Pak. j. sci. ind. res. Ser. B: biol. sci. 2023 **66B**(3) 239-248

Pathology of Experimental Velogenic Viscerotropic Newcastle Disease (VVND) in House Sparrows and Australian Parrots

Sajid Khan[a], Zaheer Ahmed Nizamani[a], Muhammad Faisal Ayoob[a,b]*, Mansoor Ayoob[c] and Jamil Ahmed Gandahi[d]

[a]Department of Veterinary Pathology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tando Jam-70060, Pakistan

[b]National Veterinary Laboratories, Ministry of National Food Security and Research, Islamabad-44000 Pakistan

[c]Department of Animal Products Technology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tando Jam, Tandojam-70060, Pakistan

[d]Department of Veterinary Anatomy and Histology, Faculty of Animal Husbandry, Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tando Jam, Tandojam-70060 Pakistan

(received March 11, 2021; revised November 22, 2021; accepted December 9, 2021)

Abstract. The pathology of experimental VVND was studied in twelve birds of house sparrows (S) and Australian parrots (P) which were divided into 6 equal (n= 4) experimental groups *viz*. S1, S2, S3, P1, P2, P3 and one group of broilers (C). The groups S1 and P1 (intramuscularly), groups S2, P2 and C (orally) were administered with 0.3 mL of VVND virus ($2.3\times10^{10.73}$ EID_{50}). The groups S3 and P3 were kept in contact exposure with group C. The susceptibility and pathology were compared by measuring parameters like clinical scores, mortality rate, mean death time (days), necropsy findings, lesion scores and histopathological findings. Moreover, anti-VVND virus antibody (HAI) titers were measured in surviving birds. Yellow greenish diarrhoea, messy feathers, torticollis and anorexia were the main clinical signs observed in groups S1, S2, P1 and P2. However, no symptoms were observed In groups S3 and P3. The mean clinical scores of the three groups were statistically non-significant ($P > 0.05$). In contrast to Australian parrots, house sparrows have a higher mortality rate. The mortality rate observed in groups S1, S2, P1, P2, S3 and P3 was 100%, 75%, 50%, 50%, 0% and 0% respectively. The difference in mean death time (days) was not statistically meaningful ($P > 0.05$). The proventriculus, small intestine, spleen and trachea of all the infected birds had histopathological lesions. Precise hemorrhages in the proventriculus, defined button like lesions of the intestinal epithelium and necrosis in the trachea and spleen are among the necropsy lesions of infected birds. Haemagglutination inhibition (HAI) titer on day 21 was found to be higher in all the experimental groups. In conclusion, both house sparrows and Australian parrots are vulnerable to experimental VVNDV infection intramuscularly and orally, with house sparrows being more susceptible. Sero-conversion was seen in contact exposed groups without VVND clinical signs.

Keywords: antibody titer, australian parrot, newcastle disease, pathology, sparrow

Introduction

Poultry is one of the fast growing industry and Pakistan is 11th largest poultry producers in the world and employs over 1.5 million people. It accounts for 35 % of the country's overall meat supply. The poultry industry is currently spending over Rs. 700.00 billion. The poultry industry has experienced strong annual growth of 8-10%, demonstrating its intrinsic promise (Ayoob *et al*., 2021).

Currently, the poultry industry in Pakistan is facing the challenges of several viral, bacterial and fungal diseases. Among viral diseases, newcastle disease (ND) is the most lethal disease which causes heavy economic losses to the industry. Newcastle disease is a highly contagious and infectious disease that affects a wide range of wild and domestic bird species . Because of their high vulnerability to the ND virus and the extreme effects of outbreaks of virulent strains on the poultry industry, the ND has the greatest impact on commercial and backyard poultry. Indeed, it has been discovered that ND may be more expensive for the global economy than any other animal viral disease (Khorajiya *et al*., 2015). The first outbreaks of newcastle disease were reported in 1926 in Java (Indonesia), Ranikhet (India) and newcastle upon-Tyne (England). The name "Newcastle Disease" was coined after the geographical location of the first outbreaks in great Britain (Ayoob *et al*., 2021).

*Author for correspondence; E-mail: ayoob.faisal@yahoo.com

Newcastle disease is synonymous with avian paramyxo virus Type 1, belonging to the family Paramyxoviridae and a member of the genus Avulavirus is a non-segmented single stranded, negative sense enveloped RNA virus (Suarez, 2013). Upto date, nine serotypes of avian paramyxoviruses have been identified i.e., APMV-1 to APMV-9. Among these, five serotypes can cause disease in poultry, which include APMV-1, APMV-2, APMV-3, APMV-6 and APMV-7. The most pathogenic serotype among these is APMV-1 and is mainly used for Newcastle disease. Based on virulence and severity of infection in chickens, these serotypes are being classified into three groups, velogenic (highly virulent), mesogenic (moderately virulent) and lentogenic (low virulence) (Costa-Hurtado *et al*., 2015). In chickens, lentogenic strains cause mild or in-apparent respiratory infections, the mesogenic strains produce low mortality, with severe respiratory disease and neurologic symptoms. The velogenic strains are further classified into either neurotropic velogenic NDV which causes high mortality in infected birds accompanied by respiratory and neurologic signs or velogenic viscerotropic NDV which produces acute fatal infections with high mortality and necro-hemorrhagic lesions which are most prominent in the gastrointestinal tract (Kabiraj *et al*., 2020). Among the total avian species, about 236 species from 27 of the 50 orders of birds are exposed to NDV (Absalón *et al*., 2019; Rehan *et al*., 2019; Kapczynski *et al*., 2013). NDV can infect different species of birds and almost all species of birds are likely at risk, but the severity of the disease varies among different species, i.e. chickens are more sensitive whereas ducks and geese are least sensitive (Hicks *et al*., 2019; Elmberg *et al*., 2017) .

