APMV-3 was specific against B37, W1, W17, KR139 and B6 but all the other inhibitions against the other APMV isolates were cross-reactions.

5.DISCUSSION

The 607 cloacal swabs were collected from apparently healthy market chickens brought to the city of Nairobi for slaughter. 162 NDV isolates were recovered from the 607 samples. One APMV-4 serotype was isolated from flamingoes; other NDV isolates were recovered from wild birds. Recovery of virulent NDV from healthy chickens concurs with work done by Khalafalla (1994) in Sudan, where he isolated NDV from chicken that showed no overt clinical signs of disease at the time of sampling. Long-term excretion of NDV from unvaccinated birds surviving challenge has been reported by Alexander (1999) and clinically normal vaccinated birds have also been reported to excrete virulent virus (Alexander 1995). Samuel and Spradbrow (1989), working with V_4 strain of Newcastle disease virus observed that the virus persisted within the flock and it spread to hatched chicks within the flock 14 months after first introduction of the virus in feed. Adu *et.al.* (1984) isolated Newcastle disease virus from outbreaks of disease in vaccinated commercial flocks and from unvaccinated rural flocks in Nigeria, thus vaccinated birds seem to excrete NDV in times of outbreaks. Subclinically infected birds and survivors of natural infections harbour the agent and may act as NDV reservoirs (Awan *et al.*, 1994). Virulent NDV may infect, replicate, and be present in the tissues and organs and be excreted from apparently healthy birds (Alexander, 1992). During the NDV outbreaks in

California in 1971 to 1973 it was recorded that vaccination did not prevent birds becoming infected with the epizootic virus and that such birds shed the virus for long periods (Utterback & Schwartz, 1973). Guittet *et al.* (1993) challenged 40-day old birds that had been vaccinated at 14 days old with velogenic NDV and showed that from 2 to 3 days up to 6 days after challenge, virus could be isolated from organs, muscles and faeces of sampled birds, although none showed any signs of disease. Excretion of NDV after challenge infection has also been reported by Parede & Young (1990), Capua *et.al.,* (1993) and Lancaster (1975). The constant introduction of susceptible birds through hatching, and the probability that some individual birds or flocks will have evaded infection during the passage of disease through the village, mean it is possible that within a village poultry population there will always be susceptible birds to whom diseased birds can transmit NDV (Martin, 1992). Thus not all birds become infected at the same time in the village and enabling the maintenance of infection in the rural population for a long time, probably in a cyclic fashion (Awan *et al.*, 1994).

Given that most of village chicken are free range, they interact with wild birds which have been shown to be reservoirs of APMV-S from which they could get infection (Adu *et al.*, 1985; Olabode *et al.*, 1992, Bawke *et al.*, 1991, Fadol, 1991, George, 1991, Ratanasethakul, 1989, Nepal Mishra, 1992, Asadullah,

1992, Lwin, 1992 and Nguyen, 1992, Ostrowski *et al.*, 1995, Panigrahy *et.al.* 1991, Alexander 1979, Waterson *et.al.* 1967). Village chickens also live longer and it is presumed that they could be harbouring viruses of low virulence (Khalafalla, 1994). All NDV pathotypes have been isolated from numerous species of wild birds (Awan *et al.*, 1994). Kaleta & Beldauf (1988) concluded that, in addition to domestic avian species at least 236 species of birds have been reported to be susceptible to natural or artificial NDV infection. Jørgensen et al., (1998) isolated APMV-1 from dead birds in a population of 77 ostriches and four emus held in quarantine. Samples from 17 of the dead ostriches examined virologically yielded three virulent APMV-1 isolates that were indistinguishable and similar to APMV-1 viruses present in a simultaneous epizootic of Newcastle disease in backyard poultry in Denmark. Isolated outbreaks in chickens in Switzerland in 1995 also showed the same binding pattern. It seemed the Ostriches and the emus were the source of infection. This indicates that in most rural setups where the chicken feed outside and the wild birds also sneak in to feed on the same food scraps they could easily infect the chicken either through the faeces that mix with the food or most likely through aerosol route.

It has been demonstrated that pathogenicity of APMV-1 is dependent on the ability of the host proteases to cleave the precursor fusion protein Fo to F1 and F2 (Rott and Klenk, 1988), so depending on the type of enzymes a host produces,

they could succumb to infection when they are infected with certain type of NDV strain. Thus the strains occurring in wild birds could be pathogenic to the rural and commercial chickens if the bird's proteases are able to cleave the precursor Fo.

Although vaccination against NDV was started between 1958-1959 (Nyaga, 1982) it seems the disease has been difficult to eradicate in Kenya because the wild birds are also reservoirs. Evidence from the current studies shows that indigenous chicken may play a significant role in keeping NDV endemic in Kenya, because they are reservoirs of virulent virus strains. Excretion of NDV from birds after challenge has also been reported. (Samuel and Spradbrow, 1989, Alexander, 1999, Alexander, 1995, Adu *et.al.*, 1984). There could be a cycling of infection from wild birds to indigenous chicken and then to the exotic-commercial birds. Moreover indigenous chicken are not usually vaccinated, thus when there is an outbreak only those which are strong and those with immunity survive. Those that recover from the infection could be the ones that act as reservoirs.

The assessment of the time taken by the isolates to elute from chicken red blood cells was carried out at $4^{o}C$. There is no relationship between the initial titre of the isolate and the elution time. Nyaga (1982) observed that isolates with a titre

of 1:2048 could elute in as short as 18 hours and as long as 144 hours, while isolates with a titre of 1:64 could take as long as 144 hours and as short as 3 hours to elute completely.

From table1 it was observed that most of the isolates were partial eluters. The rate of elution appeared unrelated to any aspect of virus history or year of isolation. This independence makes the property a valuable character for differentiating strains. Although it is known that elution is an indication of Neuraminidase activity, (Seto *et.al.*1959) there are other factors that may modulate the phenomenon, especially the type and access to the sialic acid residues. Inaccessibility of these RBC receptors may be due to the absence of congruence between the HA and N-ase subunits on a viral particle on one hand, and by the configuration of RBC surface on the other hand (Choppin and Tamm as cited by Lipkind and Tsvetkova, 1968). Data has shown that two strains of Influenza A2 virus sharply differing by their elution rate have a similar rate of N-ase activity soluble substrate, thus confirming the inferences of Choppin and Tamm (1960) thus indicating that N-ase activity and rate of elution from RBC are not necessarily correlated (Lipkind, Tsvetkova, 1968).

Receptor specificity of the isolates regarding red blood cells from 7 different species showed that out of the 87 isolates studied, 14 isolates did not agglutinate any of the mammalian RBCs, 1 isolate haemagglutinated RBCs from all the

species. From Table 3, fifty-five isolates haemagglutinated dog RBCs. It can be seen that dog RBCs were agglutinated by most of the isolates tested while equine RBCs were agglutinated by least of the isolates. Caprine and ovine RBCs were identical. Winslow *et.al.* (1950) studied 225 strains of which 19 haemagglutinated cow RBCs, 10 haemagglutinated sheep RBCs, 13 haemagglutinated horse and all of them haemagglutinated dog RBCs. This agrees with the findings in this study that dog red blood cells are agglutinated by most NDV isolates. Winslow *et al.*, (1950) also observed that RBCs from the horse, cow and goat differed in their haemagglutination ability when tested by a single strain of virus. Of the 17 strains that haemagglutinated RBCs from the horse, 9 haemagglutinated only those of certain individuals. RBCs from some cows could be haemagglutinated by a virus while others gave negative results (Brandly *et al.*, 1946b). It can be concluded from this study that chicken red blood cells remain the first choice for use in haemagglutination test to detect the haemagglutination activity of avian paramyxoviruses although Abu Elzein *et al.*, (1993) showed that camel red blood cells gave HA titres similar to those obtained with chicken red blood cells and where chicken red blood cells are unavailable, camel red blood cells could be used.

At 56^0C some isolates lost the haemagglutination activity in less than 10 minutes, while others still retained their haemagglutination activity after 10 hours. These

results are similar to those of Hanson *et al.*, (1949); Kaschula, (1952); and Lomniczi, (1975). The rate of inactivation of the haemagglutination activity has been shown to be independent of the initial HA titre (Nyaga, 1982), thus any small number of particles of NDV could lead to an outbreak of the disease. It was also observed that the place of origin of the NDV isolates did not play a role in the rate of inactivation, as shown in table7.

Virulent NDV are endemic in many tropical and subtropical countries as cited by Awan *et al.*, (1994), (Spradbrow, 1990). Velogenic strains of NDV are reported to be prevalent in rural poultry from Nigeria (Adu *et al.*, 1985; Olabode *et al.*, 1992), Ethiopia (Bawke *et al.*, 1991), Sudan (Fadol, 1991), Uganda (George, 1991), Thailand (Ratanasethakul, 1989), Nepal (Mishra, 1992), Bangladesh (Asadullah, 1992), Myanmar (Lwin, 1992) and Vietnam (Nguyen, 1992). The most common strain of NDV reported is viscerotropic velogenic (Awan *et al.*, 1994). NDV comprises a wide range of naturally occurring strains that differ in pathogenicity for their hosts (Waterson *et.al.*1967). Reports have shown that precursors exist to both glycoproteins of NDV that are converted by proteolysis into biologically active form. The susceptibility of these glycoproteins to proteolytic cleavage that is carried out by host specific enzymes differs from one NDV strain to another. Thus infection with a pathogenic strain which produces virions with cleaved glycoproteins in a wide variety of cultured cells in vitro may

spread more rapidly in the organism than infection with an apathogenic strain which produces virions with uncleaved glycoproteins in most cells (Nagai *et al.* 1976))

Pathogenicity was tested using the method of Hanson and Brandly (1955). According to the classification of Hanson and Brandly, (1955), 10 isolates were velogenic, 7 were mesogenic and 4 were lentogenic (Table 8). These isolates were from apparently healthy chicken. For the isolates to be velogenic without causing disease could be explained by the fact that the pathogenicity of Newcastle disease virus in chicken is dependent on the ability of host proteases to cleave the precursor fusion protein FO to F1 and F2; and if this does not occur then the virus will not be pathogenic (Rott & Klenk 1988), or the birds at one time had been exposed to NDV thus were able to shed the NDV without any apparent clinical signs. In a prevalence study in Mauritania, tracheal swabs from 80 rural chickens were investigated and out of these, six isolates of NDV were recovered which were either mesogenic or velogenic (Awan *et al.*, 1994). In Morocco (Bell & Moulodi, 1988) tracheal swabs were taken from 100 village chickens and forty-one isolates of NDV were made, several from each region. Characterisation of two isolates from each region revealed all to be velogenic. All strains of Newcastle disease virus occur in rural poultry, but velogenic strains are reported to be more common (Awan *et al.*, 1994)

Most of the partial eluters were velogenic, and the non-eluters were lentogenic. Virulence of the Avian paramyxoviruses could be related to elution rate with the eluters being velogenic and the non-eluters being lentogenic.

