

Original Article

Clinico-pathological assessment of virulent Newcastle Disease Virus in ducks

Avaliação clínico-patológica do Vírus da Doença de Newcastle em patos

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Abstract

Newcastle disease (ND) is an infectious, highly contagious and lethal disease of avian species. It is considered that ducks are natural reservoir or carrier for Newcastle disease virus (NDV) and are resistant against different strains of NDV. Current study was designed to evaluate the pathogenesis of Newcastle disease in domestic ducks through histopathology, immunohistochemistry (IHC) and serum biochemical changes. For this purpose, eighty ducks were reared for 42 days and divided in two groups A and B. Ducks in group A were challenged with (NDV) at rate of 0.1 ml of ELD50 (virus titer $10^{7.32}/100\mu\text{l}$) on second week of age, whereas Group B was control negative. Splenomegaly, atrophy of thymus and necrotic lesion in kidney were observed on 9th day of post infection. Hepatic degeneration and mononuclear cell infiltration were noticed in proventriculus and intestine in challenged ducks. Viral antigen detected in lungs, intestine, proventriculus and lymphoid organs of infected ducks through IHC. Albumin and total protein values were significantly low in infected groups A as compared to control group B. ALT, AST, and ALP values were significantly high in infected group A. On 5th and 7th day of post infection oropharyngeal swabs were negative for NDV and cloacal swabs were positive for NDV through Reverse transcriptase polymerase chain reaction. It is concluded that ducks are susceptible to NDV and virulent strain of NDV caused disease in ducks.

Keywords: ducks, Newcastle Disease Virus (NDV), histopathology, polymerase chain reaction, serum Biochemistry.

Resumo

A doença de Newcastle (DN) é uma doença infecciosa, altamente contagiosa e letal de espécies aviárias. Considera-se que os patos são reservatórios ou portadores naturais do vírus da doença de Newcastle (VDN) e são resistentes a diferentes cepas de VDN. O presente estudo foi desenvolvido para avaliar a patogênese da DN em patos domésticos por meio de histopatologia, imuno-histoquímica (IHQ) e alterações bioquímicas séricas. Para este propósito, 80 patos foram criados por 42 dias e divididos em dois grupos A e B. Os patos do grupo A foram submetidos ao VDN a uma taxa de 0,1 ml de ELD50 (título viral de $107,32 / 100 \mu\text{l}$) na segunda semana de idade, enquanto o Grupo B foi controle negativo. Esplenomegalia, atrofia do timo e lesão necrótica no rim foram observadas no 9^o dia pós-infecção. Degeração hepática e infiltração de células mononucleares foram observadas no proventrículo e intestino em patos infectados. Antígeno viral foi detectado em pulmões, intestino, proventrículo e órgãos linfóides de patos infectados por IHQ. Os valores de albumina e proteína total foram significativamente baixos no grupo A infectado em comparação com o grupo B. Os valores de ALT, AST e ALP foram significativamente altos no grupo A. No 5^o e no 7^o dia após a infecção, os esfregaços orofaríngeos foram negativos para VDN, enquanto os esfregaços cloacais foram positivos para VDN por meio da reação em cadeia da polimerase via transcriptase reversa. Conclui-se que os patos são suscetíveis ao VDN e à cepa virulenta de VDN que causou doenças em patos.

Palavras-chave: patos, Vírus da Doença de Newcastle (VDN), histopatologia, reação em cadeia da polimerase, bioquímica do soro.

1. Introduction

Newcastle disease (ND) is a highly fatal disease of the birds. ND virus belongs to genus *Avulavirus* and family *Paramyxoviridae* (Mayo, 2002). Its genome is a

non-segmented, single stranded and negative-sense RNA molecule. Considering poultry, turkey and chicken are highly to susceptible to disease and duck and geese are

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very less likely to susceptible to the various strains of NDV. Ducks and geese are natural reservoir for NDV. (Alexander and Senne, 2008). Many NDV strains of different virulence have been isolated from diseased ducks (Zhang et al., 2011). Some isolates were pathogenic for ducks, and ND cases in ducks have been gradually increased in recent years (Song et al., 2007; Liu et al., 2010).

