IR2.1000802.

A Comparative Study on Histopathologic Effects of Iranian Newcastle Disease Virus Isolates

Kianizadeh*[1], M., Aini, I.[2] and Gholami, G.R.[3]

1. Avian Diseases Research & Diagnosis Dept., Razi Vaccine & serum Research Institute, P.O.Box:11365-1558, Tehran, Iran.

2. Faculty of Veterinary Medicine, University of Putra Malaysia, 43400 UPM, Malaysia.

3. Pathology Dept., Razi Institute

Received 28 Mar 2002; accepted 17 Sep 2002

Summary

The histopathologic effects of Iranian Newcastle disease viruses isolated from different outbreaks across the country were studied on different organs of specific pathogen free chickens. Clinically, time and sequence of the signs' occurrence were varied among the groups receiving different isolates. Depression was the first clinical sign observed by 48h postinfection (PI) in all groups except two groups, which showed depression by 97h PI. Grossly, among the three systems, gastro-intestinal, respiratory and central nervous system that were examined in infected groups, the latter two showed less remarked lesions. Macroscopically, from 72h PI toward the end of experiment the spleen showed atrophy in all infected groups. At 96h PI and later on, the brain was slightly hyperaemic in limited infected groups. The early findings were observed in proventriculus, liver, small intestine and spleen. Generally all nine Iranian Newcastle disease viruses can affect visceral organs faster than other organs and could be placed in viscerotrop velogenic group.

Key word: Newcastle disease, histopathology, Iran

Introduction

ND viruses (NDVs) distribution in various organs, and histopathologic changes in organs have been studied as ways of revealing their pathogenicity (Shirai *et al* 1988,

* Author for correspondence. E.mail: kiian@imamreza.net

Ojok & Brown 1996, Hooper *et al* 1999). The virus enters the body via the conjunctiva, digestive and respiratory tracts. The primary target organs for attachment and replication are respiratory and intestinal tracts. After propagation at the initial sites through the first viraemia, virus may reach the other organs such as kidney, liver, bursa, spleen and lungs.

Pathological changes vary, based on several factors including infection route, tropism and virulence of the virus, immune status and age of the host. It has been shown that respiratory route is more sensitive compared to other routes of infection (McFerran & McCrackern 1988). The reason NDV is less infectious when given orally appears to be because the gizzard content is acidic (pH around 2.6), reducing the infectivity 1000 fold (Alexander 1988). Hanson and Brandly (1955) have grouped NDV strains based on tropism into velogenic, mesogenic and lentogenic groups.While lentogenic strains are present in low titres in circulation, mesogenic strains spread to some visceral organs by 24-44h PI. Virulent viruses can be found in all tissues with highest titer in thymus and lowest in muscles and brain. The reason for this feature has been described (Collins *et al* 1993) of which, the cleaveability of F_0 is the main factor.

The objective of this study was to compare the pathogenesis among Iranian NDV isolates in an experimental infection.

Materials and Methods

Viruses and inoculation procedures. Nine Iranian NDV isolates presented in table 1, were inoculated as described by Ojok and Brown (1996). Briefly, groups of ten 6-week-old specific pathogen free (SPF) chickens (Valo, Lohmann, Cuxhaven, Germany) for each isolate were inoculated with 0.05ml of 10^7 embryo-lethal doses 50% (EID_{50}) of virus by the ocular route. Additionally two birds were kept in seprate pen during the experiment as negative controls. The birds were examined clinically everyday.

Table 1. *Data on ND outbreaks in Iran*

No	Isolate	Type	Population
1	MK7	Broiler	10000
2	MK12	Bro/layer	200
3	MK13	Indigenous	200
4	MK14	Indigenous	3500
5	Krd76	Broiler	2500
6	Kasra7	Broiler	16000
7	GH77	Broiler	5000
8	KH2/78	Broiler	5000
9	ES1/99	Broiler	10000

Sample collection. At intervals of 24h, birds were killed by injection of 1.5ml of thiopental sodium (Specia, 16RUE GLISSON 75013 Paris, France) into the brachial vein; one chicken (B) was killed at 24h PI, one (C) at 48h PI, two (D and E) at 72h PI, two (F and G) at 96h PI and three (H, I, J) at 120h PI. Necropsy was carried out immediately. The gross lesions were recorded. A non-infected chicken (A) was killed at the beginning of the experiment to supply control samples.