Some isolates used in the study showed antigenic differences when the extent of their relationships were calculated using Lipkind and Archetti methods despite the fact that all of them were NDV strains. This concurs with the findings of Upton, Hanson and Brandly (1953); Alexander, (1989) who found serological differences among NDV strains. There was no cross-reaction among some of the isolates seen from table 9. In analysing the results of table 10 using the method of Lipkind (1985) and that of Archetti and Horsfall (1950), there were distinctive differences between the isolates using both methods. Vaccine strains reacted asymmetrically with some of the isolates, for example, one-sided asymmetric cross reactivity of anti-LaSota and anti-F-strain against B26, K2 and K11 isolates. This may indicate that if isolates B26, K2, and K11 could be the causative agents of an outbreak at any one time, then the birds would not be protected and they could succumb to infection. This may explain vaccine breakdown observed in vaccinated flocks that have been reported to come down with disease despite being vaccinated. Biancifiori *et al.*, (1983) observed considerable antigenic differences between pigeon isolates and LaSota strain

while in the USA, there have been reports of failure of well-tried vaccines to protect against local field viruses (David-West, 1972). The Newcastle disease virus has been reported to have remained antigenically stable, the antigenic variability shown in serological reactions and that shown by agglutination of mammalian red cells leads to the conclusion that there is potential change for NDVs. Alexander, (1989) detected small differences between some strains by kinetic neutralization test while some recent isolates from wild European ducks and racing pigeons showed antigenic differences, compared to reference strains (Lipkind and Shihmanter, 1986).

It is evident that there is no complete agreement between the results of the NI test (Table 14) and of the HI test (Table 9). Some cross-reactions evident in the HI test are absent in the NI test. For example, in the case of NI there is no cross-reaction between LaSota and Bnf6; LaSota and B16; Bnf6 and K3; K12 and B16; KRC123 and K13. The enzyme activity of an isolate is inhibited by reference antisera containing antibodies against its neuraminidase subtype. All the isolates gave high serum dilutions at 50% neuraminidase activity. A278 and K12 could be belonging to the same neuraminidase subtype.

A positive serological result is clear that the bird has been exposed to NDV at one time or another. Thirty-six sera collected from the village birds had titres

above 1:8 indicating prior exposure to NDV as conluded in the studies by Alexander (1991). The presence of sero-positive birds indicates that NDV is widespread in the rural poultry. Since none of the chickens were vaccinated, all the positive NDV titres observed must have been due to natural infection and that APMV-2, APMV-3, and APMV-4 are present in chickens in Kenya. Avian paramyxovirus-3 has been reported to infect turkeys, but chickens have been demonstrated to be susceptible to infection although no natural infections have been reported (Alexander, 2000). Since village chickens are kept together with turkeys in households where both are reared, the chickens may have contracted APMV-3 either through ingestion or inhalation as they interact day in day out.

6.CONCLUSION

In this study evidence has been presented to show the presence of NDV in village poultry populations. Isolation of NDV from wild birds and healthy market village birds is evidence of the source of interepidemic NDV. Village chickens that survive NDV outbreaks or those kept for longer periods are a potential source of infection to the birds in the next generation.

NDV were also isolated from species of wild birds in addition to domestic avian species. These birds may form a reservoir for virulent virus.

Heterogeneity within strains of NDV was also observed in this study and may play an important role in the maintenance and development of infection in village poultry populations. Field isolates, as well as the vaccine strains of NDV differed significantly from each other in their biological and physical properties such as thermostability, rate of elution from red blood cells, ability to haemagglutinate red blood cells from different species and pathogenicity. Genetic heterogeneity allows the NDV strain to survive under a variety of conditions and to adapt rapidly to a changing environment. This could explain the differences in the NDV isolates used in this study.

According to the serological surveys in conjunction with isolation studies, velogenic NDV are endemic in village poultry populations. These flocks constitute a reservoir of virulent virus throughout the country independent of commercial flocks. Cases of Newcastle disease will continue to be observed on commercial poultry farms because of the presence of village rural flocks and wild birds that are not vaccinated against Newcastle disease. The study also indicates that rural chickens harbour virulent NDV strains and some of these are antigenically different from the vaccine strains in the market. This may explain vaccine failures among other factors. APMV-3 occurs in Kenya but virus isolation has no yet been achieved while APMV-4 has been isolated.

7.TABLES

Table1. Haemagglutination-elution of the NDV isolates at 4^0C

Fast eluters.	Moderate eluters.	Partial eluters.	Non-eluters.
KRC123, KRC244, F-strain, B39, KLC3, KRI35, KRI36, KRI41, KRI42, KRI46, KRI49, KRI55, KRI59.	A278, K3, K13, TC1, WC28, NDV-L, Bf16, KRC97, KRC236, KRI37.	DV213/95,Bf3, Bf5,Bf7, K6, K8,K9,K14,K16, KRC83,KRC102, KRC103,KRC114, KRC125,KRC131, KRC134,KRC136, KRC141,KRC143, KRC181,KRC184, KRC223,KRC263, KRC290,KRI2, KRI21,KRI22, KRI39,KRI43, KRI45,KRI47, KRI53,KRI72, KTC1,KWC19, TC3,WC15,WC16, WC20,63C,76D.	B16, B26, K2, K11, K12, KRC108, KRC139, KRC144, KRC150, KRC242, KWC30, LaSota, Bf4, K1, KLC17, KRC237, KRC238, TC2, WC2, WC8, WC21, WC24.
13	10	41	23

- Fast eluters:-1-4 hours
- Moderate eluters:-4-24 hour
- Partial eluters:-Elution incomplete at 24 hours
- Non-eluters:-No elution at 24 hours

Table 2. Distribution of haemagglutination-elution patterns of NDV isolates by place of origin.

Source	Year of isolation	Fast eluters	Moderate eluters	Partial eluters	Non-eluters
Kariokor	1994	2	2	15	7
Kariokor	1997	8	1	8	-
Burma	1994	1	-	-	1
Burma	1997	1	-	-	2
Westlands	1995	-	1	3	4
Kawangware	1994	-	-	-	1
Nairobi	1995	-	2	5	4
Lake Bogoria	1995	-	1	3	1
Kiambu	1995	-	1	2	-
Show-birds	1996	-	-	3	-

Table 3: Haemagglutination titres obtained with red blood cells from 7 species

	Red blood cells										
Isolate	Chicken	Dog	Horse	Donkey	Pig	Sheep	Goat	Buffalo	Cow	Waterbuck	Totals
A278	256	-		-	-	-	-	-	-		1
B26	8	-	-	-	-	-	-	-	--	-	1
KRC150	32	-	-	-	-	-	-	-	-	-	1
TC1	16	-	-	-	-	-	-	-	-	-	1
KRC238	256	-	-	-	-	-	-	-	-	-	1
KRI2	1024	-	-	-	-	-	-	-	-	-	1
KRI21	128	-	-	-	-	-	-	-	-	-	1
KRI36	32	-	-	-	-	-	-	-	-	-	1
KRI39	64	-	-	-	-	-	-	-	-	-	1
KRI49	32	-	-	-	-	-	-	-	-	-	1
KRI47	64	-	-	-	-	-	-	-	-	-	1
KRI55	16	-	-	-	-	-	-	-	-	-	1
KRI59	64	-	-	-	-	-	-	-	-	-	1
KRI72	128										1
B16	16	16	-	-	-	-	-	-	-	-	2
WC24	128	8	-	-	-	-	-	-	-	-	2
76D	512	256	-	-	-	-	-	-	-	-	2
KRC108	64	8	-	-	-	-	-	-	-	-	2
KRC123	64	4	-	-	-	-	-	-	-	-	2
Totals	19	5	0	0	0	0	0	0	0	0	24

Table 3 continued.

	Red blood cells										
Isolate	Chicken	Dog	Horse	Donkey	Pig	Sheep	Goat	Buffalo	Cow	Waterbuck	Totals
KRC139	64	16	-	-	-	-	-	-	-	-	2
KRC242	32	4	-	-	-	-	-	-	-	-	2
KRC244	16	8	-	-	-	-	-	-	-	-	2
DV213/9	16	-	-	-	8	-	-	-	-	-	2
B39	16	-	-	-	2	-	-	-	-	-	2
Bf5	64	4	-	-	-	-	-	-	-	-	2
Bf7	128	16	-	-	-	-	-	-	-	-	2
K1	32	4	-	-	-	-	-	-	-	-	2
K9	128	32	-	-	-	-	-	-	-	-	2
KRC184	256	256	-	-	-	-	-	-	-	-	2
KRC233	128	32	-	-	-	-	-	-	-	-	2
KRC237	256	32	-	-	-	-	-	-	-	-	2
KRC263	32	64	-	-	-	-	-	-	-	-	2
KRI22	128	32	-	-	-	-	-	-	-	-	2
KRI35	128	4	-	-	-	-	-	-	-	-	2
KRI37	64	4	-	-	-	-	-	-	-	-	2
KRI42	32	16	-	-	-	-	-	-	-	-	2
KRI53	64	-	-	-	2	-	-	-	-	-	2
KTC1	128	128	-	-	-	-	-	-	-	-	2
KWC19	32	16	-	-	-	-	-	-	-	-	2
Totals	20	17	0	0	3	0	0	0	0	0	40

Table 3 continued.