In NDV, clinical symptoms observed in infected birds are asymptomatic carriers to different ranges of high morbidity and mortality (upto 100%). Disease severity depends on the virulence of the virus tropism, age of the host species, immune status, other diseases and factors such as age, environmental conditions (Susta *et al*., 2018; Brown and Bevins, 2017). The incubation period of ND is 2-3 days and, on rare occasions, 2-15 days. Typical characteristics of the NDV include rapid spread, death within 2-3 days and high mortality rate (Su *et al*., 2018). Young birds are usually more prone to infection but the disease is causing heavy losses in birds of all ages. Depending on where the virus has its affinity site and symptoms can be observed in the digestive tract, respiratory system and nervous system. When the respiratory system is involved, the common symptoms are gasping, coughing, sneezing and rales. However, with the involvement of the nervous system, several nervous symptoms are seen which include complete or partial paralyzed wings and legs, torticollis, tremors, circling and convulsion. Other frequent clinical signs mostly observed are greenish diarrhoea, depression, loss of appetite, partial or complete drop in egg production and deformities in the production of eggs (Susta *et al*., 2018).

Upon postmortem examination of the dead birds, the most predominant findings observed in affected organs are multifocal area of hemorrhages through the mucosal surface of the intestine, multifocal necrosis and ulceration of the gut-associated lymphoid tissues and disseminated foci of necrosis in the spleen. The cecal tonsils are enlarged, display hemorrhages and necrosis grossly, the proventriculus display hemorrhages and ulceration at the junction between proventriculus and gizzard (Cattoli *et al*., 2011).

Newcastle disease is highly infectious and can easily be transmitted from one bird to another through direct contact with sick birds or exposure to the virus at any route. Even vaccinated avian species that are healthy clinically can excrete virulent viruses in-apparent after they have been exposed. The NDV virus can also be transmitted indirectly by people, animals, contaminated poultry products, vehicles, feed and water. Birds can be infected by either route, i.e., inhalation or ingestion of the virus or by direct contact of the virus with mucous membranes, especially the conjunctiva. Sick birds excrete viruses in aerosols, respiratory discharge and droppings. Infected birds begin to shed viruses during incubations and continue to shed viruses for a varying but limited time during the period of convalescence (Abdisa and Tagesu, 2017).

Apart from commercial poultry, the prevalence of various pathotypes of NDV in healthy wild avian species has been reported. Wild avian species act as a primary source of the spread of NDV in the natural environment, which points towards the need for investigation of wild birds as they might be the source of the spread of NDV (Ayoob *et al*., 2021). The house sparrows and Australian parrots have been shown to be the carriers of NDV virulent strains of the same genotypes that are circulating in poultry (Rehan *et al*., 2019; Snoeck *et al*., 2013; Zhu *et al*., 2010). Therefore, it is necessary to investigate the susceptibility and possible role of house sparrows

and Australian parrots in the epidemiology of NDV under controlled experimental conditions. The present study aimed to determine the susceptibility, gross and histopathology of VVNDV infection in house sparrows and Australian parrots along with possible disease transmission from infected poultry chickens to these two species.

Material and Methods

Velogenic viscerotropic newcastle disease virus (VVNDV) culture. The VVND virus (APMV-1/chicken/Multan/19-06-2012) was obtained and taken to the Department of Veterinary Pathology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tando Jam, Pakistan where it was frozen (-40 °C) until culture. For VVNDV culture, nine days old embryonated eggs were disinfected with 70% ethyl alcohol. Two holes were pierced in each egg, one for inoculation and the other for the escape of air trapped during inoculation. 0.1mL of virus suspension was inoculated into each egg with the help of an insulin syringe by the amnio allantoic route. After inoculation, holes were sealed with melted wax. Infected eggs were kept in an egg incubator at 38 °C and 65% humidity at the Department of Veterinary Pathology for the multiplication of the virus. Eggs were candled daily to check the mortality. Seventy two hours post-inoculation, the eggs were chilled at 4 °C overnight. On the next day, eggs were disinfected with 70% ethyl alcohol and placed in a biological safety cabinet. With the help of sterile forceps, the top of the shell was broken, opened the air sac and pulled the allantoic membrane. The amino allantoic fluid (AAF) was harvested from each egg by using a micro-pipette and put in sterile 15mL falcon tubes.

VVNDV Titration by haemagglutination (HA) assay. The clumping or agglutination of RBCs caused by certain viruses, antibodies or other substances is called haemagglutination. The successful culture of VVNDV was verified by performing HA titration. HA was performed on a 96-well U bottom polystyrene micro-titer plate under the instructions described by World Organization for Animal Health WHO (2021). Briefly, a volume of 25µL of PBS was dispensed into each well of a microtiter plate with the help of a micropipette. Virus suspension 25µL was placed in the first well and two-fold serially diluted upto the 11th well, while 25µL of the 1% (v/v) freshly prepared chicken RBCs was dispensed to all the 96 wells. The solution was mixed by tapping the plate gently and incubated at 4 °C for 45 min. The HA was determined by observing the presence or absence of tear shaped streaming of RBCs.

Positive HA. Absence of tear-shaped streaming of RBCs in a suspended form in the bottom of the well.

Negative HA. A small sharply outlined button of RBCs and the presence of tear-shaped streaming of RBCs at the bottom of the well.