Only few duck and chickens can die due to NDV virus (Shi et al., 2011). When ducks get infected with virulent NDV they showed histopathological normalities in the immune system (Anis et al., 2013). NDV disease causes splenomegaly and lymphoid follicles of thymus and bursa severely damaged in chicken, mild to moderate changes observed in ducks. Newcastle disease showed symptoms in ducks more passive conjunctivitis, slight depression, neurologic signs, cloudy eyes, and blindness. Congestion observed in liver and lung tissue of ducks (Brojer et al., 2013). Hepatic tissue necrosis and degenerative changes resulted in significance increase of alanine aminotransferase (ALT), total protein and aspartate aminotransferase (AST) Mahmoud (2015). Therefore, aim of present study was to evaluate the pathogenesis of Newcastle disease in domestic ducks through histopathology and serum biochemical changes for estimation of liver and kidney damage by NDV in Punjab Pakistan.

2. Material and Methods

2.1. Newcastle Disease Virus (NDV)

The challenged virus was virulent NDV (2981533-2-ND/BK1) strain was inoculated into 9-11 days old embryonated chicken eggs through the way of allantoic cavity. Egg inoculation, candling, incubation and virus harvesting were performed in agreement with the Manual method and technique (OIE, 2012). The collected allantoic fluid was confirmed by haemagglutination (HA) test and haemagglutination inhibition (HI) test (Rasool et al., 2015). A Specific motif at the cleavage site of the F protein is considered important factor of NDV Pathogenicity. Specific primers of 238bp targeted hypervariable region F gene used for identification of NDV virulent strain. EID₅₀ of isolated NDV was calculated according to procedure as described by Reed and Muench (1938).

2.2. Experimental design

Eighty ducks were purchased from tollinton market Lahore. The ducks were divided into two groups A and B each contains forty ducks. They were reared as per standard management for feeding (grower feed with CP 20%), watering, light as well as routine care. The ducks in group A were challenged with NDV at rate of 0.1 ml of EID₅₀ (virus titer 10^{7.32}/100µl) on second week of age through eye route (Bharathi et al., 2018). Second group B was control without infection. All ducks were observed twice daily throughout the trial till 42 days and clinical signs were observed (Lu et al., 2014).

2.3. Histopathological examination

Three ducks from each group were slaughtered on 3rd, 7th, 9th and 14th days of post infection of Newcastle Disease Virus (NDV). Gross as well as histopathological lesion on lungs, kidney, proventriculus, intestine and liver were observed and tissue samples were preserved in 10% formalin for histopathological study. These tissues samples were processed with standard techniques for fixation, dehydration, clearing, embedding, sectioning and staining (Etriwati et al., 2017).

2.4. Immunohistochemistry

Immunohistochemistry of paraffin embedded tissues of liver, kidney, lungs and intestine were performed by using Mouse and Rabbit Specific HRP/DAB (ABC) Detection IHC kit (LUCERNA-Chem) according to manufacturer. Anti-NDV hyperimmune serum raised in rabbits.

2.5. Serum biochemistry

The blood samples were collected from five ducks of each group on 3rd, 5th and 7th days of postinfection. Three ml blood was collected through intra cardiac and jugular vein route by using a small needle of 23 G and syringe of 3.0 ml. Serum was separated from clotted blood and preserved at -20°C. Liver enzymes including Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Total protein (TP) and Alanine Aminotransferase (ALT) were analyzed by using Human kit according to manufacturer protocol.

