# PATHOLOGY AND TRANSMISSION OF EXPERIMENTAL VELOGENIC VISCEROTROPIC NEWCASTLE DISEASE IN WILD PIGEONS, BROILER AND ASEEL CHICKENS

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***ABSTRACT:*** *The present study has been designed to evaluate pathology and transmission of VVND virus in wild pigeons, broiler and Aseel chickens. In this regard, experimental inoculation was made in Aseel, broiler chickens and in Pigeons after APMV-1 virus culturing and harvesting. The birds were inspected for Haemagglutination inhibition assay, Haemagglutination Inhibition test assay, Embryo Infectious Dose$_{50,}$ Body temperature, Feed Intake and water intake, Mortality Rates, Post mortem lesion scores, and histopathological examination.*

*Results, revealed significantly higher HI titers on day- 10 in comparison to day-7 in all respective groups. However, differences of HI titers among different groups of pigeons were found significant. Peaked cloacal temperature was recorded from day 2 to 3 in all birds. Moreover, there was significant drop in the feed and water intake after viral inoculation. Additionally, Clinical signs appeared rapidly in intra-oral and intra -muscular inoculated birds than in exposed birds. However, pigeon did not show any clinical sign. Besides mortality was 100% at day-6 and day-5 post-inoculation of VVNDV in broiler and Aseel chickens respectively. Gross lesions observed were hemorrhages and necrotic plaques on proventriculus, hemorrhages on small intestine and caecal tonsils, congested trachea, mottled spleen, and necrosis in bursa. Histologically virus produced hemorrhages and congestion and degenerative lymphocytes on the mucosa and sub-mucosa of proventriculus, ulceration of intestinal epithelium, necrosis in the lymphoid tissue and sloughing off of the necrotic tissue of caecal tonsils. Additionally, degenerative and lymphocytic necrosis was observed in air sacs, lymphoid tissues and in spleen.*

**Key words:** APMV-1 virus, pathology, transmission. New castle Disease, wild pigeons, broilers

## INTRODUCTION

Among all the infectious viral diseases, Newcastle disease (ND) causes major economic losses. Newcastle disease is an acute, infectious, highly contagious, rapidly spreading and fatal viral disease of poultry affecting chickens, pigeons, turkeys, Guinea fowl, Japanese quail and many wild birds of all ages, worldwide. It varies widely in type and severity of symptoms [1,2]. The global impact of Newcastle disease is enormous and it is responsible for International trade barriers of poultry and poultry products [3]. It is an endemic disease of poultry in Pakistan and is a major constraint to developing poultry industry, where control remains extremely difficult or even impossible. The disease is very complex and complicated due to different pathotypes and strains of the virus that may induce enormous variation in the severity of disease. The virus has been isolated from the trachea, intestine (duodenum, caecum and cloaca), spleen and droppings of different avian species [4].

The disease is caused by a Paramyxovirus type 1 (APMV-1), which is an RNA virus called ND virus. It belongs to the family Paramyxoviridae, genus Avulavirusand the order mononegavirales [5]. (Wild pigeons are infected by a different strain of NDV, called as Pigeon Paramyxovirus (PPMV). Pigeons have been implicated as carriers of NDV [6]. However, chickens are the most susceptible hosts for NDV. On the basis of its virulence, ND has been classified into velogenic, mesogenic and lentogenic type. Among three velogenic ND causes acute lethal infection strain with high mortality rates and is divided into viscerotropic and neurotropic strains..

The clinical signs and gross or microscopic lesions of VVND are highly variable and based on the viral strain, host species, age of host, route of exposure to infection and the environment (7). In natural infection the incubation period is 2-15 days while in experimental infection it is 2-5 days. The clinical disease might range from sub-clinical infection to 100 percent mortality in a short period of time [5]. The VVND cause severe, often fatal disease in chickens. Most birds are lethargic,anorexic and other signs consist of ruffled feathers, conjunctivitis and edema. Some birds develop watery, greenish or white diarrhea, respiratory signs such as gasping and coughing and swelling of the tissues of the head and neck. Nervous signs including tremors, paralysis of the wings and legs, torticollis and circling may also be seen. Neurological signs can occur concurrently with other symptoms but are generally seen later in the course of disease [8].

