Intracerebral pathogenicity index (ICPI) for virulent strains

Procedure

1. Ten 1-day –old chicks were use.
2. The birds were examined at intervals of 24 h for 8 days.
3. At each observation, each bird was scored: 0=normal, 1=sick, 2=dead. Birds that were alive but unable to eat or drink should were killed humanely and scored as dead at the next observation. Dead individuals were scored as 2 at each of the remaining daily observations after death.
4. The index was calculated as shown in the following example. A sample method for recording results and calculating indices is shown in the Table below:

Table 9. Determination of ICPI for virulent isolates

Clinical signs	Days after inoculation Number of chickens with specific signs								Total score	
	1	2	3	4	5	6	7	8		
Normal	3	0	0	0	0	0	0	0	3X0	= 0
Sick	7	0	0	0	0	0	0	0	7X1	= 7
Dead	0	10	10	10	10	10	10	10	70X2	= 140
									Total = 147/80	
									ICPI=1.83	

10 birds observed for 80 days= 80 observations

Index= mean score per bird per observation = …../80=

Any APMV-1 yielding a value of 0.7 or greater was considered to be a virulent ND virus

The ICPI is the mean score per bird per observation over the 8-days period. The most virulent isolates have an ICPI close to 2.0, lentogenic and asymptomatic enteric viruses have values of 0.0-0.6.

Intracerebral pathogenicity index (ICPI) for avirulent strains

Procedure

1. Ten 1-day –old chicks were used.
2. The birds were examined at intervals of 24 h for 8 days.
3. At each observation, each bird was scored: 0=normal, 1=sick, 2=dead. Birds that were alive but unable to eat or drink were killed humanely and scored as dead at the next observation. Dead individuals were scored as 2 at each of the remaining daily observations after death.
4. The index was calculated as shown in the following example. A sample method for recording results and calculating indices is shown in the table below:

Table 10. Determination of ICPI for avirulent isolates

Clinical signs	Days after inoculation Number of chickens with specific signs								Total score	
	1	2	3	4	5	6	7	8		
Normal	10	10	10	10	9	8	7	7	71X0	= 0
Sick	0	0	0	0	1	1	1	0	3X1	= 3
Dead	0	0	0	0	0	1	2	3	6X2	= 12
									Total=15/80	
									ICPI=0.1	

10 birds observed for 80 days= 80 observations

Index= mean score per bird per observation = …../80=

Any APMV-1 yielding a value less than 0.7 was considered to be an avirulent ND virus.

The ICPI is the mean score per bird per observation over the 8-day period. The most virulent isolates have an ICPI close to 2.0, lentogenic and asymptomatic enteric viruses have values of 0.0-0.6.

Table (11) Fate of the virus in experimentally infected chickens

Organ from virulent strain	**Days post-inoculation**				**Organ from Avirulent strain**	**Days post-inoculation**			
	1	**2**	**3**	**4**		**1**	**2**	**3**	**4**
	Virus titer					**Virus titer**			
Bursa	-	-	64	32	Bursa	-	-	-	-
Spleen	-	-	64	8	spleen	-	-	-	-
Lung	-	-	-	128	Lung	-	-	-	-
Bone marrow	-	-	64	8	Bone marrow	-	-	-	-
Trachea	-	-	8	256	Trachea	-	-	-	-
Cecal Tonsil	-	-	256	256	Cecal Tonsil	-	-	-	-
Kidney	-	-	-	4	Kidney	-	-	-	-
Brain	-	-	32	8	Brain	-	-	-	-
Intestine	-	-		-	Intestine	-	-	-	-

Table (12) HA activity and elution time using chicken and horse RBCs at room temperature

Isolate No.	Virus titer	HA activity for chicken RBCs	HA activity for horse RBCs	Elution time for chicken RBCs per min.	Elution time for horse RBCs per min	Pathotype
1	256	+	-	15 min	-	Velogenic
2	512	+	+	130 min	240 min	Lentogenic
3	64	+	+	10 min	5 min	Lentogenic
4	256	+	+	200 min	630 min	Lentogenic
5	256	+	+	155 min	-	Lentogenic
6	1024	+	-	180 min	333 min	Lentogenic
7	128	+	+	175 min	333 min	Lentogenic
8	256	+	+	5 min	5 min	Lentogenic
9	1024	+	+	175 min	5 min	Lentogenic
10	16	+	+	5 min	5 min	Lentogenic
11	64	+	+	10 min	5 min	Lentogenic
12	512	+	+	5 min	5 min	Lentogenic
13	128	+	-	-	-	Velogenic
14	512	+	-	-	-	Velogenic
15	128	+	-	-	-	Velogenic
16	128	+	-	-	-	Velogenic
17	64	+	-	-	-	Velogenic
18	64	+	-	-	-	Velogenic

3.8. Microscopic findings

The chickens used for experimental inoculation were divided into three groups: group 1and 2 inoculated by the virulent strain; they died in day 3 and day4 p.i respectively whereas chicken in group 3 inoculated by the avirulent strain died in 7 days p.i. Virus inoculation produced a variety of pathological changes for all groups.

3.8.1. Group 1

3.8.1.1. Brain

The changes in the brain consisted of moderate proliferation of glial cells in cerebellum and cerebral hemispheres in the form of scatter gliosis, dilation of Virchow-Robin space and neuraldegenaration, shrunken and clumping of their nuclear material. In addition there was focal meningitis with detachment of piamater and mild infiltration of lymphoid cells vacuolation of cerebral cortex and cerebellum foli and peducle and extravastation erythrocytes.

3.8.1.2. Lung

Showed interstitial pneumonia; the most prominent changes included thickening of the alveoli septa with infiltration of lymphoid cells, congestion and hemorrhages.

3.8.1.3. Trachea

This organ revealed degeneration, vacuolation of the lining epithelium and focal sloughing of the mucosa. There were aggregates of lymphoid cells infiltration in subepithelial area and serosa.

3.8.1.4. Kidney

Displayed glomerulo-interstitial nephritis; there were a tropic collapsed glomerular tufts and marked dilation of Bowman' space necrosis and

fragmentation of tubule cells and intense infiltration of lymphoid cells in the interstitial connective tissue

3.8.1.5. Bursa of fabrisus

This organ revealed proliferation of the epithelial lining which later formed gland-like structure, oedema and mononuclear cell infiltration and subepithelial and interfolicular area. Degeneration with formation of central cavities in medulary zone of the follicles was scarcely observed.

3.9 Group 2

The pathological changes in the organs brain, lung, trachea and kidney were same as in group 1, with the exception of bursa of fabricus which showed oedema, lymphatic infiltration in sub-epithelial and interfolicular areas. There were mild thickening and vacuolation of the lining epithelium.

3.10. Group 3

3.10.1. Brain

The changes in the brain included proliferation of glial cells in the cerebellum and cerebral hemispheres forming focal and scattered gliosis which was more prominent; dilation of Virchow –Robin spaces and neuronal degeneration was evidenced by clumping of nuclear and shrunken of the cells in same area. There were focal infiltrations of lymphoid cells in meninges which often detached and collapsed of many blood vessels. Degeneration and vacuolation of cerebral cortex and cerebellar peduncle degeneration and vacuolation of Purkinje cell layer were regularly encountered.

3.10.2. Lung

Interstitial pneumonia with profuse infiltration of lymphoid cells in the alveolar septic around the secondary and tertiary bronchi was seen. In some

areas the lung architecture was obliterated and normal lung tissue was replaced by the infiltrated lymphoid cells which often formed lymphoid follicle. Oedema and emphysema with rupture of alveolar septa and dilation of air pouches were observed.

3.10.3. Trachea

There were aggregation of lymphoid cells in the subepithelial area and focal detachment of tracheal mucosa.

3.10.4. Kidney

Glomerulo-interstitial nephritis, changes in the kidney included accumulation of lymphoid cells in the interstitial connective tissue, detachment of tubular-epithelial from basement membrane which was discontinued in some tubules, glomerular segmentation, fragmentation and dilation of Bowman capsule. In some areas the kidney tissue was effaced by intense and diffuse lymphoid cells.

3.10.5. Bursa of fabricus

This organ displayed interfolicular and subepithelial oedema and infiltration of mononuclear cells; the lining epithelium revealed proliferation degeneration. In severely affected areas there were extensive proliferation of follicular lymphoid cells and some follicular cells coalesced together; they lost the normal structure forming large mass of lymphoid cells.