Haemagglutination inhibition (HAI) Test. The HAI test is used for the measurement of serum antibodies directed against a haemagglutination virus. The highest dilution of the serum that completely inhibits haemagglutination by standardized viral preparation is reported as haemagglutination inhibition titer. The antigen in the HAI test is simply a solution of antigenic particles (usually a virus) which can induce the reaction of haemagglutination when mixed with the suspension of RBCs. The presence and concentration of antibodies are measured by their ability to inhibit the agglutination at various dilutions. The results of the HAI test are used to assess the efficacy of the newcastle disease vaccine in the laboratory. The HAI test was performed on the sera of surviving house sparrows and Australian parrots at the end of experimental trials. The serum samples of house sparrows and Australian parrots were tested to determine the level of antibodies that developed against newcastle disease virus after exposure and induced infection by using the standard method (Okwor *et al*., 2014). For this purpose, blood was collected before infection and after infection on days 0, 7 and 21. The serum was obtained by centrifugation from clotted blood samples. The HAI test was performed as under: 25µL of PBS was dispensed into each well of the micro-titration plate with the help of a micro-pipette. Each serum sample was shaken and 25µL was dispensed in the first well of the plate. Two-fold serial dilution of 25µL was made across the micro-titer plate up to the 10th well. 25µL of the 4HA units (HAU) antigen was added to each well across the plate upto the 11th well. To mix the reagents, lightly tap the sides of the micro-titer plate and set aside for 30 min at room temperature. Then the volume of 25µL 1% of freshly prepared chicken RBCs was dispensed in all 96 wells and incubated for 45 min at room temperature. The observed pattern for each serum was recorded as follows:

Positive HAI. RBCs were settled down at the bottom of the wells and formed a compact button.

Negative HAI. RBCs were uniformly distributed and there was no button formation at the bottom of wells.

Embryo infectious dose$_{50}$ (EID$_{50}$) determination. The infectivity titer of the newcastle disease virus was determined by using embryo infectious dose$_{50}$ (EID$_{50}$), which is defined as the quantity of virus which induces 50% of inoculated embryonated eggs to die. EID$_{50}$ was determined by using the standard procedure of Reed and Muench (Ayoob *et al*., 2021). Briefly, ten fold serial dilutions of the virus suspension were prepared. A volume of 900 µL of normal saline was put in twenty sterile 1mL Eppendorf tubes in a biological safety cabinet. A volume of 100 µL of AAF having VVNDV was added to the first tube and mixed well to make 1 in 10 or 10-1 dilution. Then the volume of 100 µL was taken from 10^{-1} dilution and was added to the second tube to make 1 in 100 or 10^{-2} dilution. By repeating this process, ten fold serial dilutions were made upto 10^{-20}. After preparation of ten fold serial dilutions, a total number of fifty 9-days old embryonated eggs was disinfected with 70% alcohol. For each dilution, 0.1mL of VVNDV suspension was inoculated in five embryonated eggs. The dying embryonated eggs were chilled overnight. For the confirmation of VVND virus infection, AAF from each egg was tested first by rapid haemagglutination assay. The formula used to calculate the EID$_{50}$ index is as under.

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

Experimental design and infection with VVNDV. Twelve birds of each species house sparrows and Australian parrots were purchased from the bird's market in Hyderabad and day old broiler chicks were purchased from a hatchery. House sparrows and Australian parrots were kept separately in an experimental room allotted to the Department of Veterinary Pathology, Faculty of Animal Husbandry and Veterinary Sciences; Sindh Agriculture University Tando Jam, Pakistan. Chickens were raised for 21 days without vaccination and medication. These birds were housed in clean, disinfected and well ventilated environments. Both feed and water were given ad-libitum. The house sparrows, Australian parrots and chickens were divided into three groups named S, P and C, respectively, while S and P groups were further divided into subgroups S1, S2, S3, P1, P2 and P3 and chicken comprises only one group C. Subgroups S1, P1 and C were comprised of 4 treated (orally) birds and S2 and P2 comprised 4 treated (intramuscularly) birds which administered 0.3 mL of AAF containing EID$_{50}$ $2.3\times10^{10.73}$ particles of VVNDV. The subgroups S3 and P3 comprised of 4 birds were contact exposed by mixing with orally infected chickens.

All the procedures in the present work were approved and performed in compliance with the institutional guidelines of the ethical review committee.

Clinical evaluation and postmortem findings. The infected birds were observed daily for the development of clinical signs, morbidity and mortality. Necropsies were performed on dead birds to study various lesions on visceral organs like trachea, proventriculus, cecal tonsils, intestine and other organs that were found affected during necropsy of house sparrows and Australian parrots. On day 21 post inoculation, blood samples were taken for determination of anti-VVNDV, antibodies level in the sera of the surviving house sparrows and Australian parrots by using the HAI test. The surviving birds were then euthanized by slaughtering and were dissected to see any lesion in internal organs. During necropsy, the severity of the lesions was scored as light (+), moderate (++) or severe (+++) (Ayoob *et al*., 2021).

Histopathological examination. Tissue samples of various affected organs were collected during postmortem examination, preserved at 10% formalin. For histopathological examination, tissue was processed as described by Ayoob *et al*. (2021).

Data analysis. The data was statistically evaluated by One way ANOVA and Tukey's test (for multiple comparison between groups) using computer software named GraphPad Prism Version 9.0.2 (San Diego, California).