Samples preparation for microscopic sections. Samples were prepared as described by Ojok and Brown (1996). Briefly, brain, lungs, trachea, liver, small intestine, spleen and proventriculus from each bird were collected and fixed in 10% neutral buffered formalin for microscopic examination. Tissues were processed and embedded in paraffin wax after fixation for 24h, sectioned 4-5μm and stained with haematoxylin & eosin and observed with light microscope for histopathology changes.

Results

Clinical signs. Some generalized signs that were observed included depression, ruffled feathers, loss of appetite, huddling, listless, swelling of eyelid of inoculated eye. Diarrhoea, paralysis, head shaking, torticollis, moribund state and death were

others features, which usually occurred at the later stages. The time and sequence of the signs' occurrence were varied among the groups receiving different isolates. The negative control birds did not show any clinical signs. The summarized data are presented in table 2.

Table 2. *Clinical signs in experimental infection*

Iranian NDV isolates	h PI				
	24	48	72	96	120
MK7	1)A	2)I	3)B-4)B	5)B,F-6)B,G,D	7)H,B-8)H,B-9)G,J,K
MK12	1)A	2)I	3)B-4)B	5)H,B-6)B	7)L<120-8)E-9)E
MK13	1)A	2)B	3)B-4)B	5)F,K-6)L<96	7)L<120-8)E-)E
MK14	1)A	2)B	3)B,C-4)B,C	5)E,K-6)E,K	7)L<120-8)H,E-9)H,E
Krd76	1)A	2)B	3)B,C-4)B,C	5)E,K-6)E,K	7)L<120-8)H,E-9)H,E
Kasra97	1)A	2)A	3)B,C,I-4)B,C,I	5)G,B-6)H,G,B	7)L<120-8)L<120-9)H,G
GH77	1)A	2)B,C	3)E,C,F-4)G	5)H,L<96-6)L<96	7)L<120-8)H,L<120-9)H,L<120
KH2/78	1)A	2)A	3)B,I-4)B,I	5)H,L<96-6)H,L<96	7)H,L<96-8)H,L<96-9)H,L<96
ESI/99	1)A	2)B	3)B,C,D-4)B,C,D	5)H,L<96-6)H,L<96	7)H,L<96-8)H,L<96-9)H,L<96

1-9: Chicken number; A: Normal statue; B: Depression.C; Ruffled feathers; D: Open mouth breathing.; E: Listless; F: Head tremors; G:Paralysis; H: Diarrhoea; I: Eyelid swelling; J: Torticollis; K: Sit on the hocks; L: Death.

Gross lesions. At each interval (24h), necropsy of killed or dead bird was carried out immediately. No abnormalities were noted in the control bird (No 10). Grossly, among the three systems; gastro-intestinal, respiratory and central nervous system that were examined in infected groups, the latter two showed less remarked lesions. The only features that were observed included congestion in lungs, which began by 48hPI. In gastro-intestinal tract, the outstanding lesions were haemorrhagic foci

associated with necrosis in the proventriculus, small intestine, caeca and large intestine, which were visible mostly at 72hPI and later (Figure 1). The early lesions in proventriculus were observed by 48 hpí in birds infected with isolate ES1/99 (Figure 2).

