Pathological investigation of Newcastle disease in layers at Ramu Upazilla of Cox's Bazar

A clinical Report Submitted in Partial Fulfillment of the Requirement for the Degree of Doctor of Veterinary Medicine

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The Author

September, 2018

Biography

This is Mohammad Najir Hosain, son of Mr. Nur Ahmad and Mrs. Suna khatun. I am from Cox's Bazar district. I completed S.S.C in 2010 and H.S.C in 2011 with GPA 5.00. I got admitted into Doctor of Veterinary Medicine (DVM) degree under Chittagong Veterinary and Animal Sciences University in 2012-2013 session. As an upcoming Veterinarian I would like to dedicate my rest of the life for the welfare of animals. I am keen to be a field veterinarian as well as a skilled poultry practitioner.

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Abstract

Newcastle disease (ND) is a virulent infectious and contagious disease of domestic poultry and other bird species caused by virulent Newcastle disease virus (NDV). This study was conducted to know the partial prevalence of Newcastle Disease (ND) cases in Upazilla Veterinary Hospital, Ramu and investigation of pathological changes in ND suspected layer birds in commercial rearing platform. All the presented dead and sick birds were examined in necropsy and history of birds were recorded in questionnaire. ND suspected layer birds were sampled for histopathology. Over the study period twenty ND suspected cases were included in this study. In necropsy, observed gross lesions include liver hemorrhage 15 (75%), liver congestion 17(85%), tracheal hemorrhage 17(85%), tracheal congestion 5 (25%), pin point hemorrhage in proventriculus 13(65%), hemorrhage in cecal tonsil 17 (85%) and hemorrhage in the base of the heart 3 (15%) in ND suspected cases. In microscopy, hemorrhage in cecal tonsil 17 (83%), hemorrhage in tip of gland of proventriculus 4 (22%), hemorrhage in liver 13 (72%) were observed. Congestion in heart and hemorrhage in lung were found to be 3 (17%) and 4 (22%), respectively. Precise diagnosis and timely management can reduce the risk ND pretended mortality in commercial poultry farming in Bangladesh.

Keywords: Newcastle disease, Commercial layer, Necropsy, Histopathology

Chapter I: Introduction

Poultry industry in Bangladesh has become a significant industry by playing an outstanding role in the rural socio-economic system improving economic growth and creating numerous employment opportunities as chicken meat is relatively cheap and affordable source of animal protein (Paul *et al*., 1990).But in commercial layer farming system main constrains are various infectious and non-infectious diseases of the poultry and one of them is the new castle disease in commercial layers (Ali *et al*., 1994).

Newcastle disease (ND) is popularly known as 'Ranikhet disease' in Bangladesh, one of the most devastating diseases of poultry causes a fatal respiratory, enteric and neurological disorder leading to 100% morbidity and mortality. The etiological agent is Newcastle disease virus (NDV), also known as avian paramyxovirus type-1 (APMV-1) - a member of the genus Avulavirus of the subfamily Paramyxovirinae in the Paramyxoviridae family under the order Mononegavirales (Mayo *et al*., 2002). It is a single stranded, non-segmented, enveloped RNA virus with negative polarity. The NDV has ~15 kb RNA genome composed of six genes that codes for six corresponding viral proteins (Kurath *et al*., 2004). Although NDV infect over 250 avian species but clinical entity is most important in domestic chickens (Kaleta *et al*., 1998).