Results and Discussion

Clinical findings. Clinical signs in different groups of birds were observed on a different day of post inoculation (Fig. 1a). First clinical signs in broiler chickens were observed on 2nd day post inoculation, while in house sparrow and Australian parrot clinical signs were observed on 5th day post-inoculation. The major clinical signs recorded in infected groups were ruffled feathers, anorexia, green yellowish diarrhoea, ataxia and torticollis. The similar clinical signs in the experimental Newcastle disease virus in birds reported by (Wulan *et al*., 2 017). Clinical signs like diarrhoea, anorexia, paralysis of unilateral and bilateral wings and inability

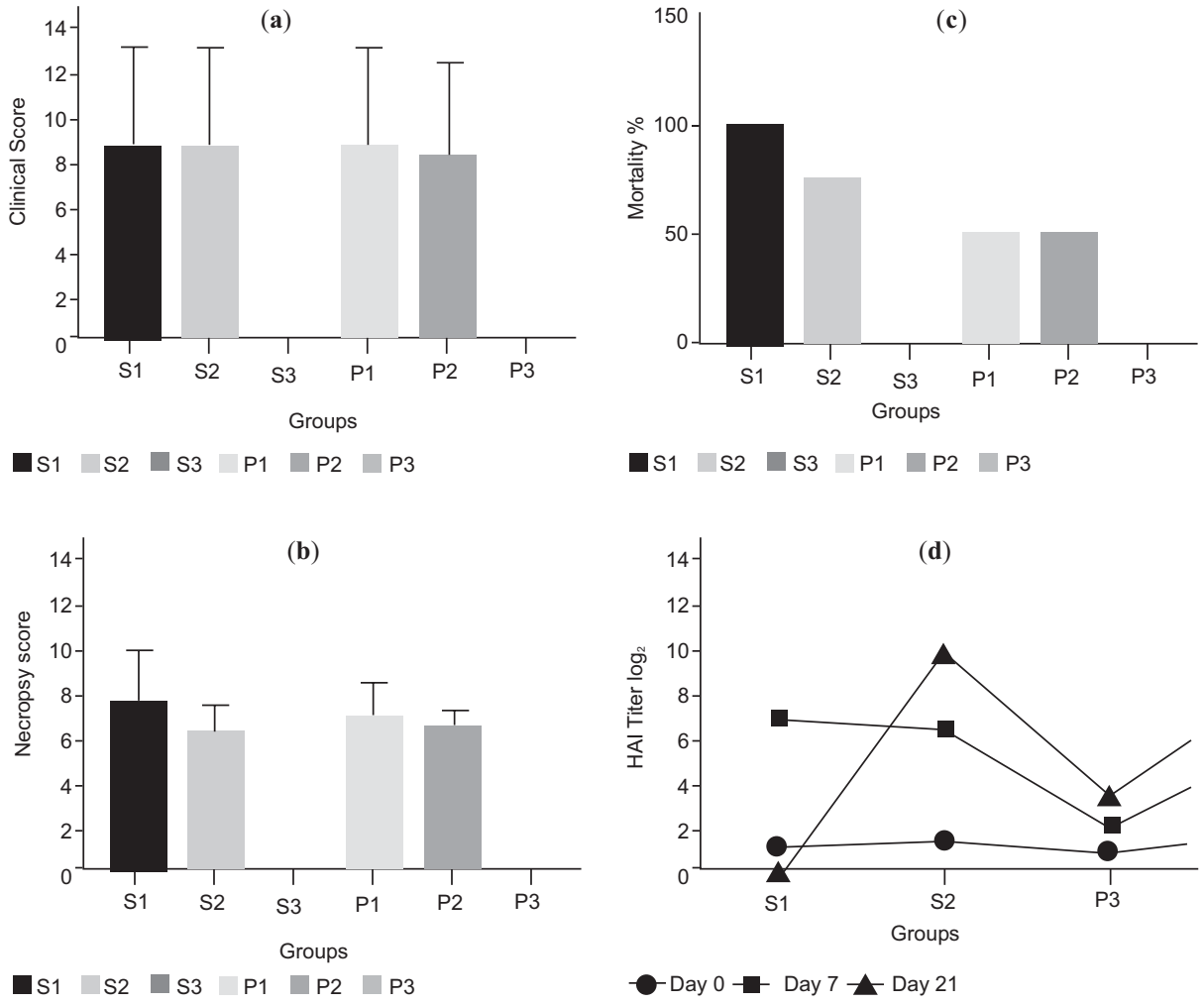

Fig. 1. (**a**) clinical scores (mean ± SD) of all the groups of house sparrow and Australian parrot; (**b**) necropsy scores (mean ± SD) with SD of all the groups of house sparrow and Australian parrot (**c**) Group-wise mortality percentage of house sparrow and Australian parrot and (**d**) day-wise HAI titers of all the house sparrow and Australian parrot groups.

to fly were recorded by Khalafalla *et al*. (1990) in house sparrows when experimentally infected with VVNDV in Sudan. Samanta and Bandyopadhyay (2017) also observed depression, ruffled feathers, anorexia, greenish diarrhea, paralysis, head shaking and torticollis as clinical signs in pet bird species.

Postmortem findings of the dead birds. The postmortem findings of dead birds were analyzed and lesions on major organs were scored. Intramuscular groups had a higher average lesion score than oral groups, while exposed groups had no lesions (Fig. 1b). Hemorrhages in the proventriculus, hemorrhagic ulcers of the intestinal epithelium and hemorrhages in the trachea and spleen were the most frequent postmortem observations in both house sparrows and Australian parrots.

These findings are in line with the findings of (Khalafalla *et al*., 1990) who recorded hemorrhages in the proventriculus, hemorrhagic ulcers in the intestinal mucosa, hemorrhages in the trachea and spleen of experimentally infected sparrows with VVNDV in Sudan. Gross pathological lesions like hemorrhages in various parts of the intestines and particularly in the mucosa of the proventriculus and trachea in Japanese quail and myna infected with VVNDV. However, contrary findings reported by Erickson who observed gross pathological lesions in parrots were mycotic airsacculitis and pneumonitis at necropsy.

Mortality rates. Mortality in house sparrows started on day 5 and in Australian parrots on day 9 post-inoculation of VVNDV. On average, a high mortality rate was recorded in house sparrows (87%) followed by Australian parrots (50%) and the group wise mortality rate was 100%, 75%, 50% and 50% in groups S1, S2, P1 and P2, respectively. Although there was no mortality in the contact exposed birds shown (Fig. 1c).