The natural infection of birds occurs by the respiratory or the intestinal routes following either inhalation or ingestion of infectious virus. Inhalation of infectious virus may occur as the result of the presence of either large droplets or fine aerosols containing virus. Since large amounts of virus particles are excreted in feces, ingestion of feces results in infection, this is likely to be the principle method of bird to bird spread without respiratory disease (5). The virus has been isolated from the trachea, intestine (duodenum, caecum and cloaca), spleen and droppings of different avian species [4].

Several epizootiological studies have shown that apparently healthy free-roaming wild birds may be important in transmission of ND virus to organized commercial poultry (9). Moreover, the virus has the ability to cross react with other species [10] Wild pigeons are free-roaming wild birds frequently found around poultry farms and Aseel chickens are

raised as backyard chicken in periphery of rural and semi urban poultry farms. Both may play a role in the transmission of ND. The present study has been designed to evaluate pathology and transmission of VVND virus in wild pigeons, broiler and Aseel chickens.

## MATERIALS AND METHODS

The present work evaluated the gross pathology and transmission of experimental VVND in wild pigeons, broiler and Aseel chickens.

### 3.1 Virus Collection and Culture

The VVND virus, APMV-1/chicken//Multan /-19-06/2012, was obtained from the Sindh Poultry Vaccine Center (SPVC) Karachi and was cultured in an allantoic cavity of ten day old embryonated chickens eggs. Virus 0.4ml of antibiotic i.e. penicillin and gentamycine (to check bacterial contamination) and 0.6ml of virus were mixed to make 01ml and then 0.1ml was inoculated in each egg and then cultured in an incubator for the multiplication of virus. After the death of embryos (72 hours post- infection), the amniotic fluid was carefully collected and stored at -40°C for future use.

### 3.2 Haemagglutinaiton Assay (HA)

The Micro HA was performed in a 96-well polystyrene micro-titer plate. Micro HA titration was performed as described by World Organization for Animal Health OIE Manual [11]. The results of HA test was determined as follows. **Positive HA:** A tear shaped streaming of RBCs in suspended form in the micro wells. **Negative HA:** A tight and sharp button of RBCs in the bottom of the micro wells.

### 3.3 Haemagglutination Inhibition (HI) Test Assay

The assay was performed by using the procedure as described by( 12). The results were recorded as follows. **Positive HI:** The red blood cells were formed clear and compact button at the bottom of wells of micro-titration plate. **Negative HI:** Red blood cells were uniformly distributed and no button formation at the bottom of wells of micro titration plate.

### 3.4 $EID_{50}$ Determination by using embryonated eggs.

$EID_{50}$ was performed by using the procedure as described by (13). Reed and Muench formula was used to calculate the EID50 Index which is as under:

$$Index = \frac{(\%\text{ infected at dilution immediately above } 50\%) - 50\%}{(\%\text{ infected at dilution above } 50\%) - (\%\text{ infected at dilution immediately below } 50\%)}$$

### 3.5 Experimental birds and management:

Twelve birds of each breed/species (day old broilers, Aseel chickens & wild pigeons) were purchased from local market and kept under standard condition of rearing . The birds of each group were divided into three groups named A, B & P with three replicates in each group.

### 3.6 Infection of Experimental birds with VVNDV:

The birds were infected intramuscularly and orally with VVND virus. The actual dose in terms of $EID_{50}$ was determined by performing $EID_{50}$ in 9-11 days embryonated eggs. The birds were observed daily for any pathological changes.