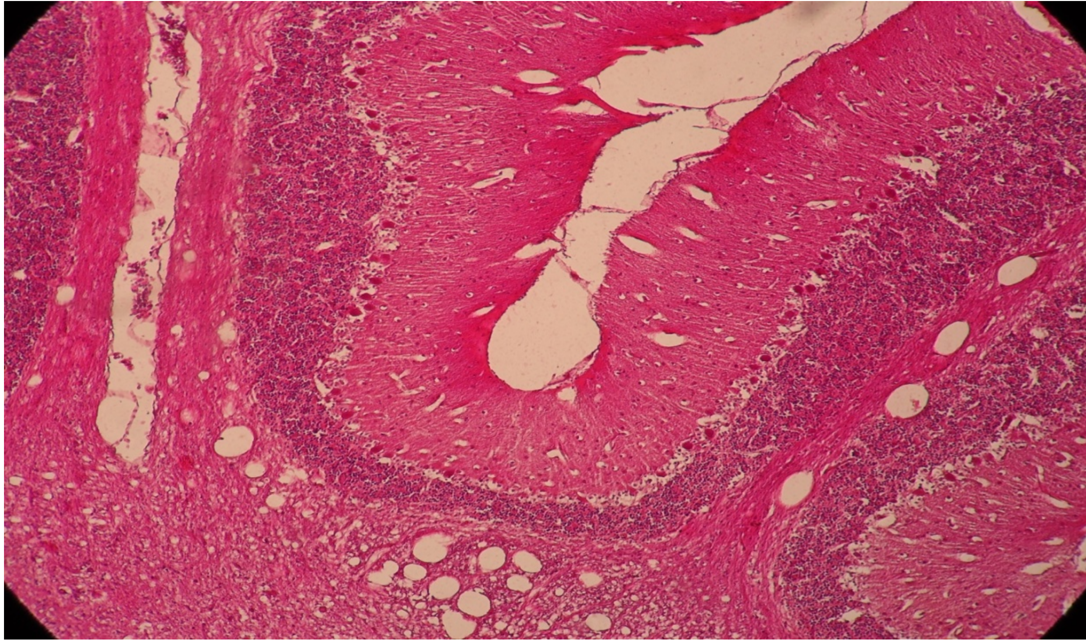

Figure 18: Section of brain in group 1, inoculated with a virulent ND strain .Note vacuolation of cerebellum .H&E x100

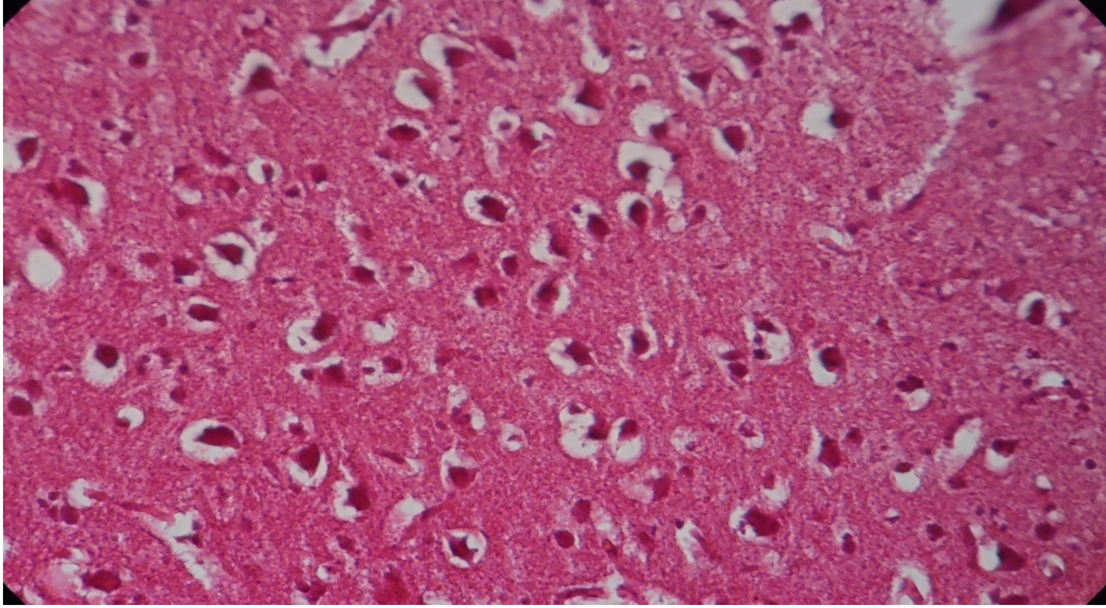

Figure 19: Section of brain in group 2, inoculated with a virulent ND strain. Note degenerated neuron and dilated Virchow –Robin space. H&Ex100

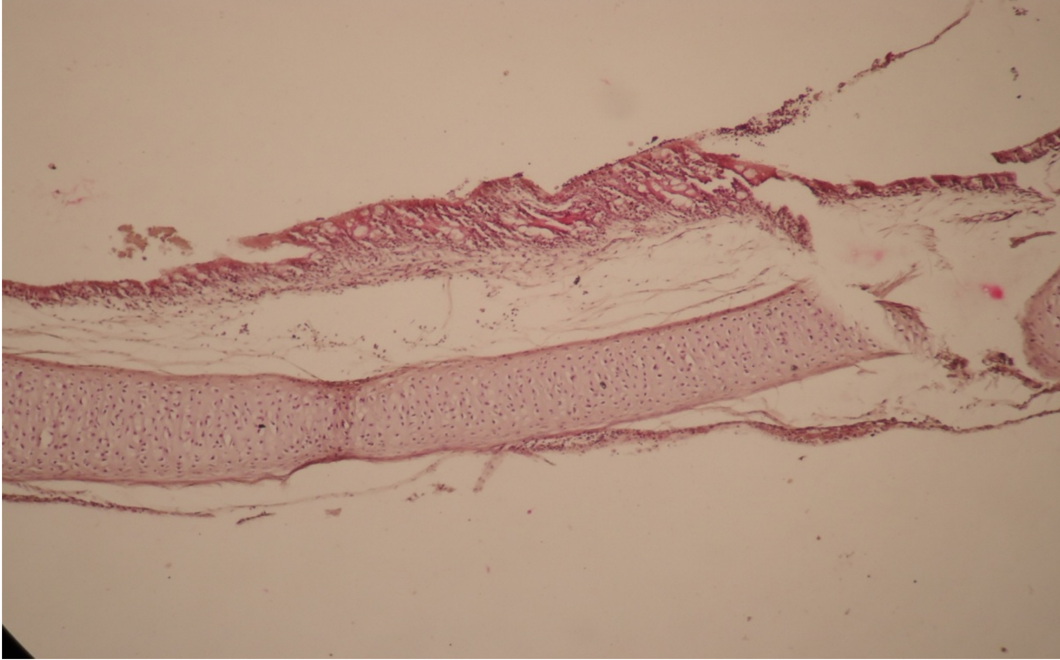

Figure 20: Section of trachea in group1, inoculated with a virulent ND strain .Note, desquamation of the mucus membrane and infiltration of aggregates of mononuclear cells .H&Ex40.

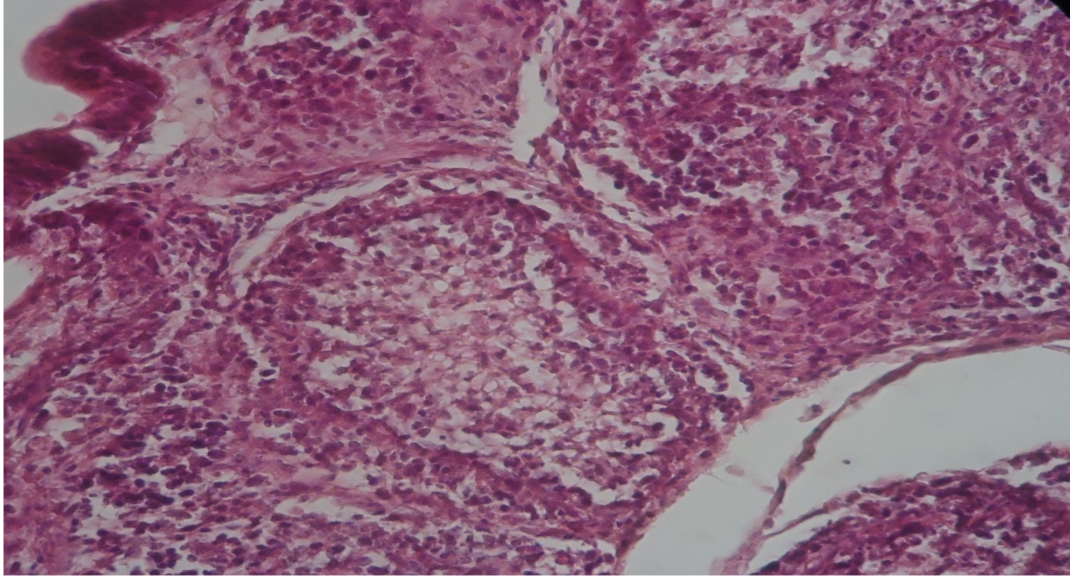

Figure 21: Section of bursa of fabrisus in group 1 inoculated with a virulent ND strain .Note degeneration and necrosis of the medulary zone of follicles. H&Ex100.