	Red blood cells										
Isolate	Chicken	Dog	Horse	Don	Pig	Sheep	Goat	Buffalc	Cow	Waterbuck	Totals
TC3	32	8	-	-	-	-	-	-	-	-	2
WC2	32	32	-	-	-	-	-	-	-	-	2
K11	16		-	-	4	-	-	4	-	-	3
K12	64	16	-	-	8	-	-	-	-	-	3
WC21	128	64	-	-	16	-	-	-	-	-	3
KWC30	8	-	-	-	-	-	4	-	4	-	3
Bf3	256	32	-	-	16	-	-	-	-	-	3
Bf4	64	32	4	-	-	-	-	-	-	-	3
KRI46	32	2	-	-	64	-	-	-	-	-	3
KRI49	64	4	-	-	2	-	-	-	-	-	3
TC2	32	16	-	-	-	-	4	-	-	-	3
KRI43	64	-	-	-	64	-	-	8	-	-	4
K3	256	4	4	16	-	-	-	-	-	4	5
K13	32	4	-	-	-	32	16	-	64	-	5
KRC144	128	16	-	-	-	4	4	-	8	-	5
WC28	256	8	-	-	-	-	4	8	-	4	5
K6	128	16		-	8	-	2	-	4	-	5
NDV-L	16	16	64	16	-	-	8	-	16	-	6
K8	128	32	-	-	-	32	32	32	16	-	6
K14	64	8'	-	-	-	4	4	4	2	-	6
Totals	21	17	3	8	8	4	8	5	7	2	83

Table 3 continued

	Red blood cells										
Isolate	Chicke	Dog	Horse	Donkey	Pig	Sheep	Goat	Buffalo	Cow	Waterbuck	Totals
KLC17	64	128	-	-	16	32	32	-	32	-	6
KRC236	256	64	-	-	-	2	2	32	2	-	6
LaSota	1024	512	512	1024	256	-	-	4	-	1024	7
F-strain	32	-	32	64	-	4	4	8	16		7
KRC114	64	-	32	64	-	8	8	4	-	2	7
KRC131	512	128	64	64	64	16	-	4	-	-	7
Bnf6	256	128	64	256	64	32	8	-	18	-	8
K2	64	8	4	8	64	2	-	8	-	32	8
K16	128	16	-	-	4	8	4	4	4	2	8
KRC103	512	16	8	64	64	4	-	-	2	4	8
KRC143	512	-	128	128	-	32	16	4	2	2	8
KRC125	128	128	32	32	8	16	-	4	-	4	8
63C	1024	256	256	256	512	32	128	-	32	128	9
KRC83	64	8	4	64	64	8	16	-	8	4	9
KRC134	512	-	32	64	16	4	16	4	2	4	9
KRC141	128	64	16	32	4	8	4	2	4	-	9
KRC136	256	32	32	64	32	128	32	64	32	64	10
Totals	17	13	14	14	13	16	12	12	12	11	134

Table 4. Frequency of NDV isolates at the maximum haemagglutination titres reached with respective chicken and mammalian red blood cells

	Haemagglutination titres reached (reciprocals)									
Red blood cell species tested	2	4	8	16	32	64	128	256	512	1024
Fowl	-	-	2	8	14	17	18	11	5	3
Equine	-	-	1	2	3	6	1	2	-	1
Bovine	5	4	2	4	3	1	-	-	-	-
Ovine	2	5	4	3	7	-	-	-	-	-
Caprine	2	8	3	4	3	-	1	-	-	-
Canine	2	9	9	12	8	6	5	3	1	-
Swine	2	3	4	4	-	8	-	1	1	-

Table 5. Time to complete heat inactivation of haemagglutinin of the isolates at 56°C

Isolate	Initial titre	Time in minutes
B39	16	10 minutes
F-strain	64	<10 "
LaSota	512	<10 "
NDV-L	16	60"
KRC139	16	60 "
KRI55	32	60 "
KRC290	2048	60 "
KWC30	8	120
KLC3	16	120 "
KRC103	16	120 "
KRC181	16	120 "
KRI36	16	120 "
KRI59	16	120 "
WC16	16	120 "
KRI41	32	120 "
KKI53	32	120 "
WC8	32	120 "
A278	128	120"
76D	2048	120
KRI42	8	180
KRI22	16	180
WC20	16	180
Bf16	32	180
KRC114	32	180
KRI46	32	180
KRC125	64	180
KRI35	64	180
KRC242	64	180
K3	128	180
KRC144	128	180
KRC143	256	180
KRC244	256	180
KRI72	256	180

Table 5 continued.

Isolate	Initial titre	56^0C
Bf3	512	180 "
K13	512	180 "
TC1	512	180 "
KRC236	1024	180 "
K12	2048	180 "
KRI37	8	240
Bf5	8	240
DV213/95	16	240 "
KRC83	16	240 "
KRC97	16	240 "
KRC141	32	240 "
K6	64	240 "
KLC17	64	240 "
KRI43	64	240 "
KRI2	128	240 "
KRC184	1024	240 "
KRC102	16	300
KRI45	32	300"
KWC19	32	300
Bf7	32	300
KRC131	64	300 "
KRC134	64	300''
KRI46	64	300''
K9	128	300''
WC28	512	300''
K8	128	360''
63C	256	360''
KRC150	64	480''
KRC263	128	480''
K2	256	480''
K11	256	480''

Table 5 continued.

Isolate	Initial titre	56^0C
K16	256	480
KRC108	256	480
KRC223	256	480
B16	512	480 "
KRC237	512	480 "
B26	2048	480 "
KWC15	2048	480 "
Bf4	64	540
K1	256	540 "
KTC1	256	540 "
KRI39	512	540 "
WC24	512	540"
KRC136	1024	540 "
KRC23	1024	540 "
WC21	2048	540"
K14	4096	540 "
WC2	512	600"
TC2	2048	600
TC31	2048	600

- Experiments run up to 7 days.
- Initial titres are expressed as reciprocals

Table 6. Frequency of NDV isolates by initial HA titre and the time to complete inactivation of haemagglutinin at 56^0C

Initial Virus titre	Time to completely inactivate haemagglutinin (minutes).												
	10	15	30	60	120	180	240	300	360	420	480	540	600
8	-	-	-	-	1	1	2	-	-	-	-	-	-
16	1	-	-	2	7	2	3	1	-	-	-	-	-
32	-	-	-	2	4	3	1	3	-	-	-	-	-
64	1	-	-	-	-	3	3	3	-	-	1	1	-
128	-	-	-	-	1	3	1	1	1	-	1	-	-
256	-	-	-	-	-	3			1	1	4	2	-
512	1					4		1	-	-	2	2	1
1024	-	-	-	-	-	1	1	-	-	-	-	2	-
2048	-	-	-	1	1	1	-	-	-	-	2	1	2
4096	-	-	-	-	-	-	-	-	-	-	-	1	-
Totals	3	0	0	5	14	21	11	9	2	1	10	9	3

Table 7. Distribution of the rate of inactivation of the NDV isolates by place of origin.

	Time in minutes									
Source	10	60	120	180	240	300	360	480	540	600
Kariokor	-	3	6	12	6	5	-	5	3	-
Burma	1	-	1	-	1	-	-	2	-	-
Westlands	-	-	2	1	-	1	-	-	2	1
Lake Bogoria	-	-	-	2	1	1	-	-	1	-
Nairobi	-	-	-	3	1	1	1	3	2	-
Show birds	-	-	1	1	-	-	1	-	-	2
Kiambu	-	1	1	-	1	-	-	-	1	-
Kawangware	-	-	1	-	-	1	-	1	-	-
Reference strains	2	-	-	-	-	-	-	-	-	-

Table 8. Mean death times (MDT) for NDV isolates)

Isolate.	MDT IN HOURS.	Remarks
KRC242	24	Velogenic
Bf3	55	velogenic
Bf7	59	Velogenic
K3	48	Velogenic
K13	54	Velogenic
KRC139	48	Velogenic
KRC144	48	Velogenic
KRC150	54	Velogenic
TC1	48	Velogenic
WC28	48	Velogenic
Bf4.	68	Mesogenic
A278	72	Mesogenic
Bf5.	80	Mesogenic
K2	60	Mesogenic
K12	60	Mesogenic
KRC123	72	Mesogenic
B26	66	Mesogenic
KRC244	126	Lentogenic
KRC108	144	Lentogenic
KWC30	148	Lentogenic
K11	168	Lentogenic

Table 9. Cross haemaglutination inhibition test of 19 randomly selected isolates and 2 reference strains.

	Isolates																				
α-sera	A278	B16	B26	K2	K3	K11	K12	K13	KRC 108	KRC 123	KRC 139	KRC 144	KRC 150	KRC 242	KRC 244	KWC 30	TC1	WC28	NDV-L	LaSota	F-strian
A278	4096	-	16	128	256	-	64	256	32	128	-	32	32	-	-	-	-	64	128	32	64
B16	32	4096	16	256	32	-	8	32	8	64	-	16	8	-	-	-	16	8	32	8	32
B26	16	-	4096	16	16	-	16	8	256	512	-	64	64	512	-	-	16	64	256	8	256
K2	16	-	8	2048	8	-	32	16	8	-	-	16	4	-	-	-	32	8	8	32	32
K3	16	-	16	16	256	-	16	16	32	-	16	16	-	-	-	-	8	256	256	8	8
K11	128	-	16	32	32	1024	8	16	32	32	32	8	-	64	-	-	8	4	8	64	8
K12	512	8	32	16	32	-	1024	128	256	512	64	512	-	128	-	-	16	8	1024	512	16
K13	128	16	64	16	128	8	-	4096	16	64	128	512	-	2048	-	-	-	4	2048	512	64
KRC108	64	-	16	16	32	-	-	64	256	32	-	64	-	32	-	-	32	2	32	8	8
KRC123	32	16	16	16	32	-	-	64	64	512	512	64	-	-	-	-	64	4	32	32	16
KRC139	256	8	16	32	64	-	-	-	256	32	1024	8	-	64	-	-	128	2	2048	512	16
KRC144	16	16	32	128	128	-	-	1024	1024	1024	64	2048	-	128	-	-	32	-	1024	16	64
KRC150	1024	256	64	-	256	-	64	1024	1024	1024	512	16	1024	512	256	256	16	-	256	64	32
KRC242	8	16	-	32	8	8	32	64	512	-	64	64	-	2048	-	-	-	-	1024	64	16
KRC244	16	8	64	128	128	256	256	256	256	-	16	32	-	16	1024	-	-	8	512	128	32
KWC30	16	32	16	8	32	256	16	16	1024	-	32	128	-	64	128	2048	-	-	32	8	16
TC1	32	8	32	32	-	4	128	512	1024	16	16	32	-	-	-	-	2048	64	2048	16	32
WC28	512	1024	-	-	256	-	64	512	256	1024	64	64	-	256	1024	256	16	1024	512	32	16
NDV-L	512	1024	8	16	512	8	128	1024	1024	1024	128	16	256	2048	1024	2048	64	512	2048	512	512
LaSota	1024	1024	-	-	2048	-	256	1024	1024	1024	64	128	32	2048	1024	2048	32	512	2048	2048	1024
F-strain	1024	64	-	-	2048	-	512	1024	1024	1024	64	256	16	2048	1024	2048	64	512	2048	512	4096

HI titres are expressed as reciprocal of the highest dilution of serum causing inhibition of 8HA units of virus.