### 3.7 Postmortem findings:

Postmortem was performed of dead birds during the diseased development for studying various lesions on visceral organs like trachea, proventriculus, caecal tonsil, intestine and other organs that were found affected during necropsy of broiler, Aseel chickens and wild pigeons. The severity of the lesions were scored as light (+), moderate (++) or severe (+++).

### 3.8 Tissue Processing

The Histopathological Examination was performed by initially fixing the samples in the 10% formalin for twenty four hours and then washing, labeling cassettes before transferring into basket of linear automatic tissue process ( model: OPTI-WAX SCILAB ).Tissues were dehydrated in a graded series of ethanol and cleared in xylene for 30 minutes before embedding them in the melted paraffin wax bath (Merck) at 65 $^{o}$C .Tissue embedding was done by using a tissue embedding center (HT company, UK) . Sectioning was made by trimming tissues into thin sections i.e. 5 µm by microtome and fixed to glass slides in warm bath. The slides with tissue sections were stained by an automatic slide stainer (HMS 70). The containers of machine were filled with required volumes and concentrations of various reagents and after processing cover slipped.

### 3.9 Statistical Analysis

On completion of the study, the data obtained were tabulated and statistically analyzed using computer software named Student Edition of Statistics (SXW), Version. 8.1 (Copyright 2005, Analytical Software, USA).

## 4. RESULTS

### 4.1 Haemagglutination inhibition assay (HI titration)

The data indicates that on day 7 the H.I. titer in pigeon was 6.75±1.25, 10.00±0.81 and 4.50±0.25 in group P1, P2 and P3, respectively. On day 10 H.I. titers were 7.75±1.18, 10.50±0.50 and 5.25±0.47, in P1, P2 and P3groups. It was further indicated that H.I. titers were significantly higher on day 10 as compared to day 7 in all respective groups. The analysis (ANOVA) indicated that significant difference was noted among the groups P1, P2 and P3 (appendix-4.1). While, significant difference in H.I. titer were seen between P1 and P2 and non-significant difference between the groups P1 and P3 on day 7 and day 10, respectively.

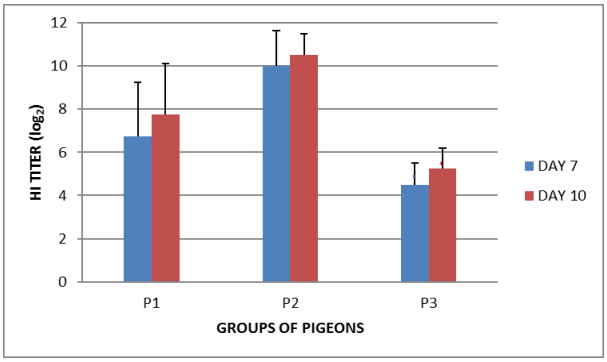


**Figure-4.1.1 H.I. titer of pigeons on day 7 and day 10, post-inoculation with VVNDV.**

**4.2 Embryo Infectious Dose$_{50}$**

The EID50 was determined by performing HA of the AAF of all the eggs and $EID_{50}$ was calculated by using Reed and Muench formula [16]. $EID_{50}$ was found to be $10^{14.3}$ / ml.

**4.3 Body temperature**

There was no statistically significant difference in cloacal temperature between birds of all groups on day 0 (before inoculation). However, it gradually increased with the passage of time and then decreased in the same fashion. Statistical analysis showed non significant difference among the groups at the final stage.

**4.4 Feed Intake and water intake**

Results showed statistically significant (P>0.05). There was no statistically significant difference in feed intake between birds of all groups on day 0 (before inoculation). However, feed intake gradually decreased in all the groups and there was no significant difference at the end of trial. Similarly there was no significant difference in the water consumption among all the groups. It decreased slowly after virus inoculation.