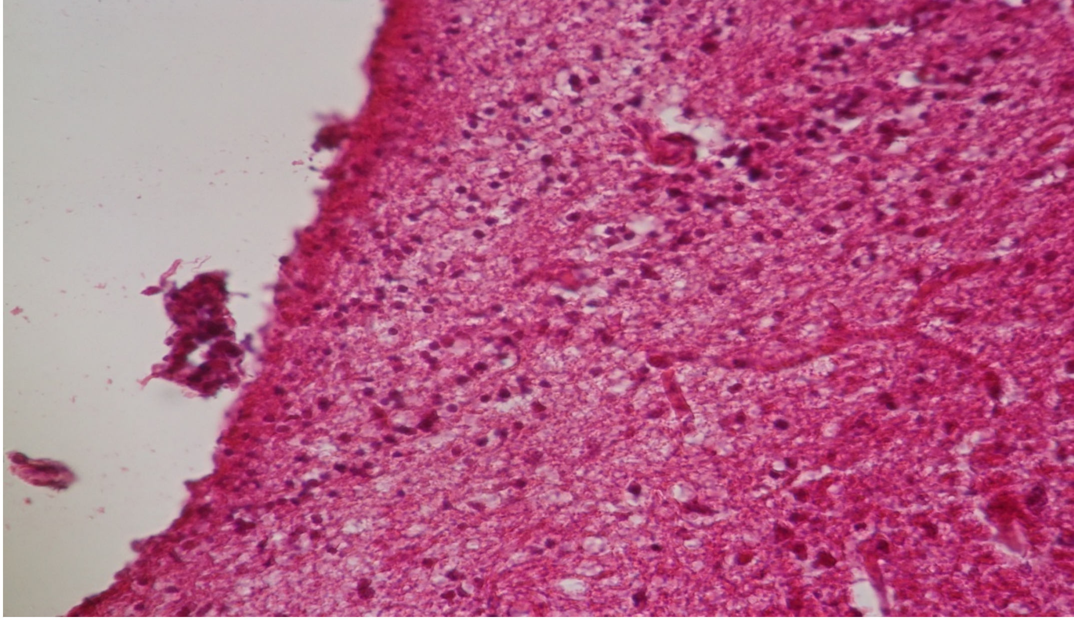

Figure 22: Section of brain in group 3 inoculated with an avirulent ND strain. Note diffuse gilais and desquamation of meninges .H &Ex 100.

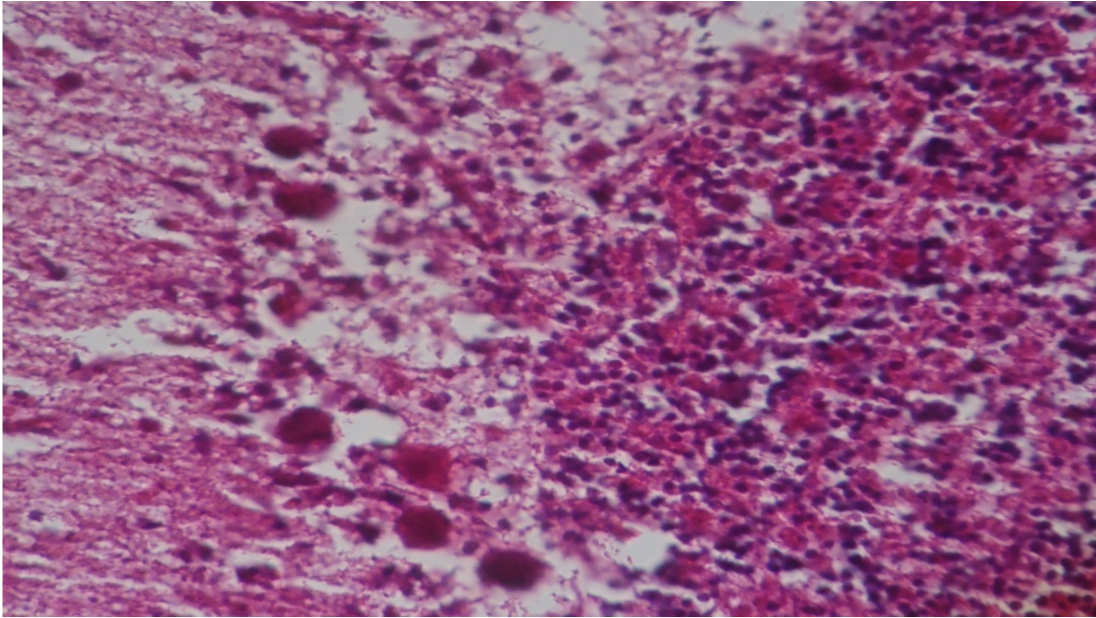

Figure 23: Section of brain in group 3 inoculated with an avirulent ND strain. Note necrosis and vacuolation of Purkinje cell layer, gliosis and degeneration of Purkinje cells. H &Ex100.

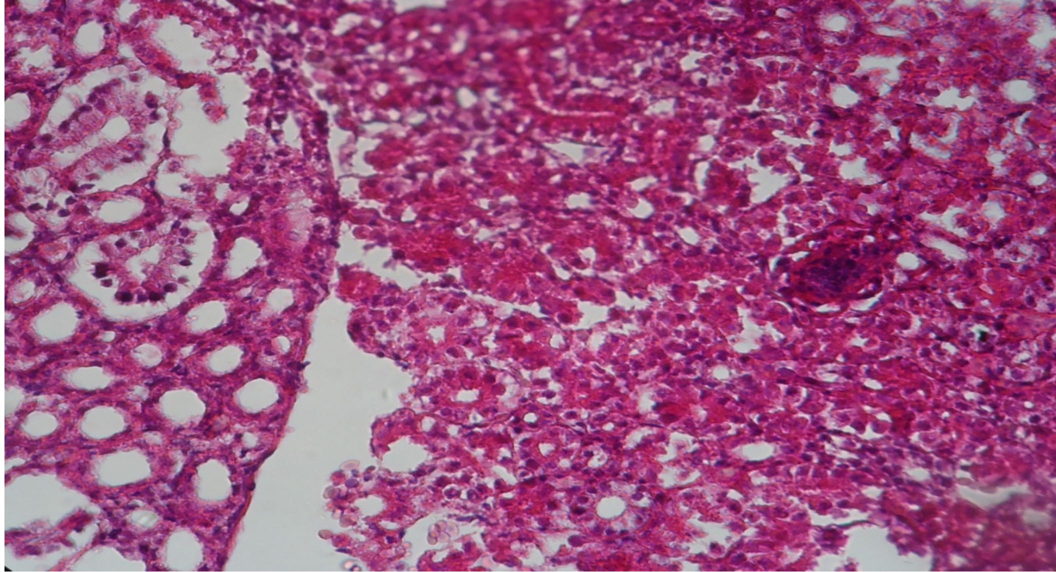

Figure 24: Section of lung in group 3 inoculated with an avirulent ND strain. Note intense infiltration of lymphoid cells, lymphoid follicles formation .H &Ex100.

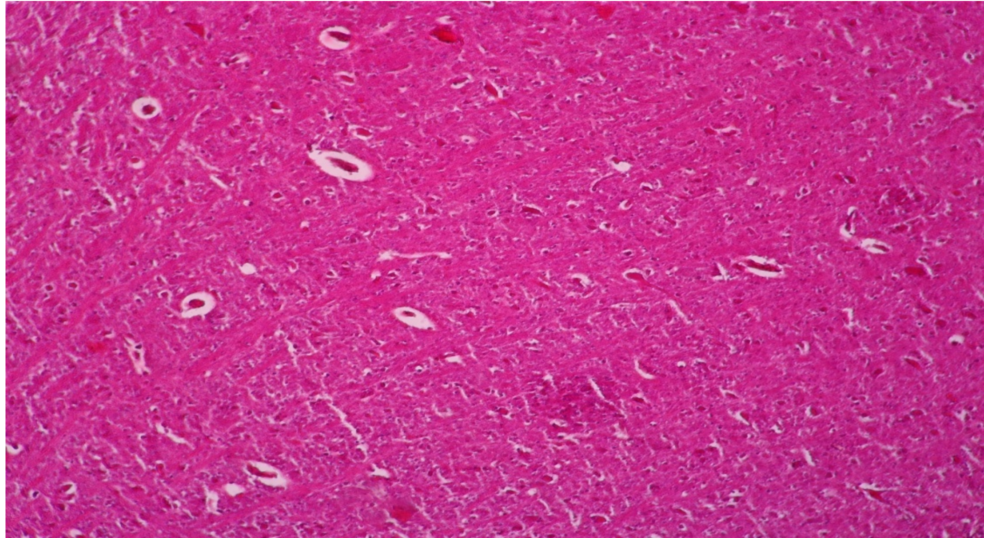

Figure 25: Section of kidney in group 3 inoculated with a virulent ND strain. Note tubular degeneration, lymphoid cell infiltration. H&Ex100.