-No visible inhibition.

Table 10: Comprehensive cross reaction haemagglutination tests by use of d values methodology of Lipkind

	Isolates									
α-sera	A278	B16	B26	K2	K3	K11	K12	K13	KRC108	KRC123
A278	0	∞	2.67±0.1 P≥95, n=3	2.23±0.2 p≥95, n=3	1.33±0.08 p≥95, n=3	∞	2.16±0.16 p≥95, n=3	1.61±0.43 p≥95, n=3	3.02±0.31 p≥95, n=3	1.79±0.10 p≥95, n=3
B16	2.53±0.24 p≥95, n=3	0	3.36±0.48 p≥95, n=3	1.46±0.01 p≥95, n=3	2.33±0.07 p≥95, n=3	∞	2.86±0.57 p≥95, n=3	2.3±0.07 p≥95, n=3	**4.5±0.5 p≥95, n=3**	2.07±0.07 p≥95, n=3
B26	2.17±0.44 p≥95, n=3	∞	0	2.67±0.03 p≥95, n=3	2.67±0.03 p≥95, n=3	∞	2.5±0.5 p≥95, n=3	3±0.57 p≥95, n=3	1.4±0.1 p≥95, n=3	1.24±0.04 p≥95, n=3
K2	2.17±0.3 p≥95, n=3	∞	2. 67±0.27 p≥95, n=3	0	2.69±0.27 p≥95, n=3	∞	1.73±0.27 p≥95, n=3	2.39±0.32 p≥95, n=3	3.72±0.14 p≥95, n=3	∞
K3	2.16±0.08 p≥95, n=3	∞	∞	2.16±0.08 p≥95, n=3	0	∞	2.0±20.13 p≥95, n=3	2.16±0.83 p≥95, n=3	1.73±0.07 p≥95, n=3	∞
K11	1.58±0.08 p≥95, n=3	∞	2.25±0.14 p≥95, n=3	2.25±0.14 p≥95, n=3	1.93±0.14 p≥95, n=3	0	2.93±0.24 p≥95, n=3	2.1±0.28 p≥95, n=3	2.25±0.144 p≥95, n=3	2.25±0.14 p≥95, n=3
K12	1.23±0.09 p≥95, n=3	2.66±0.33 p≥95, n=3	1.6±0.2 p≥95, n=3	2±0.25 p≥95, n=3	1.69±0.17 p≥95, n=3	∞	0	2.03±0.3 p≥95, n=3	1.58±0.16 p≥95, n=3	1.23±0.09 p≥95, n=3
K13	1.74±0.45 p≥95, n=3	2±0.5 p≥95, n=3	1.47±0.27 p≥95, n=3	2±0.5 p≥95, n=3	1.47±0.11 p≥95, n=3	2.17±0.17 p≥95, n=3	∞	0	2.16±0.44 p≥95, n=3	2±0 p≥95, n=3
KRC108	1.8±0.29 p≥95, n=3	∞	1.83±0.08 p≥95, n=3	1.83±0.08 p≥95, n=3	2.07±0.23 p≥95, n=3	∞	∞	1.08±0.29 p≥95, n=3	0	1.89±0.22 p≥95, n=3
KRC123	1.14±0.14 p≥95, n=3	2.64±0.61 p≥95, n=3	2.16±0.08 p≥95, n=3	2.16±0.08 p≥95, n=3	2.49±0.35 p≥95, n=3	∞	∞	**4.7±1.87 p≤95, n=3**	1.7±0.23 p≥95, n=3	0

Table 10 continued.

Comprehensive cross reaction haemagglutination tests. Mean data from all the experiments

	Isolates										
α-sera	KRC139	KRC144	KRC150	KRC242	KRC24 4	KWC30	TC1	WC28	NDV-L	LaSota	F-strain
A278	∞	2±10.15 p≥95, n=3	2.3±0.15 p≥95, n=3	∞	∞	∞	∞	2±0 p≥95, n=3	1.68±0.014 p≥95, n=3	2.13±0.133 p≥95, n=3	1.77±0.19 p≥95, n=3
B16	∞	3.22±0.22 p≥95, n=3	**9±2.5 p≥95, n=3**	∞	∞	∞	3.25±0.38 p≥95, n=3	**7±0.08 p≥95, n=3**	2.3±0.066 p≥95, n=3	3.05±0.48 p≥95, n=3	2±0.066 p≥95, n=3
B26	∞	2±0 p≥95, n=3	2.67±0.03 p≥95, n=3	1.2±40.04 p≥95, n=3	∞	∞	**5±0.1 p≥95, n=3**	2.76±0.03 p≥95, n=3	1.3±0.1 p≥95, n=3	3±0.8 p≥95, n=3	1.3±0.1 p≥95, n=3
K2	2.36±0.21 p≥95, n=3	**6.83±0.72 p≥95, n=3**	∞	∞	∞	∞	1.98±0.13 p≥95, n=3	3.72±0.14 p≥95, n=3	3.33±0.51 p≥95, n=3	1.86±0.24 p≥95, n=3	2.17±0.08 p≥95, n=3
K3	2.07±0.13 p≥95, n=3	2.02±0.12 p≥95, n=3	∞	∞	∞	∞	2.89±0.11 p≥95, n=3	1.13±0.006 p≥95, n=3	1.04±0.4 p≥95, n=3	2.64±0.21 p≥95, n=3	2.64±0.21 p≥95, n=3
K11	1.93±0.13 p≥95, n=3	2.93±0.24 p≥95, n=3	1.64±0.02 p≥95, n=3	1.6±0.0238 p≥95, n=3	∞	∞	3.22±0.11 p≥95, n=3	**6.50.176 p≥95, n=5**	3.22±0.09 p≥95, n=3	1.82±0.09 p≥95, n=3	3.5±0.76 p≥95, n=3
K12	1.1≥4±0.14 p≥95, n=3	1.23±0.09 p≥95, n=3	∞	1.14±0.14 p≥95, n=3	∞	∞	3.17±0.33 p≥95, n=3	3.44±0.05 p≥95, n=3	1.11±0.57 p≥95, n=3	1.09±0.05 p≥95, n=3	2±0.25 p≥95, n=3
K13	1.57±0.07 p≥95, n=3	2.11±0.48 p≥95, n=3	∞	1.09±0.06 p≥95, n=3	∞	∞	∞	**6±0 n=3**	1.36±0.14 p≥95, n=3	1.44±0.05 p≥95, n=3	1.67±0.16 p≥95, n=3
KRC108	∞	2.78±0.72 p≥95, n=3	∞	1.7±0.05 p≥95, n=3	∞	∞	2.87±0.63 p≥95, n=3	**7.3±0.334 p≥95, n=3**	1.7±0.08 p≥95, n=3	2.44±0.11 p≥95, n=3	2.22±0.11 p≥95, n=3
KRC123	1.37±0.18 p≥95,n=3	1.53±0.33 p≥95,n=3	∞	∞	∞	∞	1.63±0.09 p≥95, n=3	**7.17±0.36 p≥95, n=3**	1.54±0.13 p≥95, n=3	2.01±0.13 p≥95, n=3	2.17±0.08 p≥95, n=3
KRC139	0	3.2±20.48 p≥95, n=3	∞	1.48±0.15 p≥95,n=3	∞	∞	2.88±0.72 p≥95, n=3	**8.33±0.88 p≥95, n=3**	1.01±0.06 p≥95, n=3	1.6±20.26 p≥95, n=3	1.92±0.08 p≥95, n=3

An upper figure d in each cell is a mean value (with a standard error) of $\log_2$ of the ratio between homologous and heterologous HI titres when namely antiserum was the same for the two compared viruses. n is a number of experiments for each pair of the compared viruses. p is the student s coefficient of probability for the found difference d for a given number of experiments n; it is expressed as a percentage of the probability in the limits of checking of "zero hypothesis" i.e. the probability of that the difference d itself differs significantly from zero. The reliability of the d values (difference from zero) was accepted as significant if it was in agreement with the 2nd Fischer s criterion pf probablility, namely 95% probability (p=0 95). In the case of absence of the cross reactivity between certain viruses the d value was expressed as infinity (∞). Highlighted values are for isolates showing significant differences (Lipkind, 1985).

Table 10 continued.
Comprehensive cross reaction haemagglutination tests. Mean data from all the experiments.