**4.5 Clinical Findings**

The statistical analysis demonstrated significant (P<0.05) difference in clinical score between the groups. LSD pair-wise confirmed that significant (P<0.05) difference in clinical score were examined among the group B1, B2 and B3 and non-significant (P>0.05) between groups A1, A2 and A3. (Data not shown)


**Figure-4.6.2. Chicken showing clinical signs of VVNDV infection including: conjunctivitis (a), oral and nasal secretion (b) diarrhea (c),**

**.6 Mortality Rates**

Our results showed that the mortality rate in Aseel infected with VVNDV was found 0, 0, 8, 42, 25 and 25% on days 0, 1, 2, 3, 4 and 5 respectively (figure-4.7.1). The mortality rate in broiler infected with VVNDV was found 0, 0, 0, 58, 8, 17, and 17% on days 0, 1, 2, 3, 4, 5 and 6 respectively. While in pigeons, mortality was found 0. Statistical analysis of variance indicated non-significant (P>0.05) difference in death time among the groups A1, B1, A2, B2 and A3 and significant (P<0.05) in group B3. While, difference in death time between groups were significant (P<0.05).

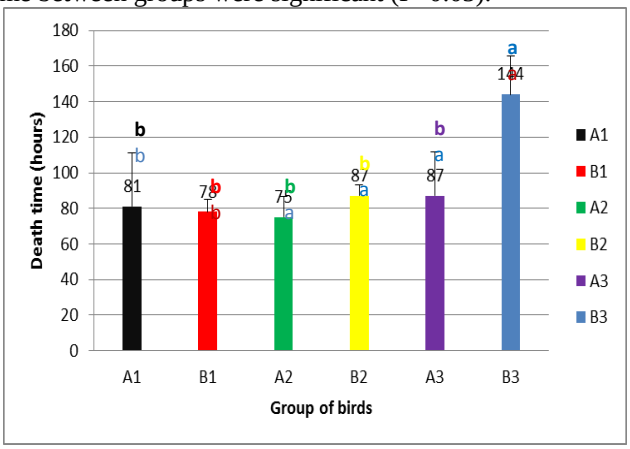


**Figure-4.7.1. Mean death time post-inoculation of VVNDV of Aseel and broiler**

**.4.7 Post mortem lesion scores:**

Our results showed prominent hemorrhagic, pateicheal, necrotic lesions filled plaques and exudates in various organs such as proventriculus, small intestine, cecal tonsils, trachea.spleen and bursa. However there was no significant difference in the post-mortem lesions of the birds due to VVNDV infection **Figure-4.8.1,**

**4.8 Histopathological Examination**

The histopathology lesions in the various organs and their frequency of occurrence were noted. It showed congestion, hemorrhages and degeneration of lymphytes in lymphoid proventriculus mucosa. The lymphoid tissues in the affected segments of the small intestine were necrotic histologicaly. There was massive destruction of intestinal lymphoid areas. Ulceration of overlying intestinal epithelium was extensive. Histopathology section of the swollen tonsils revealed haemorrhages, congestion and necrosis in the lymphoid tissue and sloughing off the necrotic tissue. Spleen showed necrotic lymphoid follicles of the spleen. Microscopic lesions found in bursa were characterized by severe lymphoid depletion along with lympho-cellular necrosis and apoptosis.

## 5. DISCUSSION

The present research was designed to study the susceptibility and transmission of VVNDV in Aseel, and broiler chicken along with wild pigeons and its comparative pathology.

In the present work, the comparative pathology was studied by measuring clinical parameters like cloacal temperature, feed and water intake and scoring intensity of clinical signs. Moreover, mean death time (hours), necropsy findings and histopathological findings were noted and compared. Finally, anti-VVND virus antibodies were measured in wild pigeons as these proved resistant to disease and showed neither significant clinical signs nor any mortality.