Clinical signs of ND are highly variable and depend on the nature of the infecting virus, dose and the degree of immunity from previous exposure or vaccination. Based on the severity of the disease in chickens, NDV has been classified into three pathotypes: lentogenic, mesogenic and velogenic. Lentogenic strains, cause subclinical infection with mild respiratory or enteric disease and are considered low virulent. Mesogenic strains are of intermediate virulence causing respiratory infection with moderate mortality (< 10%), while velogenic strains are highly virulent causing mortality rates up to 100%. Velogenic strains are further classified into viscerotropic velogenic and neurotropic velogenic strains. Viscerotropicvelogenic strains produce lethal haemorrhagic lesions in the viscera, whereas neurotropic velogenic strains cause neurological and respiratory disorders (Dortmans *et al*., 2011).The NDV outbreaks regularly occur in commercial and village chicken and rarely reported in breeders (Capua *et al*., 1993).Various researchers reported higher prevalence of clinical ND in commercial

poultry of Bangladesh. The pathology produced by NDV in chicken has variation in organs affected and their intensity. This study was intended with following objectives:

1. To know the proportionate prevalence of ND in commercial chickens at Upazila Veterinary Hospital, Ramu, Cox's Bazar
2. To know the occurrence of gross and microscopic lesions produced in Newcastle disease of poultry.

Chapter II: Materials and Methods

2.1. Study area and study unit

Cox's Bazar district is located in the south-eastern part of Bangladesh. This district is currently in environmentally critical situation due to refugee movement from Myanmar. This study was carried out in Upazilla Veterinary Hospital, Ramu Upazilla (Administrative area) of Cox's Bazar. Farmers bring their dead or sick commercial poultry to the hospital for diagnosis and treatment. Every bird brought to the hospital was considered as study unit.

2.2. Necropsy of dead birds

All birds were examined during necropsy. History of clinical signs, age, flock size, rearing system etc. were recorded by administering standard questionnaire. Gross lesions found in suspected ND cases were recorded accordingly. A total of 20 cases were suspected for ND in necropsy.

2.3. Sample collection

Trachea, lungs, liver, intestine, cecal tonsil, kidney were taken as samples and labeled for histopathology from ND suspected birds. Samples were preserved in Bouini's solution and transported to Department of Pathology and Parasitology laboratory for histopathology.

2.4. Histopathology of specimens

Fixed tissues were sectioned at 5µm thickness and stained with Hematoxylin and Eosin (H&E) stain as per standard method (Luna, 1968).

2.4.1. Collection of tissue and tissue processing

During tissue collection the following points were taken into consideration; the tissues were collected in conditions as fresh as possible. Normal and diseased tissues were collected side by side. The thickness of the tissues were as less as possible (5mm approximately). Formalin fixed tissues were processed by following protocol.

A. Fixation: 10% neutral buffered formalin was added in the plastic container (10 folds of the tissue size and weight) and fixed for 3-5 days.

B. Washing: The tissues were trimmed into a thin section and washed over night in running tape water to remove formalin.

C. Dehydration: The tissues were dehydrated by ascending ethanol series to prevent shrinkage of cells as per following schedule. The tissues were dehydrated in 50%, 70%, 80%, 95%, 100%, 100%, 100% ethanol for one hour in each.

D. Cleaning: The tissues were cleaned in chloroform for 3 hours to remove ethanol (two changes; one and half hour in each).

E. Impregnation: Impregnation was done in melted paraffin (56- 60°C) for 3 hours.

F. Sectioning: Then the tissues were sectioned with a microtome at 5-µm thickness. A small amount of gelatin was added to the water bath for better adhesion of the section to the slide. The sections were allowed to spread on warm water bath at 40-42°C. Then the sections were taken on grease free clear slides.

G. Drying: The slides containing section were air dried and kept in cool place until staining.

2.4.2. Routine hematoxylin and eosin staining procedure

The sectioned tissues were deparaffinized in three changes of xylene (three minutes in each). Then the sectioned tissues were rehydrated through descending grades of alcohol (three changes in absolute alcohol, three minutes in each; 95% alcohol for two minutes; 80% alcohol for two minutes; 70% alcohol for two minutes) followed by distilled water for five minutes. The tissues were stained with Harris hematoxylin for fifteen minutes and then washed in running tap water for 10-15 minutes. The tissues were differentiated in acid alcohol by 2 to 4 dips (1 part HCL and 99 parts 70% alcohol) and washed in tap water for five minutes followed by 2-4 dips in ammonia water until sections were bright blue. Stained with eosin for one minute and differentiated and dehydrated in alcohol (95% alcohol: three changes, 2-4 dips each; absolute alcohol: three changes 2-3 minutes for each). Cleaned by xylene and three changes were made in every five minutes in each. Tissues were mounted with cover slip by using DPX. The slides were dried at room temperature and examined under a low (10X) and high (40X, 100X) power objectives.