CHAPTER FOUR

DISCUSSION

NDV is a serious disease of birds that kills up to 80% of unvaccinated poultry .Outbreaks of the disease occurred in all over the world. In Sudan it had been reported from all regions of the country with a highly virulent nature and heavy losses among both exotic and indigenous chicken breeds (Karrar and Mustafa, 1964).

In view of the fact that the velogenic viscerotropic type of the disease predominates in the Sudan and due to the high economic losses encountered, ND

is nowadays listed as a notifiable disease. In spite of intensive vaccination still there were many outbreaks, one during 2003-2006 and another during 2006-2008 from which samples were examined in this present study.

The aim of this study was to test the biological properties of NDV strains using conventional methods as well as nucleic acid- based techniques. The HA tests were performed for detection and identification of the isolates; the positive samples were neutralized by known antisera. As shown in the results, all virulent strains were found positive for HA activity by using both chicken red blood cells (CRBCs) and horse red blood cells (HRBCs), while avirulent strains were found positive for HA activity by (CRBCs), but negative for HA activity by (HRBCs).

The virulence of these isolates was assessed by the intracerebral pathogenicity index (ICPI) test. The average index values obtained were 1.83 for virulent strains and 0.1 for avirulent ones; this method was officially recognized for APMV-1 pathotyping by the World Organization for animal health –OIE, (OIE, 2008). Moreover, the (ICPI) is considered the most sensitive "in vivo test" for pathotyping by many investigators (Terregino and Capua, 2009).

The conventional method was used for the preparation of chick-embryo fibroblasts (CEF). When confluent (CEF) monolayer was inoculated with virus dilutions containing 10^{3} ELD^{50} per 25µl, CPE was observed 24-48 hours p.i. in all isolates. For plaque production, when 10^{-6}-10^{-10} virus dilutions were inoculated in primary cell culture, plaques were not seen after incubation for many days. Hence, when secondary (CEF) monolayers were prepared and were inoculated with higher virus dilutions from 10^{8-}_10^{-12} and bovine serum albumen was added to the virus suspensions, the virulent viruses had been successfully adapted after the second passage in chick –embryo fibroblast

forming different sizes of plaques. This was in agreement with Omaima *et al.*, (2005a). In a previous similar work, small, medium and large plaques could be obtained in CEF monolayers only when virulent NDV strains were inoculated (Omaima *et al*., 2005b).

In this study the 18 positive isolates revealed amplicons of fragment size 298 bp for virulent and a virulent NDVs, (Figures (4) and (5).

Since the conventional methods of virus isolation are usually slow and depend on the viability of the virus in the sample many molecular- based techniques have been developed for diagnosis of NDV.

Real time reverse transcriptase PCR-(RRT-PCR) has several advantages such as speed and elimination of the possibility of cross contamination of new samples with previously amplified products because the sample tube is never opened after PCR. RT-PCR was used for detection of APMV-1 from extracted RNA, by using probes for the detection of NDV. Jarecki Black and King (1993) reported a radiolabelled oligonucleotide probe for distinguishing virulent viruses from avirulent ones. In this test the RRT-PCR product was detected with a sequence- specific probe and there was a grantee that the correct target was amplified which reduces the chance of false positives. Another method was used to detect the virulence of NDV strains by determination of the presence or absence of multiple basic amino acids (lysine-L, or arginine R) located at the C-terminus of the FO protein and phenylanine F at the N-terminus of the F2 protein which correspond to the cleavage site of the precursor FO glycoprotein (Position 112-117) (OIE, 2008).

In this study it was found that the cleavage site of all virulent strains contained the ^{112} RRQKRF^{117} motif, similar to the sequence in the study

conducted by Mahasin *et al* ., (2005); in Genbank AJ243391 amino acid of the fusion protein cleavage site (FO) was: 112 $RRQKRF^{117}$ and was an indication for high virulence. These findings were similar to those described by Wegdan *et al*., (2010).Other Sudanese viruses such as Chicken/Sudan/Obied/ 87 strains have the same cleavage site of $^{112}RRQKRF^{117}$. In a relative study conducted by Mahasin *et al.,* (2005), 3 samples were confirmed positive for NDV by RP-PCR analysis; two of these samples were found avirulent while one was diagnosed as virulent. After alignment and comparison of their sequences in a phylogenetic tree, the two avirulent viruses appeared to be of the same type as they were closely related to a group of avirulent viruses that include the South African Avinew vaccine strain and the Queensland V4 virus. Their FO cleavage site sequence was avirulent, and they do not cause serious disease in chickens. On the other hand, the third virus was closely related to the Kuwait C virulent strain which is responsible for disease outbreaks. It was also observed that all these viruses had a maximum sequence similarity of 94% to any other ND sequence submitted to Genbank and were therefore considered as unique to the Sudan. These facts led to both an increasing number of groups using nucleotide sequencing as their research tool, following the early work with the same amino acid motifs which was confirmed by Collins *et al*., (1993) for Essex 70 strain; others used nucleotides as tools for pathotyping viruses, with the same motif $^{112}RRQKRF^{117}$. (Zorman *et al*., 2002), for pathotyping of the Slovene PMV-1chicken isolate and for 135/93 strain (Oberdorfer and Werner, 1998)

In the present study the sequence of avirulent fusion protein cleavage site (FO) motif $^{112}GRQGRL^{117}$ was in agreement with the findings reported by Collins *et al*., (1993) and by Flavia *et al*., (2005). The Sudanese virulent strains

show close relationship to other Africa isolates (Chicken /1377-8/ Niger 2006). (Chicken/1910-11|Mauritania, 2006), (NDV-3724-6 Nigeria 2008) (FM 200808 Burkinafaso).

Field studies conducted during the period of 2003 -2006 in the Sudan investigated and used a phylogenetic analysis of the fusion protein gene of genotype 5. In this present study the phylogenetic analysis results agreed with the above findings which indicated that the velogenic isolates (no.1, 5 and from13 to18) all clustered in lineage 5.This linage appears to be the dominant linage in Asia and other areas of the world (e.g. Eastern Africa) and is the main type responsible for the current outbreaks of ND worldwide. The isolates collected in this study were very much related to isolates of sublineages 5d described previously, hence, the majority of NDVs responsible for the recent outbreaks in layer flocks in Sudan in the period 2003-2006 were caused by these viruses which belong to the same lineage, (Wegdan *et al*., 2010).These viruses included (GQ258675.1Chicken /Sudan 0812004(F), GQ258671-1 chicken/Sudan/o42003, GQ258669-1/chicken/Sudan/02 2005, GQ258670-1/chicken Sudan/03/2003(F), GQ258673-1/chicken Sudan.0612006 (F), GQ 258672.1/chicken Sudan/05/2004 (F).On the other hand, the strain GQ258674. 1/chicken/Sudan/Obeid 1987 (F) fell within the lineage 4.

This study revealed that all avirulent isolates were lentogenic or mesogenic strains (no.2, 3, 4 and from 6 to 12) and belonged to lineage 2, causing no serious disease in chickens. They were most probably the circulating strains used in live vaccines and when they were matched with NDV Sudan vaccines it was clear that all of these vaccines have been confirmed as vaccine strains because they belonged to lineage 1and 2 of class1 ND virus..

Field recent investigation by Cattoli *et al*., (2009) in which result from phylogenetic analysis indicated that there is a new genetic lineage circulating in West and Central Africa which was different from the lineage 5 described in the Eastern and Southern parts of the continent. This new novel genetic lineage assigned to lineage 7 included (GR.7C. FM 200808 chicken Burkina Faso/S, GR.7DAPMV-1CK/1377-8/Niger/2006,GR.7A.APM1/CK11910-11/ Mauritania /2006, GR. 7B. NDV-3724-6 Nigeria 2008). This occurred due to the mismatches observed in the segment of the matrix gene targeted by the primers and probe designed for the molecular detection of APMV-1, which were responsible for the false negative results in the diagnostic tests conducted.