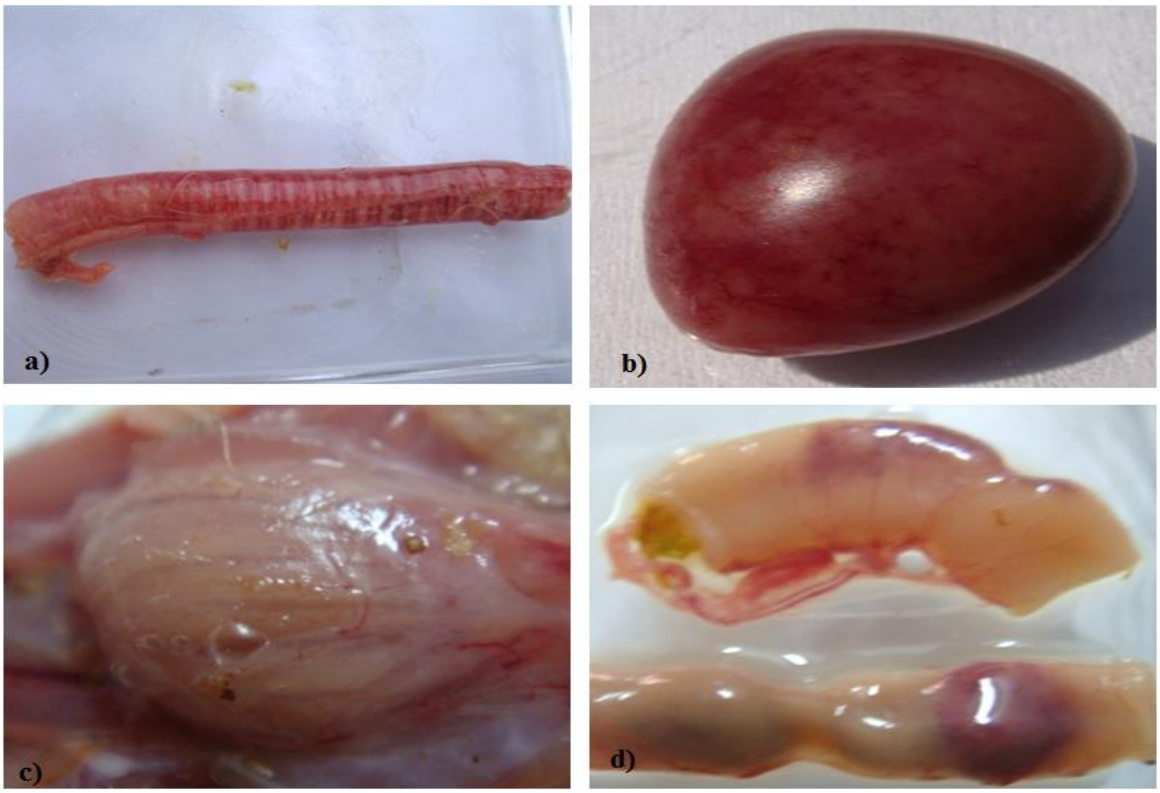


**Figure-4.8 Post-mortem lesions of the birds due to VVNDV infection: Showing hemorrhages ulceration on proventriculus (a), trachea (b), spleen (c), bursa (d), small intestine.**