2.6. Reverse transcriptase polymerase chain reaction

The post infection cloacal and oropharyngeal swabs were collected on 3rd, 5th and 7th day of postinfection from groups A and B. Reverse transcriptase polymerase chain reaction was performed. RNA was extracted from collected swabs by using Favorgen (FavorPrep™ Mini kit). cDNA was synthesized using first strand cDNA synthesis Kit® (Fermantas, USA) according to manufacturer's instructions. Specific primers of 238bp F.TGCTGATGCTGTGATA R.TGCTGATGTAGCTGCT targeted hypervariable region F gene used for amplification (Shabbir et al., 2013)

3. Results

In present study no clinical signs were observed in first twenty four hours of post infection (PI) in challenged group A. Ducks of group A showed clinical signs of listlessness, anorexia, and greenish-white diarrhea after 48hrs of (PI). All ducks challenged to Newcastle Disease Virus were dull, depressed, feed and water consumption was decreased and had ruffled feathers at 9th days post infection (DPI). Most deaths occurred during the period of day 9 to day 14 PI. Ducks were slaughtered on 3rd, 7th, 9th, 14th, day of post challenge for gross and histological lesions observation. Ducks (*AnasPlatyrhynchosdomesticus*) of group A were challenged with NDV showed severe congestion in lungs. The excessive hemorrhages were observed in mucosa of small intestine (duodenum and upper part of jejunum) on 7th day of post infection. Severe congestion were observed

on 3rd day of post infection in liver. Splenomegaly, atrophy of thymus and necrotic lesion in kidney observed on 9th day of post infection as compare to control ducks in group A.

Mild lymphoid depletion was observed in spleen on 3rd day of (PI) in duck of group A. On 7th day of PI sever congestion and leukocytic infiltration of lungs observed (Figure 1a). Hepatocytes degeneration (Figure 1b) and few hepatocytes have microvacuolation resulted in compression of sinusoidal spaces on 7th day of PI. Hemorrhages and congestion observed in liver and on 9th and 14th day of PI (Figure 1c). Moderate mononuclear cell infiltration was noticed in proventriculus and intestine (Figure 1d).

Tubular necrosis, leukocytic infiltration and congestion of kidney observed on 9th day of PI (Figure 1e). Marked leukocytic infiltration observed in spleen (Figure 1f). Immunohistochemical staining of NDV infected intestine revealed the tan color antigen on 4th day of PI with extensive necrosis and degeneration of villi (Figure 2). NDV antigen mainly virus was detected in the center of the lymphoid follicles of proventriculus in (Figure 2b). Serum biochemical parameters of Alkaline phosphatase (ALP), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and Total protein and Albumin values were evaluated in (Table 1). Albumin and total protein values were significantly