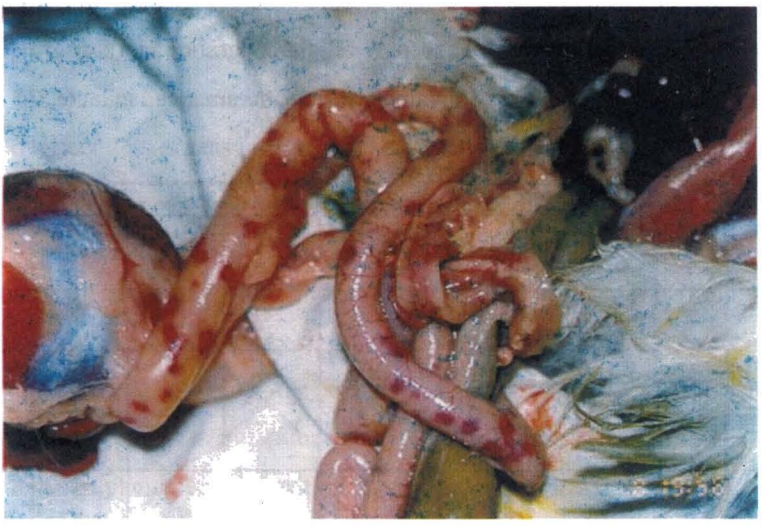

Figure 1. *Haemorrhage in small intestine of ND infected chicken*

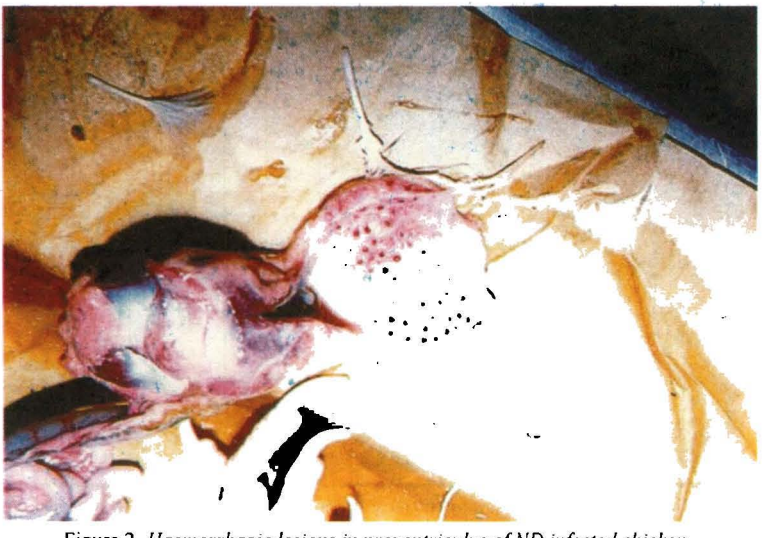

Figure 2. *Haemorrhagic lesions in proventriculus of ND infected chicken*

In some cases the spleen had a stippled appearance on both capsular and cut surfaces and was enlarged at 48hPI. Liver in all infected groups was apparently normal. Macroscopically, from 72hPI toward the end of experiment the spleen showed atrophy in all infected groups. At 96hPI and later on, the brain was slightly hyperaemic in limited infected groups. During the experiment period no noticeable effects on trachea were observed except for slight congestion in a few groups. Gross lesions in each group caused by Iranian isolates are summarised in table 3.

Table 3. *Gross lesions caused by NDV isolates*

No	HPI	Organs presented with macroscopic lesions						
		Proventri-culus	liver	Intestine	lung	Trachea	Spleen	brain
1	24	A +	A +	A +	A +	A +	A +	A +
2	48	B ES1/99	A +	A +	C KH2/78, ES1/99	A +	D MK7, GH77, MK14	A +
3,4	72	B MK12, MK13, KH2/78,GH77, ES1/99,MK14	A +	A +	C MK7, KH2/78, ES1/99	E Kasra97	D MK7	A +
5,6	96	B* MK7, GH77, K12, MK13, KH2/78,ES1/9 9, MK14, Krd76	A +	B GH77*50% KH2/78, Krd76, ES1/99*, MK12 Kasra97	C MK12, MK13, Kasra97, KH2/78, ES1/99	E MK12	F +	C Kasra97
7,8,9	120	B* MK7, GH77, MK12, MK13, Krd76, Kasra97, MK14	A +	B MK7, GH77*, MK14, Krd76, Kasra97	C MK7, MK12, MK13, Kasra97, ES1/99	E MK12, Kasra97	F +	C MK12, Kasra97, KH2/78