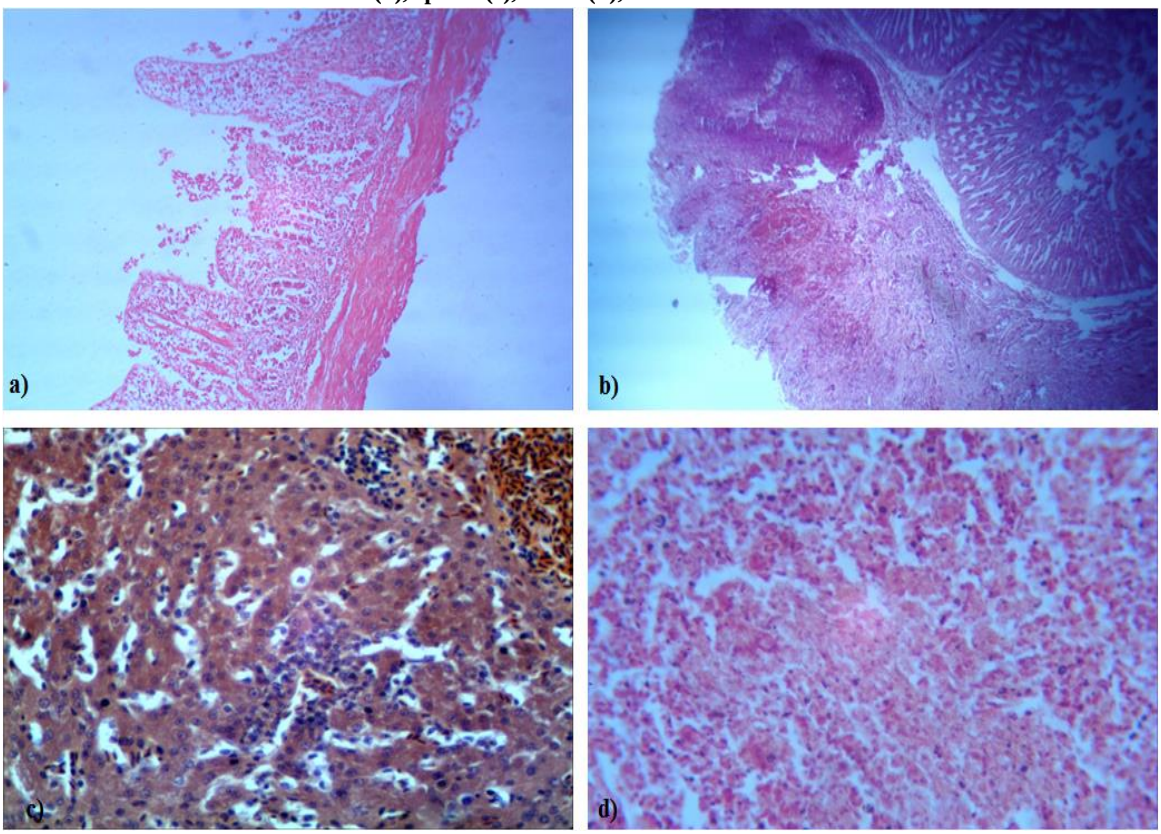


**Figure 4.9: Histological lesions on various chicken organs affected by VVNDV Infection. Intestine of broiler chicken infected with VVND virus showing necrosis of villi (arrow). Intestine of broiler chicken infected with VVND virus showing necrosis of villi (arrow). Proventriculus showing heavy infiltration of mucosa with inflammatory cells mostly heterophils and mononuclear cells. Spleen of broiler showing swelling and oedema in the vascular wall of the follicular blood vessels.**

Birds are homeotherms and in viral diseases, the body temperature rises due to the increased in viral replication and viraemia [14,15]. There was an increase in body temperature post-inoculation of VVNDV which is in line with the findings of [14, 16,17]. Moreover, the route of exposure also showed variation in the time of rise in temperature. Parenteral (intra-muscular) route of inoculation leads to viremia faster than both by oral inoculation or contact-exposure.

A reduction in feed intake during VVNDV exposure may be linked to inflammation of gastrointestinal tract virus is known to replicate primarily in the digestive tract of the birds which is evident from our gross and histopathological findings of GIT. Water intake is usually linked with feed intake. The consumption of water in birds is double of the feed intake. Deceased feed intake also reduces the water intake of the birds. A decrease in water intake of pigeon, Aseel and broiler was noted on day-2 post-inoculation of VVNDV. These results are supported by (24) who stated that after inoculation of VVNDV the birds lose their water intake capacity and water intake is reduced. The reduced water intake post-inoculation of VVNDV has also been reported by previous studies (18,19).