Chapter III: Results

3.1. Clinical signs

The spread of the disease was rapid and the mortality rate was very high (>80%). The general clinical signs consisted of depression, severe prostration, somnolence, reduction in normal vocalizations, and decrease in food and water consumption, huddling behavior, ruffled feathers, greenish diarrhea (Figure-2), prolapse (Figure-4) and soft shell eggs (Figure-3) have been found. A wide range of consistent and progressive neurological signs including tremors of head and neck, inability to stand, torticollis (Figure-1), paresis, paralysis, convulsions, rolling or circling movements, incoordination, loss of balance and recumbency with pedaling movement, flapping movements of the wings, and unusual positions of head and appendages had been noticed in the affected flock. Birds exhibited respiratory signs namely labored breathing, increased rhales, wheezing, and open-mouthed breathing and enteric signs namely watery/tenacious mucus discharge from the nostrils.

Table 1: Occurrence of gross lesions in ND cases

Gross Lesions	Total sample (N)	Percentage n (%)
Hemorrhage in liver	20	15 (75)
Congestion in liver	20	17 (85)
Tracheal in hemorrhage	20	17 (85)
Congestion in tracheal	20	5 (25)
Pin-point hemorrhage in proventriculus	20	13 (65)
Hemorrhage in cecal-tonsil	20	17 (85)
Hemorrhage in heart	20	3 (15)

3.2. Necropsy findings

In necropsy, following findings were observed in ND suspected layers such as liver hemorrhage 15 (75%) (Figure-11), liver congestion 17 (85%), tracheal hemorrhage 17 (85%) (Figure-12), tracheal congestion 5 (25%), pin point hemorrhage in proventricolus 13 (65%) (Figure-7), hemorrhage in cecal tonsil 17 (85%) (Figure-5) and hemorrhage in the base of the

heart 3 (15%) (Figure-10). In some cages abnormal follicles (Figure-8) and perihepatitis (Figure-6) have been found in the abdominal cavity of the layers. From the result of the Table 1 the proportionate prevalence is 20 (52%) where total cases were 39.

3.3. Microscopic lesions in ND

Microscopic lesions of ND in this study included hemorrhage in cecal tonsil 17 (83%), hemorrhage in tip of gland of proventriculus 4 (22%), hemorrhage in liver 13 (72%). Congestion in heart and hemorrhage in lung were found to be 3 (17%) and 4 (22%), respectively (Table-2). Mucosal surface of the trachea was stripped with numerous red streaks of congestion/hemorrhage and the lumen contained catarrhal exudates. Multiple petechiae were seen in the epicardium, surface of the proventriculus fat and serosal surface of peritoneum. Marked to severe, acute multifocal hemorrhages and accumulation of tenacious mucus had been observed on the tips of the proventriculus glands. The intestine was devoid of feed and contained tenacious mucous. The lung was one of the most severely affected organs. Severe necrosis and desquamation of the epithelial lining with consequent exposure and rupture of capillaries led to massive hemorrhage in tracheal lumen. The lesions consisted of vascular engorgement, congestion and hemorrhages in the bronchial submucosa, air and blood capillaries, smooth muscle hypertrophy in tertiary bronchus and intense peribronchial infiltration of mononuclear cells, epithelial thickening, and accumulation of fibrinous material in bronchi.