1-9: Chicken number.; A: Apparently normal; B: Haemorrhagic; C:Congestion; D: splenomegaly;
E: Restricted hyperaemic areas; F: Atrophy.;*: Multiple site; +: All.

Microscopic findings. There were no abnormalities in non-infected control bird (No.10). At 24hPI no marked changes were observed in tissue sections in all infected groups. By 48hPI some changes were observed in different organs by different isolates as follow: 1 isolate (ES1/99) affected the proventriculus by haemorrhagic necrosis, 6 isolates affected the liver by congestion and increased mononuclear cells around the blood vessels (Figure 3), 3 isolates affected the small intestine by localized heterophils infiltration. At 72hPI the number of the isolates that could affect the organs increased. The affected organs included the following: spleen, proventriculus, liver, small intestinal, and lungs that were affected by 8, 8, 6, 3 and 7 isolates, respectively. In intestine, lesions were haemorrhagic foci associated with necrosis in intestinal wall (Figure 4). By 72hPI, trachea and brain, in all infected groups did not show any significant change. At 96hPI and later on, almost all the isolates could affect all the organs with progressive lesions. The extent of lesions in spleen and proventriculus were severe. The spleen showed multifocal necrosis area, lymphoid depletion with fibrin replacing (Figure 5).

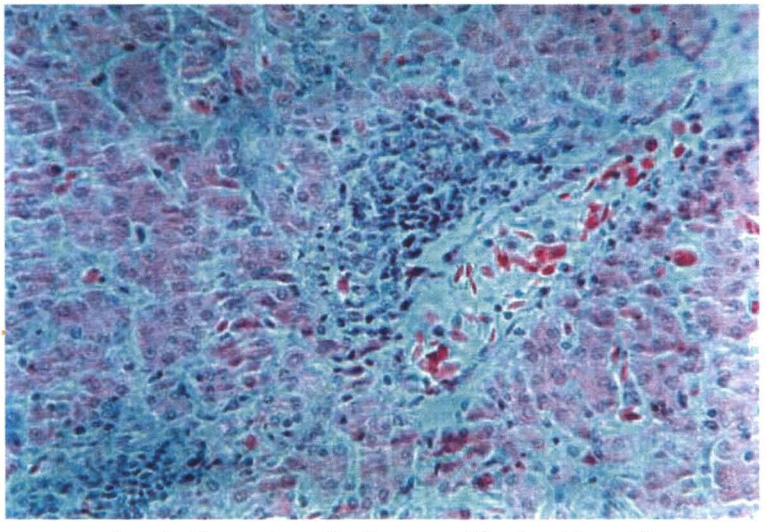

Figure 3. *Mononuclear cell infiltration in ND infected liver x400*

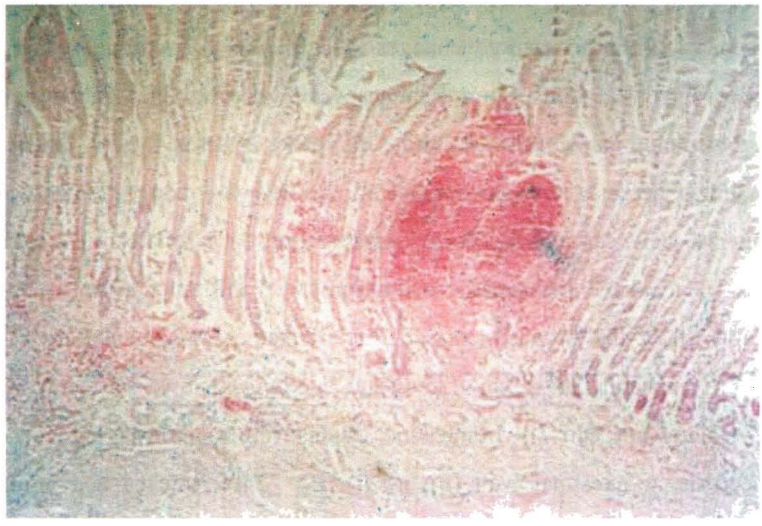

Figure 4. *Haemorrhagic intestine of ND infected chicken x400*

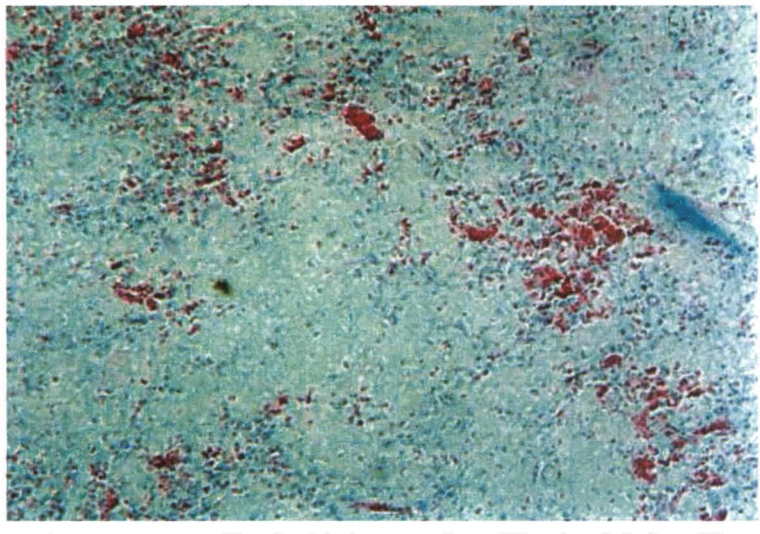

Figure 5. *Necrosis and lymphoid depletion in spleen of ND infected chicken x400*

During the experimental period the trachea in most infected groups showed lesser pathologic effects except, in 3 groups infected with MK7, MK12 and Kasra97 ND isolates that showed haemorrhage and loss of cilia in infected group

with MK12 isolate. By 96hPI and later on, in 6 groups of infected chickens some changes including endotheliosis in cerebrum and malacia in cerebellum occurred (Figures 6 and 7), but these changes were not so extensive.