Phylogenetic analysis, therefore, remains an important tool to determine the evolving status of ND in Africa and other regions, to trace the sources of infection, to these countries in taking decisions to improve their disease control efforts.

To conclude, the present investigation provided important information on the epidemiology, diagnosis and control of NDV in Sudan, and highlighted the importance of supporting surveillance in developing countries for trans-boundary animal diseases. It also proved that Sudan is free from this new novel genetic lineages, but diversification of the recent epizootic viruses and lineages has accelerated in the last few decades and suggested that vaccination could play a role in the generation and spread of the most recent virulent viruses.

CHAPTER FIVE

Conclusion and Recommendations

Conclusion

– It could be concluded that all forms of Newcastle disease virus, virulent and avirulent could be found with the highly pathogenic type predominating, causing high mortality rates in all age groups of chickens.

– The avirulent strains contain both mesogenic and lentogenic forms.

– Diagnosis of the disease was performed using conventional methods i.e. propagation and isolation in chick-embryos and identification by HA and HI tests.

– Advanced diagnosis and confirmation was performed by using novel nucleic acid-based techniques including RT-PCR, RRT-PCR. All samples diagnosed as positive by conventional methods were also confirmed and gave the same result by PCRs methods.

– Molecular characterization using genome sequencing and phylogenetic analysis revealed that the virulent –Sudan viruses belong to the velogenic viscerotropic strain that fell within linage 5d that was circulating in South Africa since late 1990s.

– It was also confirmed that Sudan is free from the newly discovered linage 7 genotype which was isolated from West and Central Africa.

– Study of vaccine and local isolates matching revealed that clone 30 vaccines LaSota strain ceva new and Komorov belonged to linage 2 and are suitable for vaccination in Sudan.

– Biological characterization investigating the LD50, MDT/MLd, ICPI, IVPI revealed the presence of both virulent and avirulent isolates.

– The viruses could be propagated readily in cell culture producing clear CPE in 24-28 hours p.i.

– Plagues in cell culture could be obtained only by virulent and mesogenic strains: hence this method could be used for differentiation between virulent and avirulent strains.

– Also hemagglutination activity by use of chicken and horse red blood cells could be used to differentiate between the two types of the virus in that only velogenic strain could agglutinate horse red blood cells.

– When spread of experimentally infected viruses in chickens was investigated it was found that the virulent type could be recovered from all organs including brain, spleen, trachea, kidney, cecal tonsils. This confirmed that the virus is pantropic in nature.

– Histopathological investigation showed that widespread changes were observed in many organs.

Recommendations

– Use of nucleic acid - based techniques are the methods of choice for confirming diagnosis of NDV; yet this does not eliminate the importance of conventional methods of diagnosis which should be available in all laboratories.

– Knowledge of the types of ND viruses is essential to decide control strategies to be applied. Many techniques can be applied for this purpose i.e. biological and molecular characterization of the virus. Hence matching between vaccines used in the country and the strains circulating is important to choose the most appropriate vaccine strains for vaccination.

– Vaccination, which is the main method of control of spread of the disease, should be enforced so that control and eradication can be made possible.

References

Abenes, G.B., Kida, H. and Yanagawi, R. (1986). Biological activities of monoclonal antibodies to hemagglutinin. Neuraminidase (HN) protein of NDV. Jap. d. Vet. Sci., **48**:353-362.

Aldous, E.W. and Alexander, D.J. (2001). Technical review: detection and differentiation of Newcastle disease virus (Avian paramyxovirus type 1). Avian Pathol, **30**(2): 117-129.

Aldous, E.W., Ynn, K.K., Banks, J. and Alexander, D.J. (2003). A molecular epidemiological study of avian paramyxovirus type 1 (Newcastle disease virus) isolates by phylogenetic analysis of a partial nucleotide sequence of the fusion protein gene. Avian Pathology. **32**(3): 239-256.

Alexander, D.J. (1991). Newcastle disease and other paramyxovirus infections. In: Disease of Poultry, 9th ed. B.W. Calnek, H.J. Barnes, C.W. Beard, W.M., Reid and H.W. Yoder, Jr. eds.

Alexander, D. J. R. J. Manvell, J .P. K .M. Frost, M.S. Collins, P. H. Russel, and J. **E. Smith (1997)**. Antigenic diversity and similarities detected in Avian paramyxovirus type 1 (Newcastle disease virus) isolates using monoclonal antibodies .Avian Pathol 26:399-418. .

Alexander, D.J. (1980). Avian paramyxoviruses. Vet. Bull., **50**:737-752.

Alexander, D.J. (1988). Newcastle disease virus –An avian paramyxovirus. In: Newcastle disease. Alexander D.J. ed. Kluwer academic Publishers. Boston MA. Pp. 11-22.

Alexander, D.J. (2000). Newcastle disease in ostriches (*Struthio camelus*). A review Avian Pathol., **29**:95-100.

Alexander, D.J. (2001). Gordon Memorial lecture Newcastle disease. Br. Poult. Sci., **42**(1):5-22.

Alexander, D.J. (2008). Newcastle disease World Organization for Animal health manual of diagnostic tests and vaccines for terrestrial animals 6th ed. chapter 2.3.14. OIE Paris pp. 576-589.

Alexander, D.J. and Allan, W.H. (1974). Newcastle disease virus pathotypes. In: Avian Pathol., **3**:269-278.

Alexander, D.J. and Collins, M.S. (1981). The structural polypeptides of avian paramyxoviruses. Arch. Virol., **67**:309-323.

Alexander, D.J. and Parason, G. (1986). Pathogenicity for chickens of avian paramyxovirus type isolated obtained from pigeons in Great Britain during 1983-1985. Avian Pathol., **15:**487-493.

Alexander, D.J. Parason, G. and Marshall, R. (1984a). Infection of fowls with Newcastle disease virus by food contamination with pigeon faeces. Vet. Rec., **115**:601-602.

Alexander, D.J. Russell, P.H., and Collins, M.S. (1984b). Paramyxovirus type infections of dancing pigeons: Characterization of isolated viruses. Vet. Rec., **114**(18): 444-446.

Alexander, D.J., Banks, J. Collins, M.S. Manvell, R.J., Frost, K.M. Speidel, E.C. and Aldous, E.W. (1999). Antigenic and genetic characterization of Newcastle disease viruses isolated from outbreaks in domestic fowl and turkeys in Great Britain during 1997. Vet. Rec., **145:**417-421.

Alexander, D.J., Kemp, P.A., Parsons, G., Collins, M.S., Brockman, S., Russell, P.H. and Lister, S.A. (1987). Used of monoclonal antibodies in the characterization of avian paramyxovirus type 1 (Newcastle disease

virus). Isolates submitted to an international reference laboratory Avian Pathology. **16**:553-565.

Alexander, D.J., Russell, P.H., Parsons, G. and Abu Elzein, E.M.E., Ballough, A., Cernik, K., Engstrom, B., fevereiro, M., Fleury, H.J.A., Guittet, M., Kaleta, E.F., Kiham, U. Kosters, J., Lomniczi, B., Meister, J., Meulemans, G., Nerome, K., Petek, M., Pokomunsle, S., Polten, B., Prip, M., Richter, R., Saghy, E., Samberg, Y., Sparoghe, L. and Tumova, B. (1985). Antigenic and biological characterization of avian paramyxovirus type 1 isolates from pigeons- an. International collaborative study. Avian Pathol., **14**:365-376.

Allan, W.H. and Gough, R.E. (1976). A comparison between the haemagglutination inhibition and complement fixation tests for Newcastle disease. Res. Vet. Sci., **20**: 101-103.

Allan, W.H., Lancaster, J.E. and Toth, B. (1978). Newcastle disease vaccines. Their production and use FAO Animal Production and Health series No. 10 FAO: Rome, Italy.

Anon (1990). Annual report of Avian Pathology Department Central Veterinary Research Laboratories, Soba, Sudan.

Anon (1951). Annual Report Veterinary Service Sudan (1950-51).

Balloh, A. Abuelezein, E.M.E. and ElMubark, A.K. (1985). Outbreak of the pigeon paramyxovirus sera-type in the Sudan. Vet. Rec., **116**: 375.

Balloh, A.A., Noyil, A. and Ali, B.H. (1983). Pathotypes of NDV isolates from the Sudan. Sud. J. Vet. Soc. And Anima, Husb. , **24**:67:78.