The results of the present study are in line with Khalafalla *et al*. (1990) who observed mortality in sparrows after experimental infection of VVND in Sudan. Similarly, Daut *et al*. (1016) carried out a study on ND interacting effects on white-winged parakeets and reported mortality (50%) in parrots (parakeets). Present study results revealed that house sparrows are more susceptible than Australian parrots as mortality starts earlier. Similarly, a high mortality rate was observed in house sparrows as compared to Australian parrots, which confirms high susceptibility to virus tropism. No mortality was reported in any exposed population of both species in the present experiment, implying that VVNDV doesn't really cause clinical infection in normal exposure of these species but serves as a carrier and shed virus.

Anti-VVNDV antibody titers in surviving birds determined by HAI test. All house sparrows and Australian parrots were tested for anti-VVNDV antibody before VVNDV inoculation and were found seronegative. Later, the HAI test for VVNDV titers was performed on days 7 and 21 post-inoculation. On day 7, post-infection HAI antibody titers in house sparrows and Australian parrots had developed and recorded as 7, 6.5, 2, 5.7, 5 and 2.33 in groups S1, S2, S3, P1, P2 and P3 respectively. Whereas on day 21, HAI titers were 0, 10, 3.5, 8.5, 6 and 4 in groups S1, S2, S3, P1, P2 and P3 respectively. It was further indicated that HAI on day 21 was significantly higher as compared to day 7 in all respective groups (Fig. 1d).

The serological findings showed statistically non-significant ($P > 0.05$) variations between the various groups. The presence of antibody titers in the serum indicates that these birds have been subjected to VVNDV by either route, i.e., inhalation, intramuscular and oral inoculation. Similar results were reported by (Daut *et al*., 1016) who studied parrots (parakeets) of Peru, indicating that these act as a reservoir and can shed viruses for extended periods. Erickson *et al.* (1977) researched VVND in six pet species of birds and recorded clinical, serological response and viral extraction. Serological observation by the HAI and neutralization tests indicated prolonged infection in five species of the total. The presence of antibodies in these birds' serum suggests that NDV infection occurs naturally in field species, especially sparrows, through inhalation or ingestion of vaccine virus particles (Silva *et al*., 2006. Similar results were recorded by Broggi *et al*. (2013) who investigated a wild Passerine's (house sparrows) immune reaction to Newcastle disease virus vaccination exposed with different doses of inactivated vaccine. House sparrows developed a high level of antibodies (HAI) titer against NDV within one week of post-vaccination, and values increased up to four weeks and remained persistent up to six weeks. In another study, Silva *et al.* (2006) investigated Newcastle disease virus infection in sparrows, captured in poultry farms, and hatchery. 10.68% of sparrows captured in the hatchery showed HAI titers ranging from 1:2 to 1:64, which suggests that sparrows were infected with stock vaccine strain which was regularly used in the hatchery and these birds could act as NDV reservoir. (Carrion *et al.*, 2014; Chang *et al*., 1999) and in comparison to the

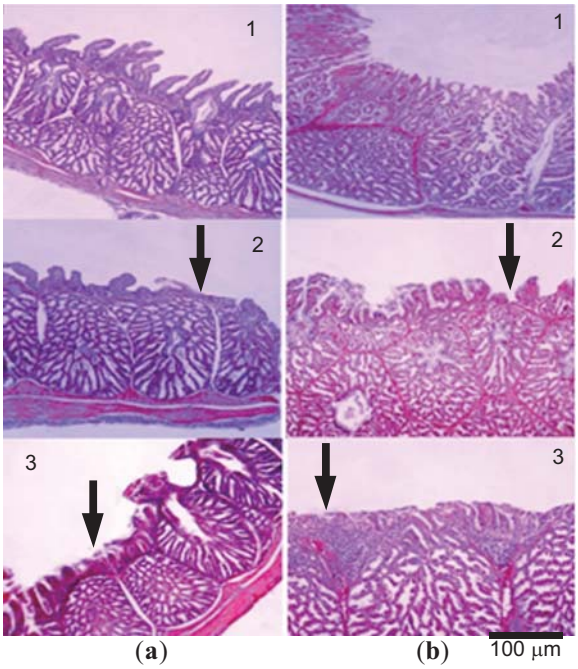

Fig. 2. (**a & b**) proventriculus of house sparrow showing excessive erosions in the mucous membrane (**a1**) normal proventriculus. (**b2 & 3**) proventriculus of an Australian parrot showing excessive erosions in the mucous membrane (**b1**) normal proventriculus.

current research, did not find any promising findings in *Passeriformes* and *Columbiformes* caught in various areas of Peru, described by This is the first research in Pakistan to look for anti-VVNDV antibodies in House sparrows and Australian parrots. The findings shed new light on the role of these species in the spread of pathogenic VVNDV variants among domestic and commercial birds.

Histopathology. Histopathological lesions in the proventriculus were mostly found in the mucosal surface of the proventriculus with excessive epithelial erosions, sporadic hemorrhages and glandular congestion (Fig. 2a 1-3 and Fig 2b 1-3). Microscopically, the small intestine showed swollen villi with various degrees of sloughed-off mucosae with irregular outlines.

Villi appeared hyperemic, ulcerated and desquamation was also noticeable (Fig. 3a and b). In sparrows (S1 and S2), histopathological observations in the trachea were more common. The surface epithelium often showed discontinuations. The damage of cilia of the epithelial lining and degradation of distinct histological layers were also noticed in both species (Fig. 4a and b). There was a massive infiltration of mononuclear lymphocytes in the spleen, particularly in the red pulp area (Fig. 5a and b).