	Isolates									
α-sera	A278	B16	B26	K2	K3	K11	K12	K13	KRC108	KRC123
KRC139	1.33±0.04 p≥95, n=3	2.78±0.29 p≥95, n=3	2.3±0.28 p≥95, n=3	1.67±0.17 p≥95, n=3	1.56±0.08 p≥95, n=3	∞	∞	□	1.42±0.10 p≥95, n=3	2.72±0.43 p≥95, n=3
KRC144	2.07±0.07 p≥95, n=3	2.25±0.25 p≥95, n=3	2.29±0.198 p≥95, n=3	1.72±0.14 p≥95, n=3	1.72±0.14 p≥95, n=3	∞	∞	1.25±0.08 p≥95, n=3	1.34±0.14 p≥95, n=3	1.43±0.14 p≥95, n=3
KRC150	0.95±0.04 p≥95, n=3	1.08±0.08 p≥95, n=3	1.69±0.17 p≥95, n=3	∞	1.25±0.41 p≥95, n=3	∞	1.56±0.08 p≥95, n=3	1.05±0.05 p≥95, n=3	1.17±0.09 p≥95, n=3	1.17±0.09 p≥95, n=3
KRC242	3.0±50.339 p≥95, n=3	3.0±80.46 p≥95, n=3	∞	3.2±0.118 p≥95, n=3	3.67±0.19 p≥95, n=3	3.6±70.19 p≥95, n=3	2.4±0.3 p≥95, n=3	2.17±0.41 p≥95, n=3	1.22±0.063 p≥95, n=3	∞
KRC244	2.94±0.24 p≥95, n=3	**4.27±0.49 p≥95, n=3**	2.14±0.24 p≥95, n=3	1.64±0.17 p≥95, n=3	1.64±0.17 p≥95, n=3	1.2±0.04 p≥95, n=3	1.85±0.36 p≥95, n=3	2±0.38 p≥95, n=3	1±.0.041 p≥95, n=3	∞
KWC30	2.67±0.083 p≥95, n=3	3.07±0.44 p≥95, n=3	2.67±0.08 p≥95, n=3	3.56±0.52 p≥95, n=3	2.48±0.15 p≥95, n=3	1.39±0.02 p≥95, n=3	2.67±0.08 p≥95, n=3	2.67±0.08 p≥95, n=3	1.14±0.03 p≥95, n=3	∞
TC1	2.57±0.18 p≥95, n=3	3.97±0.8 p≥95, n=3	2.38±0.18 p≥95, n=3	2.2±0 n=3	∞	**4.81±0.01 p≥95, n=3**	2.3±60.39 p≥95, n=3	2.23±0.51 p≥95, n=3	1.14±0.07 p≥95, n=3	3.97±0.8 p≥95, n=3
WC28	1.53±0.22 p≥95, n=3	1.03±0.033 p≥95, n=3	∞	∞	1.36±0.106 p≥95, n=3	∞	1.72±0.053 p≥95, n=3	1.37±0.139 p≥95, n=3	1.58±0.17 p≥95, n=3	1.03±0.033 p≥95, n=3
NDV-L	1.62±0.21 p≥95, n=3	1.1±0 n=3	3.67±0 n=3	2.75±0 n=3	1.26±0 n=3	3.67±0 n=3	1.57±0 n=3	1.140 n=3	1.14±0.04 p. ≥95, n=3	1.14±0.04 p. ≥95, n=3
LaSota	0.93±0.081 p≥95, n=3	1.03±0.033 p≥95, n=3	∞	∞	1.07±0.066 p≥95, n=3	∞	1.18±0.108 p≥95, n=3	0.98±0.07 p≥95, n=3	0.93±0.08 p≥95, n=3	0≥.87±0.12 p95, n=3
F-strain	1.19±0.108 p≥95, n=3	1.87±0.24 p≥95, n=3	∞	∞	0 .96±0.23 p ≥95, n=3	∞	1.4±0.12 p≥95, n=3	1.14±0.07 p≥95, n=3	1.09±0.11 p≥95, n=3	1.16±0.03 p≥95, n=3

Table 10 continued.

Comprehensive cross reaction haemagglutination tests. Mean data from all the experiments

	Isolates										
α-sera	KRC139	KRC144	KRC150	KRC242	KRC244	KWC30	TC1	WC28	NDV-L	LaSota	F-strain
KRC144	1.58±0.13 p≥95, n=3	0	∞	1.41±0.08 p≥95, n=3	∞	∞	2.07±0.07 p≥95, n=3	∞	1.13±0.01 p≥95, n=3	2.22±0.22 p≥95, n=3	1.67±0.06 p≥95, n=3
KRC150	1.18±0.13 p≥95, n=3	2.5±0.09 p≥95, n=3	0	1.2±0.068 p≥95, n=3	1.19±0.1 p≥95, n=3	1.25±0.41 p≥95, n=3	2.5±0.98 p≥95, n=3	∞	1.23±0.13 p≥95, n=3	1.8±0.098 p≥95, n=3	2.14±0.74 p≥95, n=3
KRC242	3.72±1.2 p≥95, n=3	1.83±0.09 p≥95, n=3	∞	0	∞	∞	∞	∞	1.1±0.057 p≥95, n=3	2.44±0.44 p≥95, n=3	2.38±0.22 p≥95, n=3
KRC244	3.5±0.76 p≥95, n=3	1.93±0.07 p≥95, n=3	∞	2.94±0.24 p≥95, n=3	0	∞	∞	**7.42±2.1 p≥95, n=3**	1.1±60.04 p≥95, n=3	2.06±0.32 p≥95, n=3	2.08±0.09 p≥95, n=3
KWC30	3.56±0.86 p≥95, n=3	1.52±0.05 p≥95, n=3	∞	3.5±0.091 p≥95, n=3	1.52±0.44 p≥95, n=3	0	∞	∞	2.13±0.07 p≥95 n=3	3.2±50.26 p≥95n=3	2.08±0.33 p≥95, n=3
TC1	3.97±0.8 p≥95, n=3	3.67±0.91 p≥95, n=3	∞	∞	∞	∞	0	3.05±1.2 p≥95, n=3	1.07±0.03 p≥95, n=3	3.67±0.91 p≥95, n=3	**4.28±0.61 p≥95, n=3**
WC28	1.85±0.17 p≥95, n=3	1.85±0.17 p≥95, n=3	∞	1.42±0.09 p≥95, n=3	1.21±0.11 p≥95, n=3	1.37±0.12 p≥95, n=3	2.58±0.08 p≥95, n=3	0	1.13±0.03 p≥95, n=3	2.23±0.16 p≥95, n=3	3.23±0.88 p≥95, n=3
NDV-L	1.66±0.08 p≥95, n=3	3.06±0.31 p≥95, n=3	1.32±0.05 p≥95, n=3	1.0±70.03 p≥95, n=3	1.18±0.04 p≥95, n=3	1.07±0.03 p≥95, n=3	1.95±0.12 p≥95, n=3	1.32±0.05 p≥95, n=3	0	1.22±0 n=3	1.18±0.04 p≥95, n=3
LaSota	1.41±0.21 p≥95, n=3	1.09±0.23 p≥95, n=3	1.53±0.33 p≥95, n=3	1.13±0.06 p≥95, n=3	1.03±0.03 p≥95, n=3	1.07±0.07 p≥95, n=3	1.2±0 n=3	1.09±0.11 p≥95, n=3	1.07±0.07 p≥95, n=3	0	1.1±0.06 p≥95, n=3
F-strain	1.67±0.25 p≥95, n=3	1.25±0.19 p≥95, n=3	2.5±0.301 p≥95, n=3	1.12±0.03 p≥95, n=3	1. 2±60.17 p≥95, n=3	1.56±0.33 p≥95, n=3	2.05±0.14 p≥95, n=3	1.68±0.32 p≥95, n=3	1.31±0.28 p≥95, n=3	1.24±0.04 p≥95, n=3	0

An upper figure d in each cell is a mean value (with a standard error) of $\log_2$ of the ratio between homologous and heterologous HI titres when namely antiserum was the same for the two compared viruses. n is a number of experiments for each pair of the compared viruses. p is the student's coefficient of probability for the found difference d for a given number of experiments n; it is expressed as a percentage of the probability in the limits of checking of "zero hypothesis" i.e. the probability of that the difference d itself differs significantly from zero. The reliability of the d values (difference from zero) was accepted as significant if it was in agreement with the 2nd Fischers criterion pf probablility, namely 95% probability (p=0.95). In the case of absence of the cross reactivity between certain viruses the d value was expressed as infinity (∞). Highlighted values are for isolates showing significant differences (Lipkind, 1985)

Table 11. Antigenic relationship (r-values) by the Archetti & Horsfall method of Kenyan NDV isolate

	S					e					r					a				
Antigen	A278	B16	B26	K2	K3	K11	K12	K13	KRC108	KRC123	KRC139	KRC144	KRC150	KRC242	KRC244	KWC30	TC1	WC28	NDV-L	LaSota
B16	0																			
B26	252	0																		
K2	64	0	256																	
K3	16	0	64	63																
K11	0	0	0	0	0															
K12	11.3	256	90	64	22.6	0														
K13	22.6	256	181	181	22.6	181	0													
KRC108	29.6	0	16	64	8	0	0	32												
KRC123	22.6	45	16	0	0	0	0	22	8											
KRC139	0	0	0	2.71	16	0	0	0	0	5.6										
KRC144	128	181	64	45	16	0	0	4	8	4	64									
KRC150	11.3	45	32	0	0	0	0	0	0	0	0	0								
KRC242	0	0	0	0	0	64	22.6	5.65	5.6	0	22.6	22.6	0							
KRC244	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
KWC30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
TC1	0	16	128	4	0	256	32	0	4	32	32	64	0	0	0	0				
WC28	11.3	22.6	0	0	2	0	45	45	22.6	11.3	40.9	0	0	0	11.3	0	45			
NDV-L	11.3	16	64	181	2	181	4	2	4	5.6	8	16	5.6	1.41	2	8	5.6	2.8		
LaSota	16	32	0	0	5.6	0	4	4	8	5.6	8	45	32	5.6	1.4	16	90	11.3	2	
F-strain	16	90.5	0	0	8	0	22	16	11.3	11.3	64	22.6	90	16	11.3	16	64	22.6	2.8	4

Archetti and Horsfall transformations of HI titres when 8HAunits of the antigen were used. The Archetti and Horsfall transformation using non-logarithmic serum end points was squareroot of —

Heterologous 1 x Heterologous 2

Table 12. Comparison of the d (antigenic relationship) and r values between NDV isolates derived from HI test

Close symmetrical	r-value	Close asymmetrical	r-value	Moderate symmetrical	r-value
A278&K3	16	A278&K11	0	A278&B26	256
A278&K12	11.3	A278&KRC139	0	A278&K2	64
A278&K13	22.6	B16&K2	0	A278&KRC144	128
A278&KRC123	22.6	K2&KRC244	0	B16&K12	256
A278&WC28	11.3	K &LaSota	0	B16&K13	256
A278&NDV-L	11.3	K3&K11	0	B16&KRC123	45
A278&F-strain	16	K &KRC150	0	B16&KRC144	181
K2&K12	64	K3&KRC144	0	B16&TC1	16
K3&KRC108	8	K11&KRC139	0	K2&K3	64
K3&KRC139	16	K11&KRC150	0	K2&K13	181
K3&KRC144	16	K11&KRC244	0	K2&NDV-L	181
K3&WC28	2	K11&KWC30	0	K11&K13	181
K3&NDV-L	2	K12&KRC108	0	K11&NDV-L	181
K12&NDV-L	4	K12&KRC123	0	K12&TC1	32
K12&F-strain	22	K12&KRC139	0	KRC139&TC1	32
K13&NDV-L	22	K12&KRC144	0	KRC144&TC1	64
K13&F-strain	2	K12&KRC150	0	KRC150&F-strain	90
KRC108&KRC123	8	K12&KRC244	0	B26&K2	256

Table 12. Continued

Moderate asymmetrical	r-value	Slight asymmetrical	r-value	No relationship	r-value
A278&B16	0	B16&KRC108	0	B16&K11	0
A278&KRC242	0	B16&KRC244	0	K2&KRC150	0
A278&KRC244	0	K11&WC28	0	KRC123&KRC242	0
A278&TC1	0			KRC123&KRC244	0
B16&B26	0			KRC123&KWC30	0
B16&K3	0			KRC150&WC28	0
B16&KRC139	0			KRC242&TC1	0
B16&KRC242	0			KRC244&TC1	0
B16&KWC30	0				

Table 13a. Characteristics of Bnf6 isolate recovered from Lake Bogoria flamingo faeces.