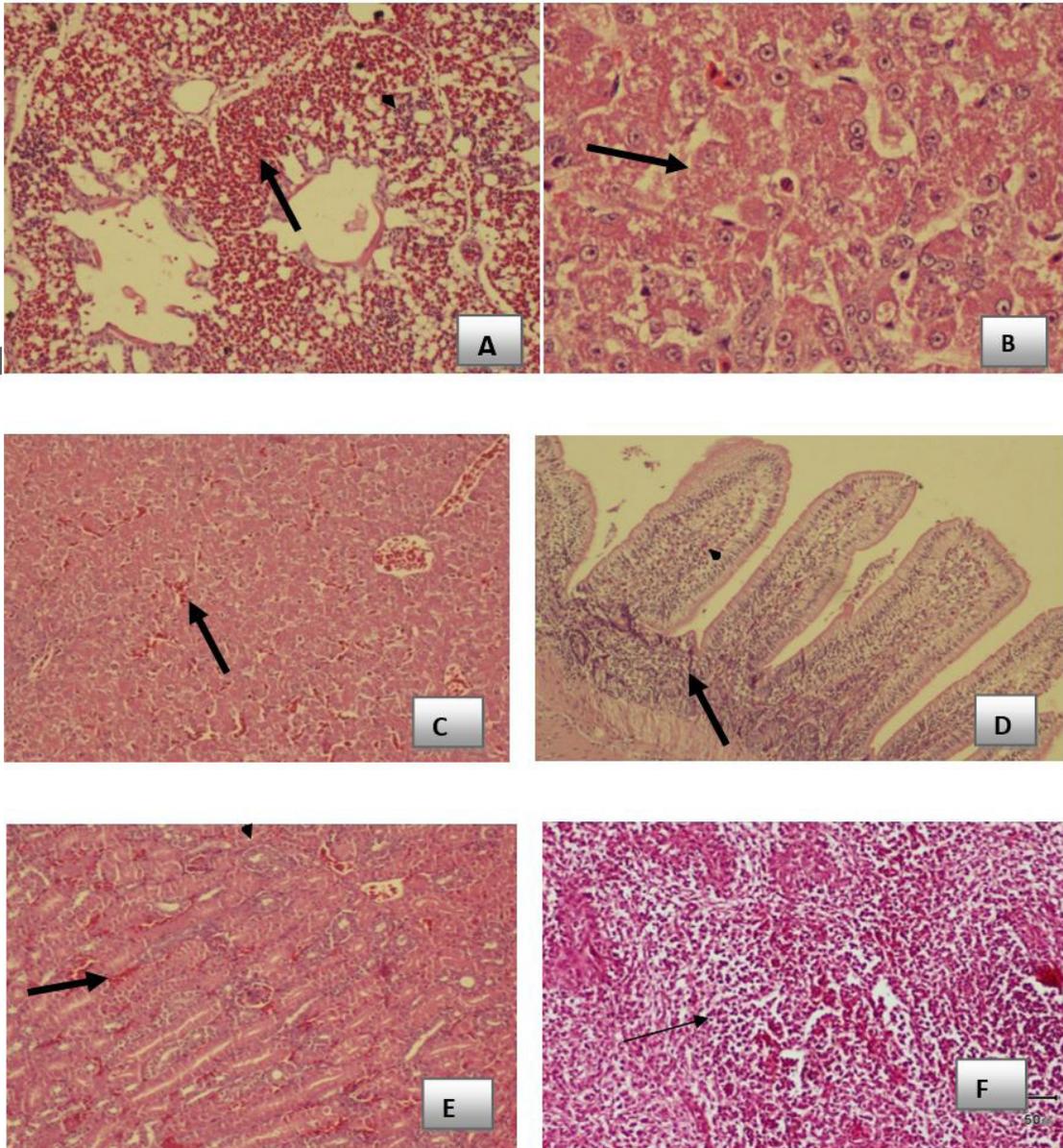


Figure 1. A. H&E staining of Lung from group A at 7th day pi. Sever congestion , Leukocytic infiltration and Bronchi epithelium is sluffed off. B. Liver from group A at 7th day pi. Hepatocytes degeneration in the liver. C. liver from group A 9th day pi. Severe congestion in liver. E. Extensive infiltration of leucocytes and few RBCs present lamina propria and epithelium of intestine. D. Kidney from group A at 9th day pi. Sever congestion, monocuclear cell infiltration and tubular necrosis in kidney. F. Marked leukocytic infiltration observed in spleen.