The histopathological lesions detected in infected sparrows and Australian parrots were similar to those seen by other researchers. (Ayoob *et al*. 2021; Kabraj *et al*., 2020) and recorded histological changes like hemorrhagic proventriculus, shortening of proventricular papillae, submucosal edema, hemorrhages, congestion and ulceration of the mucosa and villi of the intestine, and lymphocytic infiltrations in Japanese quail and myna after VVNDV experimental infection. Similarly, this study agreed with (Wakamatsu *et al*., 2006) who recorded histological changes in trachea were hemorrhages, pockets of necrotic epithelial cells and multifocal regions of lymphocytic infiltrates. This study is also familiar with the study of (Susta *et al*., 2015) who mentioned microscopic changes in the spleen, particularly in lymphoid tissues and splenic lymphoid

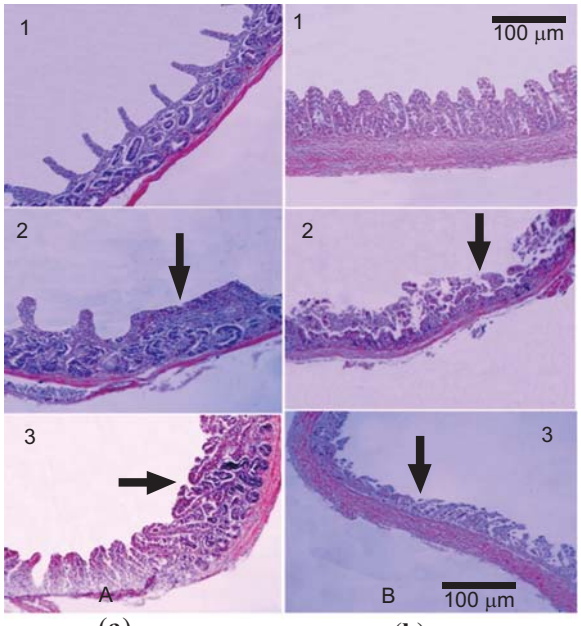

Fig. 3. (**a2 & 3**) small intestine of house sparrow showing swollen villi with various degrees of sloughed-off mucosae with irregular outlines (**a1**) normal small intestine (**b2 & 3**) small intestine of an Australian parrot showing swollen villi with various degrees of sloughed-off mucosae with irregular outlines (**b1**) normal small intestine.

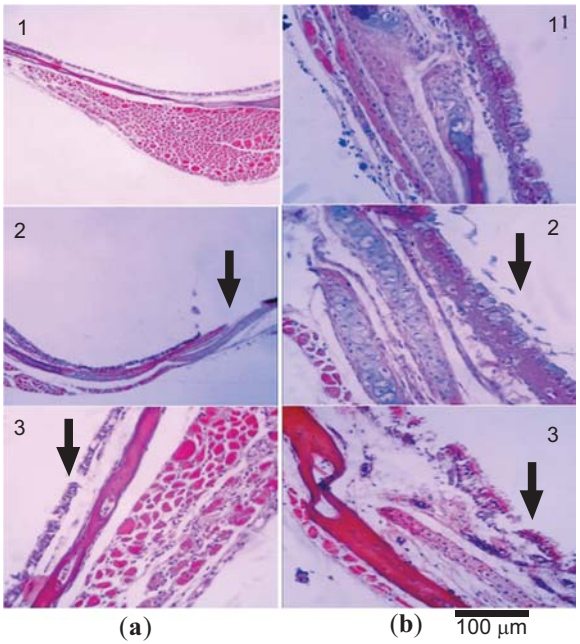

Fig. 4. (**a2 and 3**) trachea of house sparrow showing loss of lining epithelium cilia and disruption of the coherence between histological layers (**a1**) normal trachea. (**b2 & 3**) trachea of an Australian parrot showing loss of cilia and surface epithelium discontinuation (**b1**) normal trachea.

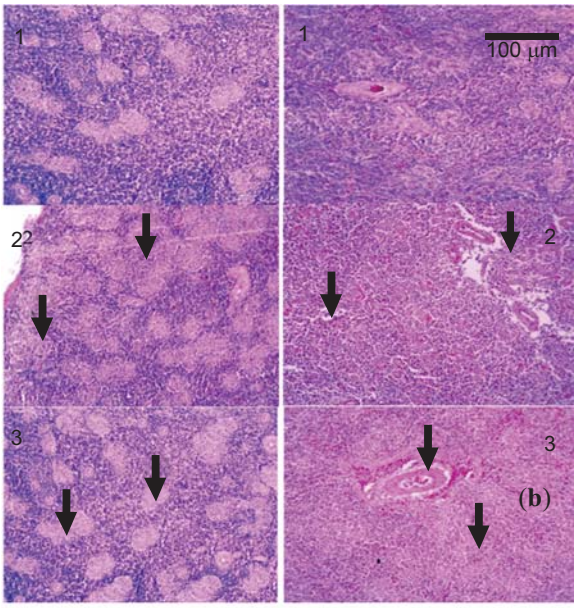

Fig. 5. (**a2 and 3**) spleen of house sparrow showing enormous infiltration with mononuclear lymphocytes (**a1**) normal spleen (**b2 & 3**) spleen of Australian parrot showing red pulp area infiltrated with lymphocytes enormously (**b1**) normal spleen.

follicles. Which showed necrosis and most lymphocytes in the medulla were degenerating.

Conclusion

It was concluded from the results that both house sparrows and Australian parrots are susceptible to VVNDV infection (intramuscularly and orally), although house sparrows are more susceptible. In both species, VVNDV causes related gross and histopathological lesions but with different incubation periods. Clinical VVND does not occur in both species when exposed VVND infected broiler chickens, but seroconversion was evident.

Conflict of interest. The authors declare they have no conflict of interest.