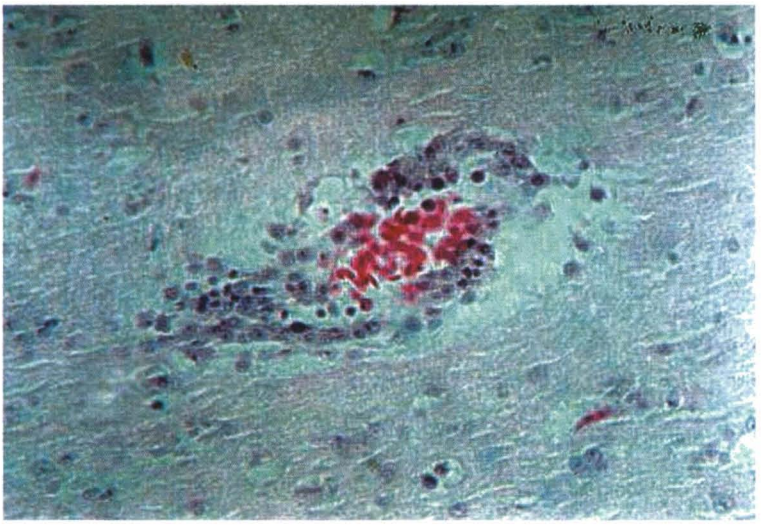

Figure 6. *Endotheliosis in cerebellum of ND infected chicken x400*

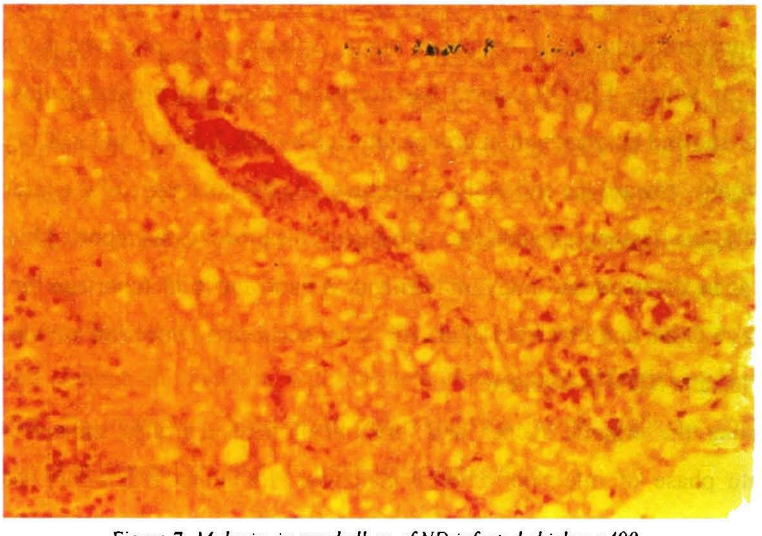

Figure 7. *Malacia in cerebellum of ND infected chicken x400*

Discussion

In this study the presence of virus in different tissues was examined by gross and microscopy findings. The pathology of NDV infection varies from strain to strain. Virulent NDV can multiply in many organs resulting in systemic infection and early death without the bird showing all the signs of the disease (Collins *et al* 1993). In all groups infected with Iranian isolates; spleen, proventriculus, liver and lung showed the effects of the viral infection in the early phase of infection although the liver was apparently normal and lesions in the lungs were not destructive and limited to congestion. Grossly, virulent viruses cause predominantly haemorrhagic lesions in various parts of intestinal tract, in particular in the mucosa, at the junction of oesophagous/proventriculus and proventriculus/gizzard. The lesions in the posterior half of the duodenum/jejunum and ileum are almost pathognomic for velogenic viscerotropic NDV (Kouwenhoven 1993). The gastrointestinal lesions that are seen following a viscerotropic ND virus infection mostly developed in lymphoid aggregates (Alexander 1988). The outstanding lesions in infected groups with Iranian NDV isolates were found in gastro intestinal tract (GIT). The first lesions in GIT (proventriculus) and lymphoid organ (spleen) were observed in 2 groups by 48hPI of which, ES1/99 caused haemorrhagic lesions in proventriculus while the spleen showed enlargement in groups infected by MK7, GH77 and MK14, followed by atrophy toward the end of the experiment. At the later stage of the infection (72-120hPI), the lesions extended to intestine. This process was reported by Kaschula (1961), in comparative study of virulent Asiatic and mild American forms of ND and by Ojok and Brown (1996), in an experimental infection of virulent and viscerotropic NDV in chicken.

Among the organs, trachea and brain in all infected groups, showed changes in the late phase of infection. This is matching with limited respiratory and nervous clinical signs during the experiment in all groups. NDVs invaded the CNS after multiplication in non-nervous tissues has ceased. However, virulent neurotropic

virus may be present in the CNS at the same time as in the respiratory or intestinal tract (Kouwenhoven 1993