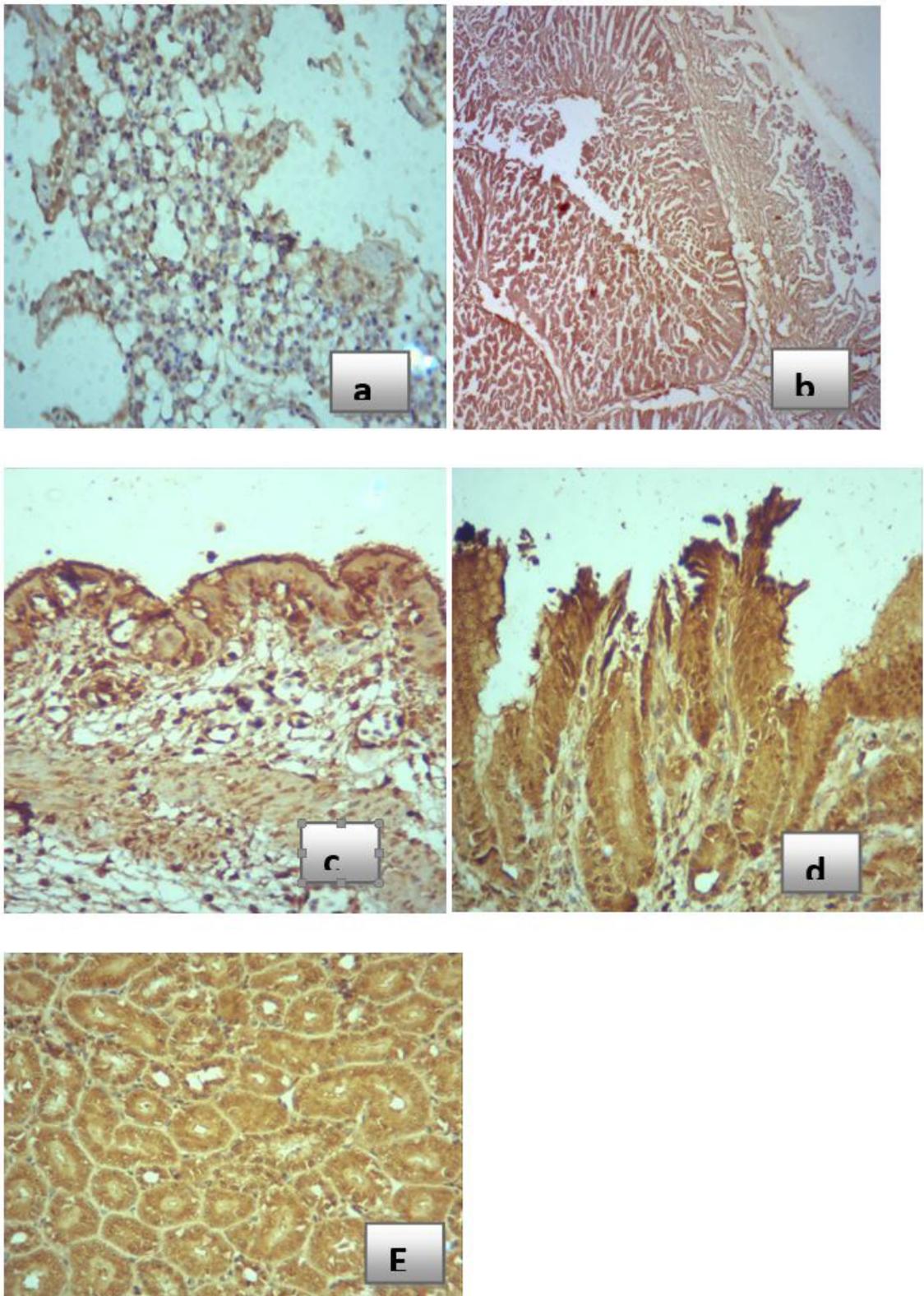


Figure 2. A.Viral antigen in both bronchiolar and alveolar lining cells of lungs of ducks 2.b. Antigen mainly virus was detected in the center of the lymphoid follicles of proventriculus. 2.c Duck Intestine showing enteritis and marked necrosis of mucosa. 2.d. Columnar Epithelium of intestine sloughed off. Antigen detected in mucosal glands. 2.e. antigen detected in renal tubules on 5th day of postinfection.

low in infected groups A as compared to control group B. ALT, AST, and ALP values were significantly high in infected group A. Cloacal and Oropharyngeal swabs were collected on 3rd, 5th and 7th day of (PI) from groups A and B. On 3rd day of PI, cloacal swabs of ducks from Group A were positive for NDV through RT-PCR (Figure 3). On 5th and 7th day of post infection oropharyngeal swabs of Group A were negative for NDV and cloacal swabs were positive for NDV as compared to control group B (Table 2).

4. Discussions

Newcastle disease is highly fatal viral infection that affecting different species of birds, including domestic and