Acknowledgment

We are thankful to Dr. Nazeer Hussain Kalhoro, Director General, Sindh Institute of Animal Health (SIAH) for providing the VVND virus isolate for conducting the research work.

References

Abdisa, T., Tagesu, T. 2017. Review on newcastle disease of poultry and its public health importance. *Journal of Veterinary Science and Technology*, **08:** 1-7. (https://www.hilarispublisher.com/open-access/review-on-newcastle-disease-of-poultry-and-its-public-health-importance-2157-7579-1000441.pdf)

Absalón, A.E., Cortés-Espinosa, D.V., Lucio, E., Miller, P.J., Afonso, C.L. 2019. Epidemiology, control, and prevention of newcastle disease in endemic regions: latin America. *Tropical Animal Health and Production*, **51:** 1033-1048. (https://dx.doi.org/10.1007/s11250-019-01843-z.)

Ayoob, M.F., Nizamani, Z.A., Kamboh, A.A., Ayoob, M., Vistro, W.A., Baloch, A.S. 2021. Pathology of induced velogenic viscerotropic newcastle disease (VVND) in Japanese Quail and Myna. *Pakistan Journal of Zoology*, **53:** 521-527. (https://dx.doi.org/10.17582/journal.pjz/20190925150957)

Broggi, J., García, O., Miranda, F., Pagès, A., Soriguer, R.C., Figuerola, J. 2013. Immune response to newcastle disease virus vaccination in a wild passerine. *Journal of Wildlife Diseases*, **49:** 1004-1008. (https://doi.org/10.7589/2012-10-266)

Brown, V.R., Bevins, S.N. 2017. A review of virulent newcastle disease viruses in the United States and the role of wild birds in viral persistence and spread. *Veterinary Research*, **48:** 68. (https://dx.doi.org/10.1186%2Fs13567-017-0475-9)

Carrión, C.A., Icochea, D.A.E., Falcón, P.N. 2014. *Seroprevalencia* del virus de la enfermedad de newcastle en aves silvestres del orden columbiforme en baños de boza, distrito de aucallama, provincia de huaral. *Revista de Investigaciones Veterinarias del Perú*, **11:** 88-91. (https://doi.org/10.15381/rivep.v11i1.6822)

Cattoli, G., Susta, L., Terregino, C., Brown. C. 2011. Newcastle disease: a review of field recognition and current methods of laboratory detection. *Journal of Veterinary Diagnotic and Investigation*, **23:** 637-656. (https://doi.org/10.1177/1040638711407887)

Chang B.P., Icochea, D.A.E., Alba, Ch.M. 1999. Detección de anticuerpos contra el virus de la enfermedad de newcastle en aves silvestres paseriformes y columbiformes en la provincia de chancay. *Revista de Investigaciones Veterinarias del Perú*, **10:** 79-81. (https://doi.org/10.15381/rivep.v10i2.6759)

Costa-Hurtado, M., Afonso, C.L., Miller, P.J., Shepherd,

E., Cha, R.M., Smith, D., Spackman, E., Kapczynski, D.R., Suarez, D.L., Swayne, D.E., Pantin-Jackwood, M.J. 2015. Previous infection with virulent strains of newcastle disease virus reduces highly pathogenic avian influenza virus replication, disease, and mortality in chickens. *Veterinary Research*, **46:** 97. (https://doi.org/10. 1186/s13567-015-0237-5)

Daut, E.F., Lahodny, G., Peterson, M.J., Ivanek, R. 2016. Interacting effects of newcastle disease transmission and illegal trade on a wild population of white-winged parakeets in peru: A modeling approach. *PLoS ONE*, **11:** e0147517. (https://doi.org/10.1371/journal.pone.0147517)

Elmberg, J., Berg, C., Lerner, H., Waldenström, J., Hessel, R. 2017. Potential disease transmission from wild geese and swans to livestock, poultry and humans: a review of the scientific literature from a one health perspective. *Infection Ecology and Epidemiology*, **7:** 1300450. (https://dx.doi.org/10.1080%2F20008686.2017.1300450)

Economic Survey of Pakistan. 2020. Poultry In: *Agriculture*, Chapter **2**, pp. 37 http:// 111.68.102.42: 8080/xmlui/handle/123456789/17015.

Erickson, G.A., Maré, C.J., Gustafson, G.A., Miller, L.D., Proctor, S.J., Carbrey, E.A. 1977. Interactions between viscerotropic velogenic newcastle diseases virus and pet birds of six species. I. clinical and serologic responses, and viral excretion. *Avian Diseases*, **21:** 642-654. (https://pubmed.ncbi.nlm.nih.gov/606222/)

Erickson, G.A. 1976. Viscerotropic velogenic newcastle disease in six pet bird species: clinical response and virus-host interactions. Ph.D. Dissertation, 110 pp., Iowa State University, Ames, Iowa. (https://doi.org/10.31274/rtd-180813-2807)

Hicks, J.T., Dimitrov, K.M., Afonso, C.L., Ramey, A.M., Bahl, J. 2019. Global phylodynamic analysis of avian paramyxovirus-1 provides evidence of inter-host transmission and intercontinental spatial diffusion. *BMC Evolutionary Biology*, **19:** 1-15. (https://doi. org/10.1186/s12862-019-1431-2)

Kabiraj, C.K., Mumu, T.T., Chowdhury, E.H., Islam, M.R., Nooruzzaman, M. 2020. Sequential pathology of a genotype XIII newcastle disease virus from Bangladesh in chickens on experimental infection. *Pathogens*, **9:** 539. (https://doi.org/10.3390/pathogens 9070539)

Kapczynski, D.R., Afonso, C.L., Miller, P.J. 2013. Immune responses of poultry to newcastle disease virus. *Developmental and Comparative Immunology*, **41:** 447-453. (https://doi.org/10.1016/j.dci.2013.04.012)