Banerjee, M., Reed, W.M., Fitzgerald, S.D. and Panigrahg, B. (1994). Neurotropic velogenic Newcastle disease in cormorants in Michigan: Pathology and virus characterization. Avian Dis., **38**:873-878.

Barahona, H. H. and R. P. Hanson. (1968). Plaque enhancement of Newcastle Disease virus (Lentogenic strains) by magnesium and diethylaminoethyl dextran. Avian Dis 12: 151-158.

Beach, J.R.C. (1942). Avian pneumonencephalitis. Processing: of the annual meeting of the US livestock sanitary association **46**: 203-223.

Beach, J.R.C. (1944).The neutralization in vitro of avian pueumonencephalitis virus by Newcastle disease immune serum. Science 100 (2599):361-362.

Beard, C.W. and Hanson, R.P. (1984a). Newcastle disease, In: Disease of poultry. 8th ed. Edited by Hofstad, M.S., John, H., Calnek, B.W., Reid, W.M., Yoder, H.W. Editorial Board for the American association avian pathologists, pp. 452-470.

Beard, C.W. and Hanson, R.P. (1984b). Newcastle disease. In M.S. Ofstad, H.J. Barnes, B.W. Calnek, W.M. Reid, H.W. Yoder (eds). Disease of poultry. 8th ed., Iowa State university press: Ames, IA, 452-470.

Beard, P.D. Spalatin, P.D. and Hanson, R.P. (1970). Strain identification of NDV in tissue culture. Avian Dis., **14**:636-545.

Beaudette, F.R. and Black, J.J. (1946). Newcastle disease in New Jersey. Proc. Annu. Meat US livest. Sanit. Assoc., **49**:49-58.

Beer, J.V. (1976). Newcastle disease in the Pheasant, Phasianus colchicus, in Britain. In I.A. page (ed.) Wildlife Diseases plenum Press: NY, 423-430.

Biancifiori, F. and Firooni (1983). An occurrence of Newcastle disease in pigeons: virological and serological studies on the isolates. Comp. immunol. Microbiol., infect. Dis., **6:**247-252.

Box, P.G., Helliwell, B.I. and Halliwell, P.H. (1970). Newcastle disease in turkeys Vet. Rec., **86**:524-527.

Bruning-Fann, C., Kaneene, J. and Heamon, J. (1992). Investigation of an outbreak of velogenic viscerotropic Newcastle disease in pet birds in Michigan, Indiana, Illinois, and Texas. J. A. V.. M.. A., **2011**:1709-1714.

Burnet, F.M. (1942). The affinity of Newcastle disease virus to the influenza virus group. Aust. J. Exp. Biol. Med. Sci., **20**:81-88.

Capua, I. Scacchia, M. Toscani, T. and Caporale, V. (1993). Unexpected isolation of virulent Newcastle disease virus from commercial fowl's eggs J. Vet. Med. B., **40**:609-612.

Cattoli, G., and Monne, I. (2009). Molecular diagnosis of Newcastle disease virus. In: Capua, I., Alexander, D.J. (Ed), Avian influenza and Newcastle disease, field and laboratory manual. Springer-Verlag. Italy. Pp. 127-132

Cattoli G., Fusaro A., Monne I., Molia S., Le Menach.,A .,Menach B., Nchaer A., Bangana I., Garba Maina A., N› Goran Koffi J-N., Thiam., Bezeid O.E.M.A., Salviato A., Nisi R., Terregino C., Capua I.(2009). Emergence of a new genetic linage of Newcastle disease virus in West and Central Africa –Implications for diagnosis and Control .VETMIC -4623: No of Pages 9.

Collin ,**M.**S., **Bashiruddin, J.B. and Alexander, D.J. (1993**).Deduced amino acid sequences at the fusion cleavage site of Newcastle disease viruses

showing variation in antigenicity and pathogenicity .Archives of virol.128,363-370.

Collins, M.S., Strong, I. and Alexander, D.J. (1994). Evaluation of the molecular basis of Pathogenicity of the variant Newcastle disease viruses termed pigeon PMV-1 viruses Arch Virol., **134**:403-411.

Czegled, A., Vjvari, D., Somogyi, E. Wehmann, E., Wemer, O., and Lomniczi, B. (2006). Third genome size category of avian paramyxovirus serotype 1 (Newcastle disease virus) and evolutionary implications virus Res., **120**(1-2):36-48.

Daniel, M.D, and Hanson, P, R. (1968).Differentiation of representative Newcastle disease viruses strain by their plaque forming ability on monolayer of chick embryo fibroblast .Avian Dis .12,423-433.

Deleeuw, O. and Petters. B. (1990). Complete nucleotide sequence of Newcastle disease virus evidence for the existence of a new genus within the subfamily paramyxovirinae Journal of General Virol., **80**:131-136.

Doyle, T.H. (1935). Newcastle disease of fowls. J. Comp. Pathol. Therap., **48**:1-20.

Doyle, T.M. (1927). Ahithert, unrecorded disease of fowls due to a filter-passing virus. J. Comp. Pathol. Therap., **40**:144-169.

Eisa, M and Omer, E.A. (1984). A natural outbreak of Newcastle disease in pigcon in the Sudan. Vet. Rec., 114-297.

Estupinan, J. Spalatin and R.P. Hanson (1968). Use of yolk sac route of inoculation for titration of lentogenic strains of NDV. Avian Dis., **12**:135-138.

Fawi, M.T. and Babiker H.A. (1970). Disease of poultry in the Sudan. Proc. Of the 5th Vet. Conf. of the Sudan Vet. Assoc.

Flavia Zanetti, Analia Berinsstein, Ariel Pereda, Oscar Taboga, and Elisa Carrillo (2005). Molecular characterization and phylogenetic analysis of Newcastle disease virus isolates from healthy wild birds. Avian Disease 49:546-550

Fijii, Y., Ssakaguchi, T., Kiyotani, K. and Yoshida, T. (1999). Comparison of substrate specificities against the fusion glycoprotein of virulent Newcastle disease virus between a chick embryo fibroblast processing protease and mammalian subtilisin-like proteases. Microbiol. Immunol., **43**:133-140.

French, E.I., T.D. St. George and J. J. Percy (1967). Infection of chicks with recent isolated Newcastle disease viruses of low virulence. Aust. Vet. J., **43**:404-409.

Ghumman, J.S. and R.A. Bankowski (1975). *In vitro* DNA synthesis in lymphocytes from turkeys vaccinated with LaSota. TC and inactivated Newcastle disease vaccines. Avian Dis., 29:479-487.

Gough, R.E. and Alexander, D.J. (1973). The speed of resistance to challenge induced in chickens vaccinated by different routes with a BI strain of live NDV. Vet. Rec., **92**:563-564.

Halasz, F. (1912). Contributions to the knowledge of fowlpest. Vet doctoral dissertain, comun. Hungor. Roy Vet. SchI: Patria, Budapest, 1-36.

Hanson, R.P. (1980). Newcastle disease, in isolation and identification of avian pathogen 2nd ed. Ed. By Hikchner S.B., Domermuth, C.H. Purchase, H. G. and Williams J.E.P. publ. by the American Association of Avian pathologist 63-66.

Hanson, R. P. and Brandly. C. A. (1955). Identification of vaccine strains of Newcastle disease virus. Science, **122**:156-157.

Hanson, R. P., Spalatin, J. and Jacobson, G.S. (1973). The viscerotropic pathotype of Newcastle disease virus. Avian disease. **17**:354-361.

Hanson, R.P. (1978). Newcastle disease. In M.S. Hofstad, B.W. Calnek, C.E. Helmboldt, W.M. Reid and H.W. Yoder (eds) Disease of poultry, Iowa State University Press. 513-535.

Haroun, M., Khalafalla, A.J. and Hajer, I. (1992). Some properties of Newcastle disease viruses field isolated in Sudan Bull. Anim. Prod. Afr., **40**:107-110.

Heller, E.D., Nathan, D.B. and Perek, M. (1977). The transfer of Newcastle disease serum antibody from the laying hen to the egg and chick. Res. Vet. Sci., **22:**376-379.

Henzege, J. Wehmann, E. Bragy, R.R. Travassos, P.M. Dias, G. Hadjiev, O. Werner and Lomnizzi, B. (1999). Two novel genetic groups (VII b and VIII). Responsible for recent Newcastle disease outbreak in South Africa, one (VIIb) of which reached southern Europe Archiv Virol., **144**:2087-2099.

Higgins, D.A. (1971). Nine disease outbreaks associated with myxoviruses among ducks in Hong Kong, Trop. Health Prod., **3:**232-240.