wild birds. It has a considerable economic impact on poultry industry. It considered that ducks are natural reservoir or carrier for NDV and having resistant against different strains of NDV. But new cases of Newcastle disease in ducks are gradually increasing. So, present project was designed to evaluate the pathogenesis of Newcastle disease in domestic ducks through histopathology and serum biochemical changes. Ducks challenged with NDV in group A showed clinical signs, gross lesions and histopathological lesions of NDV as compared to control group B. These findings were similar to previous study of (Anis et al., 2013). Disease produced in challenged ducks presents following clinical signs of anorexia, greenish diarrhea, depression, weight loss, ruffled feathers, and prostrations. These findings were matched with previous study of Dai et al (2014). Ducks of group A were challenged with NDV and lungs of this group showed severe congestion on ventral side. The excessive hemorrhages were observed in mucosa of small intestine on 3rd day of post infection. Spleen was little atrophic and enlarged observed on 7th day of post infection. Severe hemorrhages and atrophy of thymus and bursa observed. Small petechial hemorrhages were seen in kidney. Necrotic lesions were observed in lungs on 9th day post infection in infected group A as compared to control group Shi et al (2011). Severe congestion was observed in liver on 9th day of post infection. Many birds show congested lungs on 9th day of post infection. These findings are justified with finding of Anis et al (2013) and Dai et al (2014). Serum biochemical parameters like alanine aminotransferase (ALT) total protein, aspartate aminotransferase (AST) and alkaline phosphates (ALP) were

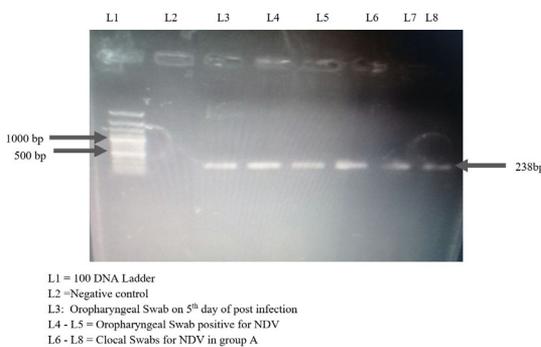


Figure 3. NDV virus detection in different swabs samples of infected Ducks.

Table 1. Serum concentration of Albumin, Total Protein, ALT, AST and ALP in different treatment groups on 3, 5 and 7 day of post infection.

Days	Groups	3 day	5 day	7 day
Albumin (g/dl)	A	1.59 ^a	1.47 ^b	1.39 ^b
	B	1.83 ^a	1.86 ^a	1.84 ^a
Total protein g/dl	A	4.10 ^a	3.88 ^b	3.51 ^b
	B	4.43 ^a	4.12 ^a	4.54 ^a
Alanine Aminotransferase (ALT) u/L	A	254 ^a	278 ^a	290 ^a
	B	180.2 ^b	184 ^b	181 ^b
Alkaline Phosphatase (ALP) u/L	A	235 ^a	239 ^a	245 ^a
	B	152 ^b	155 ^b	158 ^b
Aspartate Aminotransferase (AST) u/L	A	310 ^a	329 ^a	353 ^a
	B	174 ^b	179 ^b	188 ^b

Group A: Ducks Infected with NDV Group B: Control Ducks . Values Showing Different Superscripts within a Column Differ Significantly (P<0.05).

Table 2. NDV detection from swabs samples of ducks on 3rd, 5th and 7th days of Post infection.

Days	Swab Samples GROUP A		SWAB SAMPLES GROUP B	
	Oropharyngeal Swabs	Cloacal Swabs	Oropharyngeal swabs	Cloacal swabs
3 rd Day of PI	++	+	-	-
5 th Day of PI	-	++	--	---
7 th Day of PI	-	+	---	----

measured on 3rd, 7th, 9th, day of post infection in infected groups. Total protein values were reduced in NDV infected group on 7th day of postinfection as compared to control group. Total protein level decreased due to impaired liver function and kidney damaged also caused to protein loss (Kaslow, 2011). This may be due to insufficient take of protein in diet or diarrhea (Ihedioha and Chineme, 2005). The AST and ALP were significantly increased in challenged birds, as in NDV infection liver and kidney was adversely affected that leads to increase of serum enzymes. Similar findings were reported by (Chekwube et al., 2014).

5. Conclusion

The results of present study concluded that ducks are not only reservoir and carrier for Newcastle disease virus, but they are also susceptible to NDV and virulent strain of NDV caused diseased in ducks as pathological lesions in lymphoid and non lymphoid organs were observed through histological and immunohistochemical techniques. It is also concluded that ducks played important part in epidemiology of Newcastle disease. This situation indicated that prevention of spread of NDV in ducks should give more attention.

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