Khalafalla, A.I., Hajer, I., Nimir, A.H. 1990. Role of some passeriformes birds in transmission of newcastle disease. II. pathogenesis of newcastle disease virus in Sudan house sparrows (Passer domesticus arborius). *Bulletin of Animal Health and Production in Africa*, **38:** 51-54. (https://www.cabdirect.org/cabdirect/abstract/19912217330)

Khorajiya, J.H., Pandey, S., Ghodasara, P.D., Joshi, B.P., Prajapati, K.S., Ghodasara, D.J., Mathakiya, R.A. 2015. Patho-epidemiological study on genotype-XIII newcastle disease virus infection in commercial vaccinated layer farms. *Veterinary World*, **8:** 372-381. (https://dx.doi.org/10.14202%2Fvetworld.2015.372-381)

Miller, P.J., Koch, G. 2013. Newcastle disease, other avian paramyxo-viruses and avian metapneumo virus infections. In: *Disease of Poultry*, Swayne., E, Glisson, J.R., McDougald, L. R., Nolan, L.K., Saurez, D.L., Nair, V.L. (ed.), pp. 87-138, 13th edition, John Wiley and Sons, Inc, Ames. (https://doi. org/10.1002/9781119421481.ch3)

WHO. 2021. Newcastle disease (infection with Newcastle disease virus). In: *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2021*, Chapter pp. New York, USA.

Okwor, G.O., El-yuguda, A., Saka, S.B. 2014. Profile of maternally derived antibody in broiler chicks and *in-ovo* vaccination of chick embryo against newcastle disease. *World Journal of Vaccines*, **4:** 72-80. (http://dx.doi.org/10.4236/wjv.2014.42009)

Rehan, M., Aslam, A., Khan, M.R., Abid, M., Hussain, S., Umber, J., Anjum, A., Hussain, A. 2019. Potential economic impact of newcastle disease virus isolated from wild birds on commercial poultry industry of Pakistan: a review. *Hosts and Viruses*, **6:** 1-15. (http://dx.doi.org/10.17582/journal.hv/2019/6.1.1.15)

Samanta, I., Bandyopadhyay, S. 2017. Infectious diseases. In: *Pet Bird Diseases and Care*, pp.13-166pp. Springer Singapore. (https://doi.org/10.1007/978-981-10-3674-3_2)

Seal, B.S., King, D.J., Sellers, H.S. 2000. The avian response to Newcastle disease virus. *Developmental and Comparative Immunology*, **24:** 257-268. (https://doi.org/10.1016/s0145-305x(99)00077-4)

Silva, J., Mota, R., Vilela, S., Doretto, J.L., Pinheiro, J.001, J., Silva, L. 2006. Newcastle disease virus infection in sparrows (Passer domesticus, Linneaus, 1758) captured in poultry farms of the agreste

region of the State of Pernambuco. *The Revista Brasileira de Ciência Avícola*, **8:** 125-129. (https://doi.org/10.1590/S1516-635X2006000200009)

Snoeck, C.J., Marinelli, M., Charpentier, E., Sausy, A., Conzemius, T., Losch, S., Muller, C.P. 2013. Characterization of newcastle disease viruses in wild and domestic birds in luxembourg from 2006 to 2008. *Applied and Environmental Microbiology*, **79:** 639-645. (https://dx.doi.org/10.1128%2FAEM.02437-12)

Su, Q., Li, Y., Zhang, Y., Zhang, Z., Meng, F., Cui, Z., Chang, S., Zhao, P. 2018. Newcastle disease virus-attenuated vaccine LaSota played a key role in the pathogenicity of contaminated exogenous virus. *Veterinary Research*, **49:** 80. (https://doi.org/10.1186/s13567-018-0577-z)

Suarez, D.L. 2013. Newcastle disease, other avian paramyxoviruses, and avian metapneumovirus infections. In: *Diseases of Poultry*, 87-138 pp. Wiley and Sons, USA. Wiley. (https://doi.org/10.1002/9781119371199.ch3)

Susta, L., Jones, M.E.B., Cattoli, G., Cardenas-garcia, S., Miller, P.J., Brown, C.C., Afonso, C.L. 2015. Pathologic characterization of genotypes XIV and XVII newcastle disease viruses and efficacy of classical vaccination on specific pathogen-free birds. *Veterinary Pathology*, **52:** 120-131. (https://doi.org/10.1177/0300985814521247)

Susta, L., Segovia, D., Olivier, T.L., Dimitrov, K.M., Shittu, I., Marcano, V., Miller, P.J. 2018. Newcastle disease virus infection in quail. *Veterinary Pathology*, **55:** 682-692. (https://doi.org/10.1177/0300985818767996)

Wakamatsu, N., King, D.J., Kapczynski, D.R., Seal, B.S., Brown, C.C. 2006. Experimental pathogenesis for chickens, Turkeys and pigeons of exotic newcastle disease virus from an outbreak in California during 2002-2003. *Veterinary Pathology*, **43:** 925-933. (https://doi.org/10.1354/vp.43-6-925)

Wulan, O.H., Yunita, N., Wuryastuty, H., Wasito, R. 2017. Detection of newcastle disease virus by immuno-histochemistry on the brains of laying birds with clinical signs torticollis and curled toe paralysis. *KnE Life Sciences*, **3:** 286-285. (https://knepublishing. com/index.php/KnE-Life/article/view/1137/2913)

Zhu, W., Dong, J., Xie, Z., Liu, Q., Khan, M.I. 2010. Phylogenetic and pathogenic analysis of newcastle disease virus isolated from house sparrow (Passer domesticus) living around poultry farm in southern China. *Virus Genes*. **40:** 231-235. (https://doi.org/10.1007/s11262-009-0436-0)