Hitchner, S.B. and Johnson, E.P (1948). A virus of low virulence for immunizing fowls against Newcastle disease (Avian pneu**mo-**encephalitis)**. Vet. Med., 43:525-530.**

Hugh-Jones, M., Allan, W.H., Dark, F.A. and Harper, G.J. (1973). The evidence for the airborne spread of Newcastle disease J. Hug. Camb., **71**:325-339.

Jarecki Black and King (1993). An oligonucleotide probe that distinguishes isolates of low virulence from the more pathogenic strains of Newcastle disease virus. Avian Disease. 37: 724-730.

Jestin, V., **and M .Cherbonne (1992**) .Use of monoclonal antibodies and gene amplification (PCR) for characterization of A –PMV-1 trains. Proceeding CEC Workshop on Avian Paramyxoviruses, Rauishholhausen. Institut Gefugelkrankheiten, Giessen, 157-166.

Jestin, V., M. Cherbonnel, and Arnauld, C. (1994). Direct identification and characterization of A-PMV-1 from suspicious organs by net PCR and automated sequencing. Proceedings of the joint first annual meetings of the national Newcastle disease and avian influenza laboratories of the European communities: Russels, 1993, 89-97.

JQrgensen, P.H., Jensen, K. Handberg, P. and Ahrens, R.J., Manvel, Frost, K.M. and Alexander, D.J. (2000). Similarity of avian paramyxovirus serotype 1 isolates of low virulence for chickens obtained from contaminated poultry vaccines and from poultry flocks. Vet. Res., **146**:665-668.

Kaleta, E.F., Alexander D.J. and Russell, P.H. (1985). The first isolation of the PMV-1 virus responsible for the current panzootic in pigeons-2 Avian Pathol., **14**:553-557.

Karrar, G. and Mustafa, E. (1964). Bull. Int. Epid., **62**(1-6): 891.

Khalafalla, A.I. (1994). Isolation and characterization of lentogenic Newcastle disease viruses from apparently healthy chickens in the Sudan. Bull. Anim. Hlth. And Prod. Afri., **42:** 179-182.

Khalafalla, A.I., Fadol, M.A., Hameid, O.A., Hussein, Y.A. and Mahasin, E.A/Rahman (1992). Pathogenic properties of Newcastle disease virus isolates in the Sudan. Acta Veterinaria Hungaria, **40**(4): 329-333.

Khalafalla, A.I., Nimir, A.H., and Hajer, I. (1990). Role of some Passeriformes birds in transmission of Newcastle disease III- Transmissibility of Newcastle disease virus in Sudan House sparrow (Passer domesticus arbonust) Bull. Anim. Hlth. And Prod. Afri., **38**:55-58.

King, D.J. and Seal, B.S. (1998). Biological and molecular characterization of Newcastle disease virus field isolates with comparisons to reference NDV strains. Avian Disease. **42**:507-516.

Koleta, E.F. and C. Baldauf (1988). Newcastle disease in Free-living and pet birds. In DJ. Alexander (ed.). Newcastle disease. Kluwer Academic Publishers: Boston, MA, 197-246.

Kranveld, F.C. (1926). A poultry disease in the Dutch East Indies. Ned indisch BI Diergeneesked, **38**:448-450.

Lamb, R., Collins, O.L., Kolakofsky, P., Melero, J.V., Nagai, Y., Oldstone, M.B.A. Pringle, C.R., Rima, B.K., (2005). The negative sense single stranded RNA viruses. In fauquet, C.M., Mayo, M.A., Maniloft, J., Desselberg, U., Ball, L.A. (eds.). Virus taxonomy. Eight report of the international committee on Taxonomy of viruses Elsevier Academic Press, San Dieyo, CA, pp. 607-738.

Lancaster, J.E. (1966). Newcastle disease: a review, 1920-1964. Monograph No. 3 Can. Eep. Agri. Ottawa.

Lancaster, J.E. and D.J Alexander. (1975). Newcastle disease virus and spread, monograph No. 11 Canadian department of agriculture, Ottawa.

Lockaby, S.B., Hoer, F.J., Ellis, A.C. and Yw, M.S. (1993). Immunohitochemical detection of Newcastle disease virus in chickens. Avian Dis., **37**:433-437.

Lomniczi, B., Wehmann, E. Herczeg, J., Ballagi, A., Pordang, E., Kaleta, F., Werner, O. Menlemans, A., Jorgensen, P.H., Mante, A.P., gielkens, A. L. J., Copua, F. and Damoser, J. Western Europe were caused by an old (VI) and novel **(1998).** Newcastle disease outbreaks in recent years in genotype (VII) archive Virol., **143**:49-64

M. A Gaffar Elamin, S.A.M.Kheir, and S.H Babiker. (1981). Studies on Newcastle Disease in the Sudan. Sudan J. Vet. Sci. and Anim. Hasb Vol 22.no(1).

Macpherson, I. W. (1956).Some observation on the epizootiology of Newcastle Disease. Canad J Comp Med 20: 155-168.

Mahasin E. A. Rahman, Celia O and Wegdan, H.A. (2001). Group specific, type- specific and pathotype identification of some Newcastle disease virus Sudan isolates.Sud. J. Vet. Sci, Anim Husb. Vol 44, (1, 2) 1-15.

McFerran, J.B. and R.M. McCraken (1988). Newcastle disease, in D.J. Alexander (ed.) Newcastle disease, Kluwer Academic Publisher: Boston. M.A. 161-183.

McFerran, J.B. Gordon, W.A.M., Finaly, J.T.T. (1968). Vet. Rec., **82**: 589-592.

McGinnes, L.W. and Morrison, T.G. (1986). Nucleotide sequence of the gene encoding the Newcastle disease virus fusion protein and comparisons of paramyxovirus fusion protein sequences virus Res., **5**:343-356.

McMillan, B.C. and Hanson, R.P. (1980). RMA oligonuclootide Finger-printing: A proposed method of identifying strains of Newcastle disease virus. Avian Disease. **24**:1016-1020.

McMillan, B.C., Rehmani, S.F. and Hanson, R.P. (1986). Lectin binding and carbohydrate moieties present on Newcastle disease virus strain Avian dis., 340-344.

Meulemanes, G., Lettellier, C., Espion, D., Le long, and Burny, A. (1988). Importance de La proteine F dans I immunite an virus de LA, maladie de Newcastle, Bull. Acad. Vet. France. **61**:51-62.

Meulemans, G., Letellier, C. Gonze, M., Carler, M.C.and Burny, A. (1988). Newcastle virus F glycoprotein expressed from a recombinant vaccinaia virus vector protects chickens against live vaccine virus challenges. Avian Pathol., **17**: 821-827.

Millar, N.S. and Emmerson, P.T. (1988). Molecular cloning and nucleotide nucleotide sequencing of Newcastle disease virus. In D.J. Alexander (Ed). Newcastle disease. Kluwer Acadaic Publishers: Boston, M.A., 79-97.

Millar, N.S., Chambers, P. and Emmerson, P.T. (1986). Nucleotide sequence analysis of the haemagglutinin neuraminidase gene of Newcastle disease virus. J. Gen. Virol., **67:**1917-1927.

Murphy, F.A., Gibbs, E.P.J., Horzinek, M.C., and Studdert, M.J. (1999). Paramyxoviridae. In: Vet. Virology. 3rd edition. Academic Press, pp. 411-420.

Murphy, F.A., Gibbs, E.P.J., Horzinek, M.C., Studdest, M. J. (1999). Paramyxoviridae. In: Vet. Virology. 3rd ed. Academic Press, pp. 411-420.

Nagai, Y. (1993). Protease dependent virus tropism and pathogenicity. Trends in microbiology. **1**:81-87.

Nagy, E. and Lomniczi, B. (1984). Differentiation of Newcastle disease virus strains by one-dimensional peptide mapping. J. virol. Methods**. 9:**227-235**.**

Nantha kumar, T. Kataria, R. S., Tiwari, A. k., Butchaiah, G. and Kataria, J.M. (2000). Pathotyping of Newcastle disease viruses by RT-PCR and restricted enzyme analysis**.** Veterinary Research Communication **,** 24**,** 275-286**.**

OIE, (2008).Manual of Diagnostic Tests and vaccines for Terrestrial Animal. Available on the Website: http: // WWW.oie .int/ eng /normes/ mmanual/ A.Summry.htm.