We also observed more severe clinincal signs Infection of chickens with velogenic Viscerotropic Newcastle Disease viruses produced severe systemic illness, with marked depression, ruffled feathers, whitish-greenish diarrhea, nasal and oral secretion and conjunctivitis. Similar clinical signs have been described by previous researchers in chickens infected with VVNDV (17,20)

In the present study the clinical signs were more severe in those groups of birds which were exposed orally with VVNDV infection than intramuscular and sentinel exposure. Clinical signs were slower in the sentinel birds as they can only get virus from the infected birds once shedding starts as the number of viruses entering sentinel birds is much lower in contact-exposure than the dose inoculated via intra-muscular or oral route. The results are similar to those of previous researchers which described that the nature route of the virus shows the severe clinical signs (1).The clinical signs, are similar to what have been previously reported in birds infected by the Viscerotropic Velogenic ND virus [12,16,21,23].

The mortality rate is the percentage of animals which are found dead compared with the total number of animals exposed to infection. In the current study after challenging pigeon, Aseel and broiler with VVND virus infection, the disease was successfully reproduced and mortality occurred among Aseel and broiler and no mortality was recorded in pigeon. It is important to emphasize that all the pigeon groups did not show any clinical signs of the disease by the VVND virus stain used in the present study throughout the whole experimental period and no mortality occurred. This type of result was also reported in previous studies in pigeons (21). The maximum mortality (100%) was recorded in Aseel and broiler at day-5 and day-6 post infection. The results were conformed with the findings of (22). Moreover, mortality started earlier in Aseel than in broiler chickens which points towards greater susceptibility of Aseel to VVNDV infection.

It was further indicated that H.I. titers were significantly higher on day 10 as compared to day 7 in all respective groups. All the pigeons that had contact with VVNDV either infected or sentinels showed subclinical infection. Same results of transmission were also reported in previous studies in several avian species, such as chickens [24, 6, 25,8] and domestic pigeons [24,6]. Thus our results are not in congruity with [34]. Results obtained are very important to compare the difference among strains of NDV, responsible for outbreaks in commercial poultry from different countries, evaluating the virulence for many avian species. In some aspects, our results are in agreement with previous studies [6, 27, 26] that showed viral excretion, detected by virus isolation.

Gross lesions observed in most of the chickens infected with VVNDV by using different routes of inoculation in this experiment produced severe hemorrhagic lesions in the proventriculus and intestine and all chickens died by D6 post-inoculation. These results are in line with the findings of (17), which described the systemic infection and acute death of chickens infected with VVND virus. The post-mortem lesions in infected chickens included the enlargement and atrophy of the lymphoid organs, swelling of the inner lining of the eyelid, reddening and haemorrhage on the trachea and gastrointestinal tracts ulceration. Same lesions were reported in lymphoid and other organs by [19,28].

Histological lesions which have been previously described in the chickens infected with VVND virus were almost similar to those of chickens infected with VVND virus in this study. Most chickens infected with VVND virus in this experiment had lymphoid cellular necrosis of spleen and bursa of Fabricious which have been previously described in the literature (29). Therefore, lymphoid cellular necrosis of immune organ may be common histological lesions of chickens infected with VVND viru (30). There were haemorrhages in the proventriculus, intestines and caecal tonsils of chickens, which are the findings similar to observations of [31[.

Conclusively,VVNDV can infect both broiler and Aseel chickens leading to 100% mortality. Intra-oral route is the natural route of all the birds. That is way, HI titers, post-mortem scores and clinical finding scores are higher in those groups which were intra-orally inoculated VVND virus. VVNDV infection produces lesions on proventriculus, small intestine, trachea, spleen and bursa of infected broiler and Aseel chickens. Susceptibility to the VVND virus varies among wild pigeons, broiler and Aseel chickens. All the pigeons that had contact with VVND virus either infected or sentinels might be infected without showing any clinical sign of the disease. Wild pigeons may carry highly virulent VVNDV without overt signs of clinical disease. With other transmitting factor, Wild pigeons and Aseel chickens can play a great role for the transmission of VVNDV infection.

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