	Anti-Bnf6	Anti-APMV-4	HA with Red blood cell from different species		MDT	Elution rate	Thermostability
			Red blood cell type	Titre			
Bnf6	4096	1024	chicken	256	>168	Non-eluter	180
A278	-	-	Dog	128			
B16	-	-	Horse	64			
B26	8	2	Donkey	256			
K2	64	64	Pig	64			
K11	-	-	Sheep	32			
KRC108	32	16	Goat	8			
KRC139	-	-	Buffalo	-			
KWC30	-	-	Cow	8			
TC1	-	-	Waterbuck	-			
WC28	32	16					
NDV-L	8	8					
LaSota	8	8					

MDT:-Mean death time. Pathogenecity determined by mean death time in hours.

Thermostability of haemagglutinins at 56°C given in minutes.

Table 13b. Antigenic relationship between the NDV isolates and Bnf6 (APMV-4)

Isolate	Relationship	D_1	D_2	r-value
Bnf6 & K13	Close asymmetrical	1.7±0.08	∞	0
Bnf6 & A278	Moderate asymmetrical	2.45±0.03	∞	0
Bn6 & KRC150	"	1.97±0.02	∞	0
Bnf6 & B26	"	3.17±0.42	∞	0
Bnf6 & K2	"	2.31±0.22	∞	0
Bnf6 & K3	"	2.11±0.3	∞	0
Bnf6 &K12	"	2.45±0.16	∞	0
Bnf6 &KRC108	"	2.45±0.16	∞	0
Bnf6 &KRC123	"	2.83±0.08	∞	0
Bnf6 & WC28	"	3.2±0.43	∞	0
Bnf6 & LaSota	Slight symmetrical	4±0	4.73±0.6	512
Bnf6 & F-strain	Slight asymmetrical	∞	4.33±0.6	0
Bnf6 & B18	No relationship	∞	∞	0
Bnf6 & K11	"	∞	∞	0
Bnf6 & KRC139	"	∞	∞	0
Bnf6 & KRC242	"	∞	∞	0
Bnf6 & KRC244	"	∞	∞	0
Bnf6 & KWC30	"	∞	∞	0
Bnf6 & TC1	"	∞	∞	0

Table 14. Comparative reciprocal neuraminidase inhibition test for eleven PMV isolates

α-sera	Isolates										
	LaSota	A278	Bnf6	B16	K2	K3	K11	K12	K13	KRC108	KRC123
LaSota	360	12	0	0	8	8	2	36	78	8	32
A278	76	320	36	8	50	260	308	200	76	148	226
Bnf6	2	4	160	0	24	0	2	2	74	52	20
B16	12	8	0	100	44	8	2	64	28	20	100
K2	12	72	72	0	368	240	128	268	44	140	168
K3	192	240	60	52	8	24	372	18	112	8	320
K11	6	10	0	8	180	240	400	10	52	32	32
K12	4	4	20	0	220	2	2	200	40	16	12
K13	28	60	32	17	160	84	56	84	240	36	224
KRC108	8	4	20	0	56	1	0	0	22	320	24
KRC123	160	42	16	28	204	168	10	132	0	210	400

- NI titres are expressed as the reciprocal of the highest dilution of serum causing 50% inhibition of Nase activity of the virus dose giving spectrophotometric reading equal to 0.85 OD_{549} (Lipkind *et.al.*, 1985)
- 0:-Non detectable inhibition.

Table 15. Antigenic relationship of the isolates (r-values) by the Archetti and Horsfall method as derived from Neuraminidase inhibition titres

	Antisera									
Isolates	A278	B16	K3	K13	K2	K11	K12	KRC108	Bnf6	KRC123
A278										
B16	22.36									
K3	2.85	2.4								
K13	4.109	7.1007	**1.27**							
K2	5.719	∞	2.14	3.541						
K11	6.446	50	3.04	4.059	2.527					
K12	8.994	∞	11.547	3.779	**1.117**	63.245				
KRC108	9.26	0	31.25	9.84	3.88	0	0			
Bnf6	18.856	∞	∞	4.026	5.83	∞	28.28	6.99		
KRC123	3.64	3.77	**0.42**	0	**2.07**	22.2	7.09	5.05	5.29	

Highlighted values show r values less than 2 i.e the isolates are not significantly different.

Table 16. Seroprevalence of Newcastle disease in village chickens. Maximum haemagglutination inhibition titres detected from field sera by APMV1-9 and two vaccine strains.

Titre	LaSota	F-strain	APMV-1	APMV-2	APMV-3	APMV-4	APMV-6	APMV-7	APMV-8	APMV-9
1:2	5	4	-	1	2	2	1	3	2	-
1:4	4	11	6	2	2	4	8	14	5	3
1:8	4	9	5	8	5	2	4	6	1	2
1:16	6	3	1	5	3	3	5	3	-	-
1:32	3	3	5	-	-	1	-	1	-	-
1:64	4	3	2	-	2	-	2	-	1	-
1:128	-	-	-	-	2	-	-	-	-	-
1:256	-	1	-	-	2	-	-	-	2	-
1:512	-	-	-	-	-	-	-	-	-	-
1:1024	-	-	-	-	-	-	-	-	-	-
1:2048	-	-	-	-	-	-	-	-	-	-
1:4096	-	-	-	-	-	-	-	-	-	-
Totals	26	33	19	16	18	12	20	27	11	5

Table.17. Haemagglutination inhibition after absorption of NDV with field sera

Titre	APMV-2	APMV-3	APMV-4	APMV-5	APMV-6	APMV-7	APMV-8	APMV-9
0	-	-	-	-	-	-	-	-
1:2	-	-	-	-	-	-	-	-
1:4	-	-	-	-	-	-	-	-
1:8	-	-	-	-	-	-	-	-
1:16	-	2	-	-	-	-	-	-
1:32	-	2	-	-	-	-	-	-
1:64	-	2	-	-	-	-	-	-
1:128	-	-	-	-	-	-	-	-
1:256	-	-	-	-	-	-	-	-

- No antisera gave that titre

Table 18. Prevalence of antibodies to Avian paramyxoviruses 1-9 (APMV1-APMV9).

	Region		
	Burma	Kariokor	Westlands
Total samples tested	145	141	32
Number positive	37	45	4
Percent positive	25.5	31.9	12.9

APPENDICES

Appendix 1. Reagents

a) Normal saline (0.85%)

Normal saline was prepared by dissolving Sodium Chloride in deionised water and autoclaving for 15 minutes at 15lb pressure. It was used to prepare red blood cells and diluting antisera.

b) Phosphate Buffered saline (PBS).

The PBS was prepared to comprise: 8.0 grams Sodium Chloride, 0.2 grams Potassium Chloride, 1.15 grams anhydrous disodium hydrogen phosphate per liter of deionised water and pH 7.2.

c) Alsever's solution.

The Alserver's solution consisted of Dextrose (20.5gms), Sodium Citrate (8.0gms), Sodium Chloride (4.2gms) prepared in distilled water(1000ml). The pH was adjusted to 6.1 with a newly prepared 10% solution of citric acid.

d). Fetuin

Fetuin powder was reconstituted with distilled water to give a final concentration of 50mg protein per ml required for use. Freshly prepared fetuin was stored at $4^{o}C$ for no longer than 1 week.

e). Periodate Reagent.

The periodate reagent was prepared with 4.28gm Sodium metaperiodate ($NaIO_4$),

62ml Syrup orthophosphoric acid dissolved in 38ml Distilled water in a fume chamber.

f). Arsenite Reagent. The arsenite reagent was prepared with 10mg sodium arsenite($NaAsO_2$), 7.1gm sodium sulphate (anhydrous), 0.3ml concentrated sulfuric acid dissolved in 100ml distilled water.

g). Thiobarbituric Acid Reagent.

The thiobarbituric acid reagent was prepared with 3.0gm thiobarbituric acid, 35.5gm sodium sulfate (anhydrous) dissolved in 500ml-distilled water. The solution was stored in a brown bottle.

h). Butanol Reagent.

This consisted of 1 litre 1-Butanol (n-butyl alcohol) mixed with 50ml Concentrated hydrochloric acid.

(The reagents were prepared as per the methods described by Hoskins, J.M. 1967., Gradwohl R. B. H., 1963; Knight C.A., 1975).