OIE, (2007): List of countries by disease situation http: / / WW-Waieint / Wahid. Prod/ public php? Page disease –status –list accessed 20th september 20007

OIE, (2010) Newcastle Disease, Terrestrial Manual, pp 19-26.Commfssion Decision 2006 -437-EC (Diagnostic Manual for Avian Influenza**).**

Oberdorfer, A. and Werner, O. (1998). Newcastle disease virus detection and characterization by PCR of recent German isolates differing in pathogenicity .Avian pathol.27.237-243.

Omaima, H. N.; Mahasin E. A/Rahman and A/ Gaffar A.I. (2005a). Studies on velogenic NDV. 1- Plaque formation and isolation of velogenic

Newcastle disease virus in primary chick embryo fibroblast. Sud. J **Vet** Res. (Accepted)

Omaima, H. N.; Mahasin E. A/Rahman and A/Gaffar A. I. (2005b). Studies on velogenic NDV II. Biological and pathological characteristics **of** plaque variants of Newcastle disease virus compared to parent virus. Sudan J Vet Res. (Accepted)

Omojola, E. and Hanson, R.P. (1986). Collection of diagnostic specimens from animal in remote areas. World Animal Rev., **60:**38-40.

Panigrapy, B., Senne, D.A., Pearson, J.E., Mixson, M.A. and Cassidy, D.R. (1993). Occurrence of velogenic viscerotropic Newcastle disease in pet and exotic birds in (1991). Avian Dis., **37**:254-258.

Person, J.E. Sene, D.A., Alexander, D. J., Taylor, W.D. Peterson, I.A. and Russell, P.H. (1987). Characterization of Newcastle disease virus avian paramyxovirus-1) isolated from pigeons. Avian Dis., **31**:105-111.

Posada, D., Buckly, T.R. (2004). Model selection and model averaging in phylogenetic: advantage of akaike Information criterion and Bayesian approaches over likehood ratio tests. Sys. Biol. 53 (5): 793-808.

Posada, D., Crandall, K.A. (2001). Selecting the best-fit model of nucleotide substitution. Sys. Biol. 50, 580-601.

Pospisil, Z., Zendukova, D. and Smid, B. (1991). Unexpected emergence of Newcastle disease virus in very Newcastle disease virus in very young chicks Acta Vet. Brno., **60**:263-270.

Razzewska, H. (1964). Occurrence of the LaSota strain NDV in the reproductive tract of laying hens. Bull. Vet. Inst. Pulary. **8:**130-136.

Reeve, P and G. Poste (1971). Studies on the cytopathogenicity of Newcastle disease virus. Relationship between virulence polykaryocytosis and plaque size J. Gen. Virol., **11**:17-24.

Reynolds, D.L. and Maraqa, A.D. (2000). Protective immunity against Newcastle disease: the role of cell-mediated immunity. Avian Dis., **44**:145-154.

Rima, B.R., Alexander, D.J., Biller, M.A., Collins, P.L. Kingshury, D.W., Lipkind, M.A., Najoi Orvell, C. Pringles C.R. and Ter Meulen, V. (1995). The paramyxovirdae. In Virus Taxonomy. Sixth Report of the International Committee on Taxonomy of Viruses. Pp. 268-275.

Russell, P.H. (1988). Monoclonal antibodies in research diagnosis and epizootiology of Newcastle disease. In D.J. Alexander (ed.) Newcastle disease. Kluwer academic publishers: Boston, MA, 131-146.

Russell, P.H. and Alexander, D.J. (1983). Antigenic variation of Newcastle disease virus strains detected by monoclonal antibodies. Arch Virol., **75**:243-253.

Sato, H., M. Oh-Hita, N. Ishida, Y. Imamura, S. Hatlovi, and M. Kawakita (1987). Molecular cloning and neucleotide sequence of P., M. and F. genes of Newcastle disease virus a virulent strain D26. Virus Res**.,** **7:**241-255**.**

Schloer, G. and R.P. Hanson (1968). Plaque morphology of Newcastle disease virus as influenced by cell type and environmental factors. Am. J. Vet. Res., **29**:883-895.

Seal, B.S., King, D. J., Bennett, D.J. (1995). Characterization of Newcastle disease virus isolates by reverse transcription PCR couple to direct

nucleotide sequencing and development of sequence database for pathotype prediction and molecular epidemiological analysis. J. Clinic. Microbiol., **33**:2624-2630.

Senne, D.A., Pearson, J.E., Niller, I.D. and Gustafson, G.A. (1983). Virus isolation from pet birds submitted from importation into the United States. Avian Dis., **27**:731-744.

Simmons, G.C. (1967). The isolation of Newcastle disease virus in **Queensland. Aust. Vet. J., 43:29-30.**

Spalatin, J. and Hanson, R.P. (1975). Avian disease, **19:** 593-582.

Spalatin, J., Hanson, R.P. and Beard, P.D. (1970). The haemagglutination. Elution pattern as a marker in characterizing Newcastle disease. Avian Dis., **14**:542-549.

Spalatin, J. and Hanson, R.P. (1966). Recovery of a Newcastle disease virus strain in distinguishable from Texas, G.B. Avian Dis., **10**:327-374

Tamara, K., Dudley, J., Nei, M., Kumar, S. (2007). MEGAA: Molecular Evolutionary Genetics Analysis (MEGAA) software version 4.0 Mol. Biol. Evol. 24, 1596-1599.

Terregino, C., and I. Capua. (2009). Conventional diagnosis of Newcastle disease virus infection. In: Capua. I., Alexander, D. J. (Eds.). Avian influenza and Newcastle disease, afield and Laboratory Manual. Springer-Verlag. Italy pp.123-126.

Toyoda, T.T., Sakaguchi, K.tmai, M. Mendoza Inocencio, B. Gotoh, M. Hamaguchi, and Y Nagi (1987). Structural comparison of the cleavage activation site of the fusion glycoprotein between virulent and a virulent strain of Newcastle disease virus. Virology. **158**: 242-247.

USAHA (1993). Report of the committee on transmissible disease of poultry and other avian species Proc. 96th Annu. Meet. US Anim. Health Associ., 1992. United States Animal Health Association: Richmond, VA, 348-360.

Utterback, W.W. and Schwartz, J.H. (1973). Epizootiology of velogenic viscerotropic Newcastle disease in southern California 1971-1973. J. A. V. M. Assoc. **163:**1080-1090.

Vindevogel, H. and Duchatel, J.P. (1988). Panzootic Newcastle disease virus in pigeons in D.J. Alexander (ed.). Newcastle disease. Kluwer Academic Publishers: Boston, MA, 184-196.

Vindevogel, H., Pastorat, P.P., Thiry, E. and Peeters, W. (1982). Annales de Medecine Veterinaire, **126**:5-7.

Walker, J. W., Haron, B. R. and Mixon, M. A. (1973). Exotic Newcastle disease eradication program in the United States of America. Avian Disease. **17**:486-503.

Wegdan, H., Sobhi, **Ahmed. ,and Celia Abolnik (2010).**Newcastle disease outbreaks in the Sudan from 2003 to 2006 were caused by viruses of genotype 5d .Virus Genes (2010) 40:106 -110.

Wemers, C.D., S. de Henau, C. Neyt, O. Espion, C. Letellier, G. Meulemans and A. Burng (1987). The haemaglutinin- neuramindase (HN) gene of Newcastle disease virus strain Italian (2nd v Italian: comparison with HNS of other strains and expression by a vaccine recombinant, Arch Virol., **97**:101-113.

Williams, J. E. and Dilard, I. (1968). Penetration patterns of *Mycoplasma gallisepticum* and Newcastle disease virus through the outer structures of chicken eggs. Avian Dis., **12**:656-659.

Winslow, N.S., Hanson, R.P., Upton, E. and Brandly, C.A. (1950). Agglutination of mammalian erythrocytes by Newcastle disease virus. Proc. Soc. Exp. Biol., **74**:174-178.

Wise, M. G., Suarez, D.L., D.L., Seal, B.S., King, D. J., Kapczynski, D. R., Spack-man, E. (2004). Development of a real-time reverse transcription PCR for detection of Newcastle disease virus RNA in clinical samples. J. Clin. Microbial. 42(1), 329-338.

Wobeser, G., Leighton, E.A., Norman, R., Myers, D.J., Onderka, D., Pybus, M.J.M Neufeld, J.I., Fox, G.A. and Alexander, D.J. (1993). Newcastle disease in wild water birds in western Canada, 1990. Can. Vet. J., **34**:353-359.

Zorman, O. Rojs, U. Krapex, F, Grom, D, Barlic Maganja. (2002). Molecular detection and pathotyping of Paramyxovirus type 1isolates (Newcastle disease virus) Slov. Vet. Res, 39(1):39-45.