Respiratory disturbances were not observed except in groups infected with ES1/99 and MK7, which showed open mouth breathing by 96hPI. The reason for lack of respiratory signs in most groups may be related to the virulence of virus causing fast progress of the disease or to the route of inoculation. The route of inoculation may influence initial site of viral replication. Mortality varies greatly depending on the property of the virus. Viruses of some strains reached vital organs like liver and kidney very rapidly so that the birds may die before disease symptoms are overt (Kouwenhoven 1993). The earliest death occurred in less than 96hPI in 4 groups of which 2 groups were infected with KH2/78 and ES1/99. The only NDV isolate that did not cause death in the group even by 120hPI, was MK7. In this group, torticollis was recorded as well as the respiratory disturbance. Thus, the slow trend of the disease progress has provided sufficient time for the birds to show all possible features of the disease. Waterson *et al* (1967) mentioned that the virulent strains might kill so quickly that neurological signs may be seen for only a brief period before death. The presence of microscopic changes since the early stages of infection (48hPI) in most selected organs, represent the ability of Iranian NDV isolates in inducing systemic infection. Based on microscopical findings, the outstanding feature was the involving of the lymphoid cells or organs in virus multiplication after first viraemia. Congestion and focal necrosis in spleen were recorded in 7 infected groups. Congestion and mononuclear lymphoid cells infiltration was seen in liver in 5 groups by 48hPI. Infiltration of heterophils was present in intestinal sections. Ojok and Brown (1996) found lymphoid areas in spleen and thymus in infection with virulent viscerotropic NDV where they were represented by massive destruction with extensive lymphocytolysis and fibrin replacing and in proventriculus with lymphoid aggregates.

Congestion was the only lesions found in the lungs. This feature is matching with lack of any clinically significant respiratory disturbance in most of the infected

groups. Lesions in brain included endotheliosis and neuronal degeneration occurred late (96hPI) in comparison to the other organs. Such lesions are presented in birds infected commonly with viscerotropic and mesogenic pathotypes (Alexander 1988).

Generally, during the experimental infection with all Iranian NDV isolates, time was the only variant regarding the pathology effects. Based on the results obtained, all isolates showed velogenic viscero neurotropic characteristics, which is in agreement with the results of pathogenicity indices (kianizadeh, *et al* 1999).

References

Alexander, D.J. (1988). *Newcastle Disease*. Massachusetts, Kluwer Academic Publishers.

Collins, M.S., Bashiruddin, B.J. and Alexander, D.J. (1993). Deduced amino acid sequences at the fusion protein cleavage of Newcastle disease viruses showing variation in antigenicity and pathogenicity. *Archives of Virology* 128:363-370.

Hooper, P.T., Hansson, E., Young, J.G., Russell, G.M. and Della-Porta, A.J. (1999). Lesions in the upper respiratory tract in chickens experimentally infected with Newcastle disease viruses isolated in Australia. *Australian Veterinary Journal* 77: 50.

Kouwenhoven, B. (1993). Newcastle disease. In: J.B. McFerran and M.S. McNuty (Eds.), *Virus Infections of Birds*. Pp:341-361. Amsterdam, The Netherland, Elsevier Science Pubs.BV.

Kianizadeh, M., Ideris, A., Shahrabadi, M.S., Kargar,R., Pourbakhash, S.A., Omar, A.R. and Yusoff, K. (1999). Biological and Molecular Characterization of Newcastle Disease Virus Isolates from Iran. *Archives of Razi Institute* 50:1-9.

McFerran, J.B. and McKracken, R.M. (1988). Newcastle Disease pathogenesis. In: D.J. Alexander (Ed.), *Newcastle Disease*. Pp:161-183. Massachusetts, Kluwer Academic Publishers.

Ojok, L., Brown, C. (1996). An immunohistochemical study of the pathogenesis of virulent viscerotropic Newcastle disease in chicken. *Journal of Comparative Pathology* 115:221–227.

Shirai, J., Hihara, H. and Maeda, M. (1988). Virus distribution and histopathologic changes in organs of chicken inoculated with Newcastle disease virus (Avian Paramyxovirus-1) isolated from racing pigeon. *Avian Diseases* 3:544-547.

Waterson, M.D., Pennington, T.H. and Allan, W.H. (1967). Virulence in Newcastle disease virus. *British Medical Bulletin* 23:138-143.