Appendix 2. Source of isolates, year of isolation and their codes

Isolate	Year of isolation	Source
NDV-L	1991	Limuru
KRC83	1994	Kariokor, Chicken
KRC97	"	"
KRC102	"	"
KRC103	"	"
KRC108	"	"
KRC114	"	"
KRC123	"	"
KRC125	"	"
KRC131	"	"
KRC134	"	"
KRC136	"	"
KRC139	"	"
KRC141	"	"
KRC143	"	"

Appendix 2 continued

Isolate	Year of isolation	Source
KRC144	"	"
KRC150	"	"
KRC181	1994	Kariokor, Chicken
KRC184	"	"
KRC223	"	"
KRC236	"	"
KRC237	"	"
KRC238	"	"
KRC242	"	"
KRC244	"	"
KRC263	"	"
KRC290	"	"
A278	1995	Eldoret
Bnf6	"	Lake Bogoria Flamingo faeces
Bf3	"	Lake Bogoria water
Bf4	"	"

Appendix 2 continued

Isolate	Year of isolation	Source
Bf5	"	"
Bf7	"	"
Bf 16	"	"
K1	1995	Nairobi, Wild bird
K2	"	Nairobi, Wild bird
K3	"	"
K6	"	"
K8	"	"
K9	"	"
K11	"	"
K12	"	"
K13	"	"
K14	"	"
K16	"	"
KWC19	"	Kawangware, Chicken
KWC30	"	"
KLC3	"	Kaloleni, Chicken
KLC17	"	"

Appendix 2 continued

Isolate	Year of Isolation	Source
WC2	1995	Westlands, Chicken
WC8	"	"
WC15	"	"
WC16	"	"
WC20	"	"
WC21	"	"
WC24	"	Westlands, Chicken
WC28	"	"
DV213/95	"	Kiambu
63C	1996	Chicken, Show bird
76D	"	Duck, Show bird
TC1	"	Turkey, show bird
TC2	"	"
B16	1997	Burma, Chicken
B26	"	"
B39	"	"
KRI1	"	Kariokor, Chicken
KRI21	"	"

Appendix 2 continued

Isolate	Year of Isolation	Source
KRI22	1997	"
KRI35	"	"
KRI36	"	"
KRI37	1997	Kariokor, Chicken
KRI39	"	"
KRI41	"	"
KRI42	"	"
KRI43	"	"
KRI37	1997	Kariokor, Chicken
KRI39	"	"
KRI41	"	"
KRI42	"	"
KRI43	"	"
KRI45	"	Kariokor, Chicken
KRI46	"	"
KRI47	"	"
KRI49	"	"
KRI53	"	"
KRI55	"	"
KRI59	"	"
KRI72	"	"

Appendix 3: Heat inactivation of haemagglutinins at 56^{0}C

	Time in minutes											
Isolate	0	10	15	30	60	120	180	240	300	360	420	480
A278	128	128	64	64	32	32	-	-	-	-	-	-
Bnf6	512	512	512	256	256	128	-	-	-	-	-	-
B16	512	512	512	512	512	512	512	512	256	256	16	-
B26	2048	2048	1024	512	256	256	256	256	256	256	16	-
K2	256	128	128	128	64	64	64	64	32	32	16	-
K3	128	64	32	4	4	2	-	-	-	-	-	-
K11	256	128	128	128	128	64	64	64	32	32	32	-
K12	2048	4	4	4	4	4	-	-	-	-	-	-
K13	512	128	64	64	32	32	-	-	-	-	-	-
KRC108	256	256	512	512	512	512	256	128	64	64	32	-
KRC123	128	32	16	8	4	-	-	-	-	-	-	-
KRC139	16	16	16	8	-	-	-	-	-	-	-	-
KRC144	128	128	64	64	32	8	-	-	-	-	-	-

Appendix 3 continued

	Time in minutes											
Isolate	0	10	15	30	60	120	180	240	300	360	420	480
KRC150	64	64	64	64	64	64	64	64	64	64	32	-
KRC242	64	64	64	64	64	64	-	-	-	-	-	-
KRC244	256	128	64	64	32	4	-	-	-	-	-	-
KWC30	8	8	8	8	4	-	-	-	-	-	-	-
TC1	512	128	64	64	32	32	-	-	-	-	-	-
WC28	512	512	512	512	128	64	32	-	-	-	-	-
NDV-L	16	4	4	-	-	-	-	-	-	-	-	-
LaSota	512	-	-	-	-	-	-	-	-	-	-	-
F-strain	64	-	-	-	-	-	-	-	-	-	-	-
DV213/95	16	8	8	8	4	2	-	-	-	-	-	-
B39	16	-	-	-	-	-	-	-	-	-	-	-
Bf3	512	512	128	64	8	-	-	-	-	-	-	-

Appendix 3 continued

	Time in minutes											
Isolate	0	10	15	30	60	120	180	240	300	360	420	480
Bf4	64	64	64	64	64	64	64	32	32	16	8	-
Bf5	8	8	4	4	2	-	-	-	-	-	-	-
Bf7	32	32	16	16	16	8	4	-	-	-	-	-
Bf16	32	16	16	16	4	-	-	-	-	-	-	-
K1	256	256	128	32	32	32	32	16	8	8	8	-
K6	64	64	32	32	8	4	-	-	-	-	-	-
K8	128	128	128	64	64	16	4	2	-	-	-	-
K9	128	128	128	128	64	32	16	-	-	-	-	-
K14	4096	4096	4096	1024	1024	512	512	512	512	128	16	-
K16	256	256	256	256	256	64	64	32	16	16	-	-
KLC3	16	16	4	-	-	-	-	-	-	-	-	-
KLC17	64	32	32	32	16	8	-	-	-	-	-	-
KRC83	16	8	4	4	4	2	-	-	-	-	-	-

Appendix 3 continued

	Time in minutes											
Isolate	0	10	15	30	60	120	180	240	300	360	420	480
KRC97	16	16	16	16	4	-	-	-	-	-	-	-
KRC102	16	16	8	8	8	8	2	-	-	-	-	-
KRC103	16	16	8	4	-	-	-	-	-	-	-	-
KRC114	32	32	32	32	8	-	-	-	-	-	-	-
KRC125	64	16	8	8	8	-	-	-	-	-	-	-
KRC131	64	64	64	64	64	16	8	-	-	-	-	-
KRC134	64	32	32	32	8	4	4	-	-	-	-	-
KRC136	1024	1024	512	512	512	64	64	16	16	8	4	-
KRC141	32	16	16	8	2	2	-	-	-	-	-	-
KRC143	256	128	128	32	4	-	-	-	-	-	-	-
KRC181	16	16	16	8	-	-	-	-	-	-	-	-
KRC184	1024	128	64	64	32	32	-	-	-	-	-	-
KRC223	256	256	256	128	128	128	128	32	8	-	-	

Appendix 3 continued

	Time in minutes											
Isolate	0	10	15	30	60	120	180	240	300	360	420	480
KRC236	1024	512	512	256	256	-	-	-	-	-	-	-
KRC237	512	128	128	128	128	32	16	16	16	16	-	-
KRC238	1024	1024	1024	256	256	256	256	32	32	32	32	-
KRC263	128	128	64	32	32	16	16	8	8	-	-	-
KRC290	2048	8	8	-	-	-	-	-	-	-	-	-
KRI2	128	128	128	64	64	32	32	-	-	-	-	-
KRI21	32	4	4	4	-	-	-	-	-	-	-	-
KRI22	16	16	16	16	8	-	-	-	-	-	-	-
KRI35	64	64	64	64	64	-	-	-	-	-	-	-
KRI36	16	16	8	4	-	-	-	-	-	-	-	-
KRI37	8	16	8	4	4	2	-	-	-	-	-	-
KRI39	512	512	512	512	512	128	128	32	8	8	-	-
KRI41	32	32	8	8	-	-	-	-	-	-	-	-
KRI42	8	8	8	4	4	-	-	-	-	-	-	-

Appendix 3 continued

	Time in minutes											
Isolate	0	10	15	30	60	120	180	240	300	360	420	480
KRI43	64	32	16	16	16	16	-	-	--	-	-	-
KRI45	32	16	16	8	8	8	8	-	-	-	-	-
KRI46	32	32	32	32	4	-	-	-	-	-	-	-
KRI47	64	64	64	64	64	64	8	-	-	-	-	-
KRI53	32	32	32	4	-	-	-	-	-	-	-	-
KRI55	32	16	8	-	-	-	-	-	-	-	-	-
KRI59	16	16	16	8	-	-	-	-	-	-	-	-
KRI72	256	512	128	128	32	-	-	-	-	-	-	-
KTC1	256	128	128	128	32	16	16	16	16	4	-	-
KWC19	32	32	32	32	8	8	4	-	-	-	-	-
TC1	2048	2048	1024	1024	1024	1024	128	128	32	32	16	-
TC3	2048	2048	512	512	256	256	128	64	64	8	4	-
WC2	512	512	512	512	512	512	512	64	64	16	8	-
WC8	32	32	16	8	-	-	-	-	-	-	-	-
WC15	2048	1024	1024	1024	128	128	64	64	32	-	-	-

Appendix 3 continued.

	Time in minutes											
Isolate	0	10	15	30	60	120	180	240	300	360	420	480
WC16	16	4	4	4	-	-	-	-	-	-	-	-
WC20	16	16	16	16	8	-	-	-	-	-	-	-
WC21	2048	1024	1024	256	256	256	256	256	64	64	-	-
WC24	512	256	128	128	128	128	64	64	32	32	-	-
63C	256	256	64	32	32	16	16	16	-	-	-	-
76D	2048	256	64	16	-	-	-	-	-	-	-	-

Appendix 4. Comparison of the d (antigenic relationship) and r values between NDV isolates

Isolate	Relationship	D_1	D_2	r-value
A278 & K3	Close symmetrical	1.13±0.08	2.16±0.08	16
A278 & K12	"	2.16±0.16	1.23±0.09	11.3
A278 & K13	"	1.16±0.43	1.74±0.45	22.6
A278 & KRC123	"	1.79±0.1	1.14±0.14	22.6
A278 & WC28	"	2±0	1.53±0.22	11.3
A278 & NDV-L	"	1.68±0.014	1.62±0.21	11.3
A278 & F-Strain	"	1.77±0.19	1.19±0.108	16.0
K2 &k12	"	1.73±0.27	2±0.25	64
K3 & KRC108	"	1.73±0.07	2.07±0.23	8
K3 & KRC139	"	2.07±0.13	1.56±0.08	16
K3 & KRC144	"	2.02±0.12	1.72±0.1	16
K3 & WC28	"	1.13±0.006	1.36±0.16	2
K3 & NDV-L	"	1.04±0.4	1.26±0	2
K12 & NDV-L	"	1.11±0.57	1.18±0.108	4
K12 & F-strain	"	2±0	1.4±0.12	22
K13 & NDV-L	"	1.36±0.14	1.14±0	22
K13 & F-strain	"	1.67±0.16	1.14±0.07	2
KRC108 &KRC123	"	1.89±0.22	1.7±0.23	8
KRC108 &KRC242	"	1.7±0.05	1.22±0.063	5.6
KRC108 & NDV-L	"	1.7±0.08	1.14±0.04	4
KRC123 & KRC144	"	1.53±0.33	1.43±0.14	4

Appendix 4 continued

Isolate	Relationship	D_1	D_2	r-value
KRC123 & NDV-L	Close symmetrical	1.54±0.13	1.14±0.04	5.6
KRC139 & NDV-L	"	1.01±0.06	1.66±0.08	8
KRC139 & LaSota	"	1 62±0.26	1.41±0.21	8
KRC139 & F-strain	"	1.92±0.08	1.67±0 25	64
KRC144 &KRC244	"	1 41±0.08	1.83±0.09	22.6
KRC144 & F-strain	"	1.67±0.06	1.25±0.19	22.6
KRC150 & NDV-L	"	1.23±0.13	1.32±0.05	5.6
KRC150 & LaSota	"	1.8±0.098	1.53±0.33	32
KRC242 & NDV-L	"	1.1±0.057	1.07±0.03	1.41
KRC244 & NDV-L	"	1 16±0 04	1.18±0.04	2
TC1 & NDV-L	"	1.07±0.03	1.95±0.12	5.6
WC28 &NDV-L	"	1.13±0.03	1.32±0.05	2.8
NDV-L & LaSota	"	1.22±0	1.07±0.07	2
NDV-L & F-strain	"	1.18±0.04	1.31±0.28	2.8
LaSota & F-strain	"	1.1±0.06	1.24±0.04	4
A278 & K11	Close asymmetrical	∞	1.58±0.08	0
A278 &KRC139	"	∞	1.33±0.04	0
B16 & k2	"	1.46±0.01	∞	0
K2 & KRC244	"	∞	1 64±0.17	0
K2 & LaSota	"	1.86±0.24	∞	0
K3 & K11	"	∞	1 93±0.14	0
K3 & KRC150	"	∞	1.25±0.4	0
K3 & KRC144	"	∞	1 64±0.17	0

Appendix 4 continued

Isolate	Relationship	D_1	D_2	R -value
K11 & KRC139	"Close asymmetrical	1 93±0.13	∞	0
K11 & KRC150	"	1 64±0.02	∞	0
K11 & KRC244	"	∞	1.2±0 04	
K11 & KWC30	"	∞	1.39±0.02	0
K12 & KRC108	"	1.58±0.16	∞	0
K12 & KRC123	"	1 23±0.09	∞	0
K12 & KRC139	"	1 14±0.14	∞	0
K12 & KRC144	"	1 23±0.09	∞	0
K12 & KRC150	"	∞	1.56±0.08	0
K12 & KRC244	"	∞	1.85±0.36	0
K11 & LaSota	"	1.82±0.09	∞	0
K13 & KRC139	Close asymmetrical	1.57±0.07	00	0
K13 & KRC150	"	∞	1.05±0.05	0
KRC108 & KRC139	"	∞	1.42±0.10	0
KRC108 & KRC150		∞	1.17±0.05	0
KRC108 & KRC244	"	∞	1 2±0.041	0
KRC108 & KWC30	"	∞	1.14±0.03	0
KRC123 & KRC150	"	∞	1.17±0.09	0
KRC139 & KRC150	"	∞	1 18±0.13	0
KRC144 & KRC242	"	∞	1.83±0.09	0
KRC144 & KWC30	"	∞	11.52±0.05	0
KRC144 & WC28	"	∞	1 85±0.17	0

Appendix 4 continued

Isolate	Relationship	D_1	D_2	r-value
KRC150 & KRC242	Close asymmetrical	1.2±0.068	∞	0
KRC150 &KRC244	"	1 19±0.1	∞	0
KRC150 &KWC30	"	1 25±0.41	∞	0
KRC244 & KWC30	"	∞	1.52±0.44	0
B26 &KRC242	"	1.24±0.04	∞	
B26 & LaSota	"	3±0.8	∞	
B26 & F-strain	"	1.3±0.1	∞	
KWC30 & NDV-L	"	∞	1.37±0.12	0
A278 & B26	Moderate symmetrical	2.67±0.1	2.17±0.44	252
A278 & K2	"	2.23±0.2	2.17±0.3	64
A278 & KRC144	"	2.1±0.15	2.07±0.07	128
B16 & K12	"	2.86±0.57	2.66.1±0.33	256
B16 & K13	"	2.3±0.07	2±0.5	256
B16 & KRC123	"	2.07±0.07	2.64±0.61	45
B16 & KRC144	"	3.22±0.22	2.25±0.25	181
B16 & TC1	"	3.25±0.38	3.97±0.8	16
K2 & K3	"	2.69±0.03	2.16±0.08	64
K2 & K13	"	2.39±0.32	2±0	181
K2 & NDV-L	"	3.3±0.5	2.75±0	181
K11 & K13	"	2.1±0.28	2.17±0.17	181
K11 & NDV-L	"	3.22±0.09	3.67±0	181
K12 & TC1	"	3.17±0.33	2.36±0.39	32
KRC139 & TC1	"	2.88±0.72	3.97±0.8	32

Appendix 4 continued

Isolate	Relationship	D_1	D_2	r-value
KRC144 & TC1	Moderate symmetrical	2.07±0.07	3.67±0.91	64
KRC150 & F-strain	"	2.14±0.74	2.5±0.30	90
B26 &K2	"	2.67±0.03	2.67±0.27	256
B26 &K3	"	2.67±0.03	2.16±0.08	64
B26 &KRC144	"	2±0	2.29±0.198	64
TC1 & WC28	"	3.05±1.2	2.58±0.08	45
A278 & B16	Moderate asymmetrical	∞	2.53±0.24	0
A278 & KRC242	"	∞	3.05±0.339	0
A278 & KRC244	"	∞	2.94±0.24	0
A278 & TC1	"	∞	2.57±0.18	0
B16 & B26	"	3.36±0.48	∞	0
B16 & K3	"	2.33±0.07	∞	0
B16 & KRC139	"	∞	2.78±0.29	0
B16 & KRC242	"	∞	3.08±0.46	0
B16 & KWC30	"	∞	3.07±0.44	0
K2 & K11	"	∞	2.25±0.14	0
K2 & KRC123	"	∞	2.16±0.08	0
K2 & KRC144	"	∞	3.2±0.18	0
K2 & KWC30	"	∞	3.56±0.52	0
K2 & WC228	"	3.72±0.14	∞	0
K2 & F-strain	"	2.17±0.08	∞	0

Appendix 4 continued

Isolate	Relationship	D_1	D_2	r-value
K3 & KRC123	Moderate asymmetrical	∞	2.49±0.35	0
K3 & KRC242	"	∞	3.67±0.19	0
K3 & KWC30	"	∞	2.48±0.15	0
K3 & TC1	"	2.89±0.11	∞	0
K11 & K12	Moderate asymmetrical	2.93±0.24	∞	0
K11 & KRC108	"	2.25±0.144	∞	0
K11 & KRC123	"	2.25±0.144	∞	0
K11 & KRC144	"	2.93±0.24	∞	0
K11 & F-strain	"	3.5±0.76	∞	0
K12 & K13	"	2.03±0.03	∞	0
K12 & KWC30	"	∞	2.67±0.08	0
K13 & KRC244	"	∞	2±0.38	0
K13 & KWC30	"	∞	2.67±0.08	0
K13 & TC1	"	∞	2.23±0.51	0
KRC139&KRC244	"	∞	3.5±0.76	0
KRC139&KWC30	"	∞	3.56±0.56	0
KRC144&KRC150	"	∞	2.5±0.09	0
KRC150 & TC1	"	2.5±0.98	∞	0
KRC242&KRC244	"	∞	2.94±0.24	0
KRC242&KWC30	"	∞	3.5±0.09	0
B26 & K11	"	∞	2.25±0.14	0
B26 & KRC139	"	∞	2.3±0.28	0
B26 & KWC30	"	∞	2.67±0.08	0

Appendix 4 continued

Isolate	Relationship	D_1	D_2	r-value
B26 & WC28	Moderate asymmetrical	2.67±0.03	∞	0
B26 & KRC244	"	∞	2.14±0.24	
B16 & KRC108	Slight asymmetrical	4.5±0.5	∞	0
B16 & KRC244	"	00	4.27±0.49	0
K11 & WC28	"	6.5±0.176	∞	0
B16 & K11	No relatioship	∞	∞	0
K2 & KRC150	"	∞	∞	0
KRC123&KRC242	"	∞	∞	0
KRC123&KRC244	"	∞	∞	0
KRC123&KWC30	"	∞	∞	0
KRC150&WC28K	"	∞	∞	0
KRC242 & TC1	"	∞	∞	0
KRC244 & TC1	"	∞	∞	0

Appendix 5: Seroprevalence of Newcastle disease in village poultry in Kenya

			Avian Paramyxoviruses							
Titres	LaSota	NDV-L	1	2	3	4	6	7	8	9
1:2	B33 B80 KR46 KR129 KR137	B80 B117 B136 KR5	-	B37	B31 KR129	B49 B67	KR27	B60 B64 B136	B73 W32	-
1:4	B52 B60 B88 KR57	B31 B60 KR46 KR50 KR53 KR64 KR66 KR68 KR78 KR82 KR86	B3 B67 B71 B136 W17 W32	B67 KR70	B3 KR123	KR7 KR46 KR63 KR98	B105 B142 KR32 KR63 KR59 KR74 KR81 KR85	B17 B20 B21 B31 B33 B53 B67 KR5 KR20 KR27 KR28 KR46 KR59	B67 KR122 KR123 KR129 W19	B6 B67 B105
1:8	B3 B64 B67 B73	B3 B20 B21 B67 B73 B81 B83 KR13 KR70	B20 B72 B76 B110 KR32	KR7 KR32 KR66 KR68 KR109 KR128 KR129 KR137	B17 B67 B73 B110 KR32	KR5 KR101	B60 B73 KR19 KR46	B49 B51 B52 B133 KR7 KR142	B72	B20 KR7
1:16	B20 B21 B31 B34 KR70 W17	B69 B72 W17	W19	B72 KR46 KR78 KR82 W19	B20 B142 KR5	B85 KR55 KR108	B64 B67 B136 KR7 KR28	B37 B134 KR120	-	-

Appendix 5 continued

			Avian Paramyxoviruses							
Titres	LaSota	NDV-L	1	2	3	4	6	7	8	9
1:32	B37 B104 W19	B37 W1 W19	B37 B104 KR4 KR131 W1	-	-	B139	-	KR57	-	-
1:64	B6 KR15 KR131 W1	B56 B104 KR131	B6 KR15	-	B37 W17	-	B17 B31	-	B31	-
1:128	-	-	-	-	KR139 W1	-	-	-	B17	-
1:256	-	B6	-	-	W19 B6	-	-	-	B21	-

-:No sera inhibited the virus at that titre.

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