Mucosal surface of the trachea was stripped with numerous red streaks of congestion/ hemorrhage and the lumen contained catarrhal exudates. The lungs exhibited severe and diffuse bilateral pneumonia and pleurisy. Multiple petechiae were seen in the epicardium, surface of the proventriculus fat and serosal surface of peritoneum. The air sacs were opaque, thickened and exhibited increased vascularity. Marked to severe, acute multifocal hemorrhages and accumulation of tenacious mucus could be observed on the tips of the proventriculus glands.

Table 2: Occurrence of microscopic lesions in ND cases

Microscopic Lesions	Total sample (N)	Percentage n (%)
Hemorrhage in cecal tonsil	18	17 (83)
Liver hemorrhage	18	13 (72)
Pin point hemorrhage in proventriculus	18	4 (22)
Congestion in heart	18	3 (17)
Lung hemorrhage	18	4 (22)
Tracheal hemorrhage	3	3 (17)

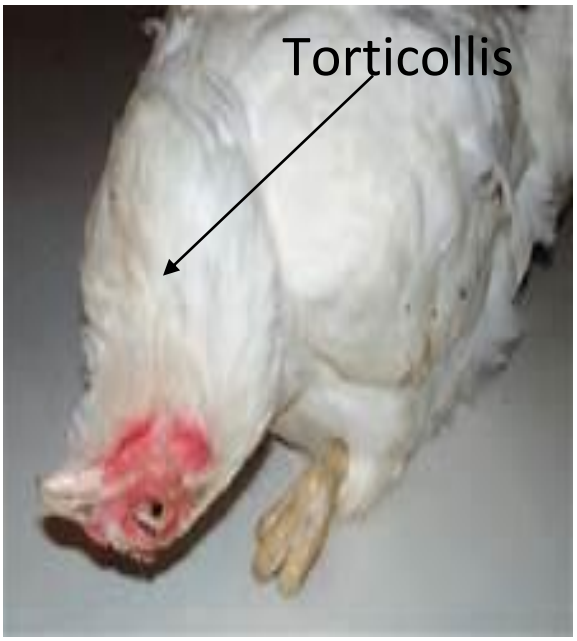

Figure 1: Torticollis in layer

Figure 2: Greenish feces in layer farm

Figure 3: Soft shell eggs of layer

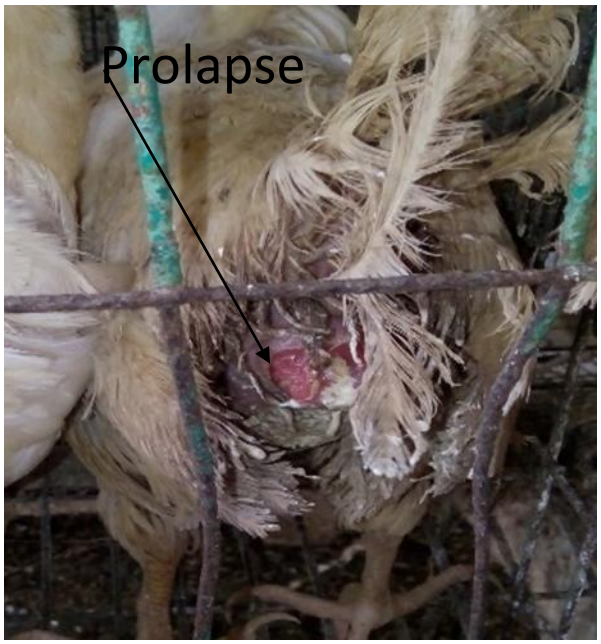

Figure 4: Prolapsed of anus of layer

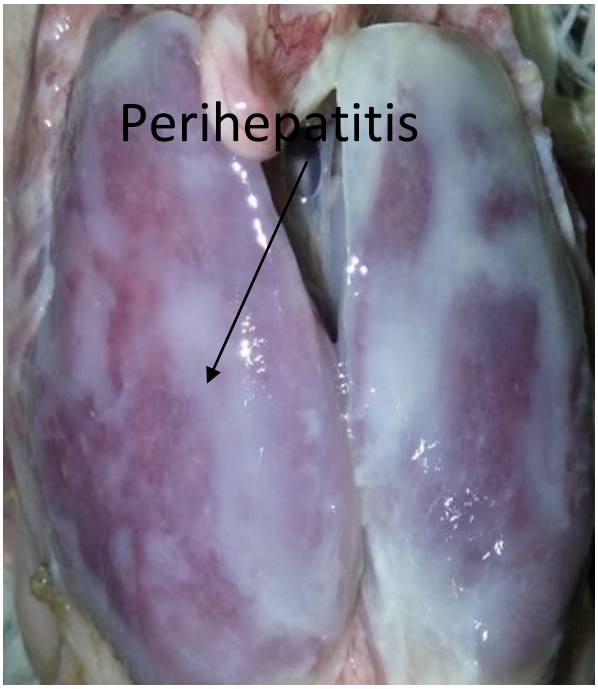

Figure 5: Perihepatitis in liver

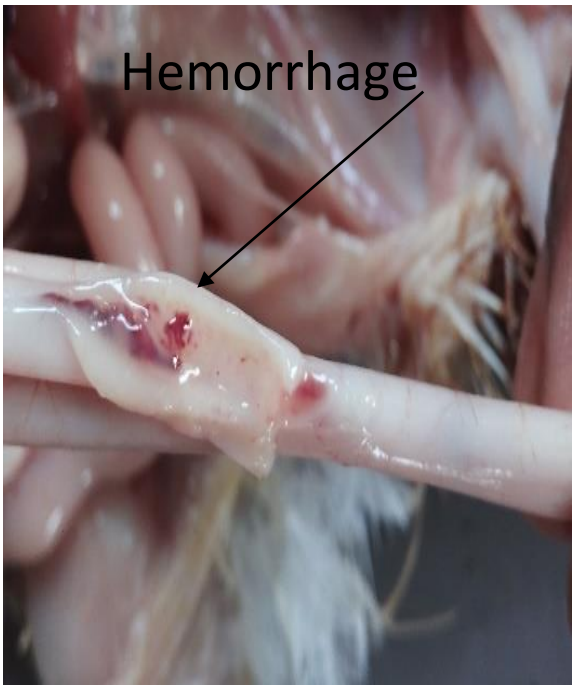

Figure 6: Cecal tonsil hemorrhage

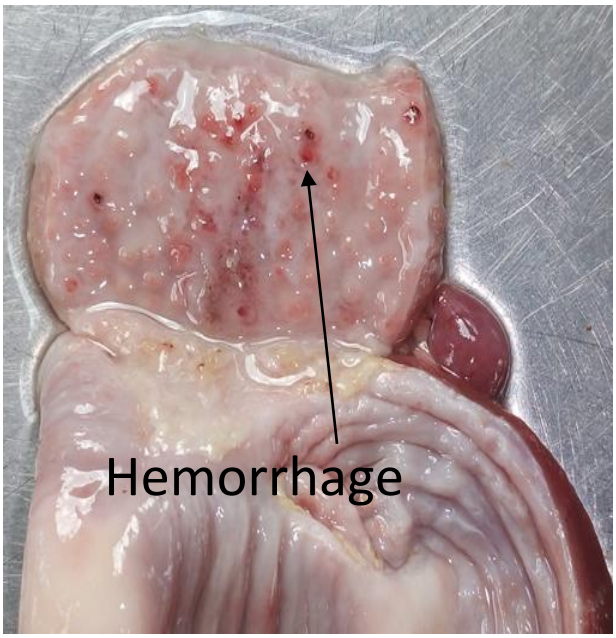

Figure 7: Hemorrhage on tip of the proventriculus

Figure 8: Abnormal follicle of layer

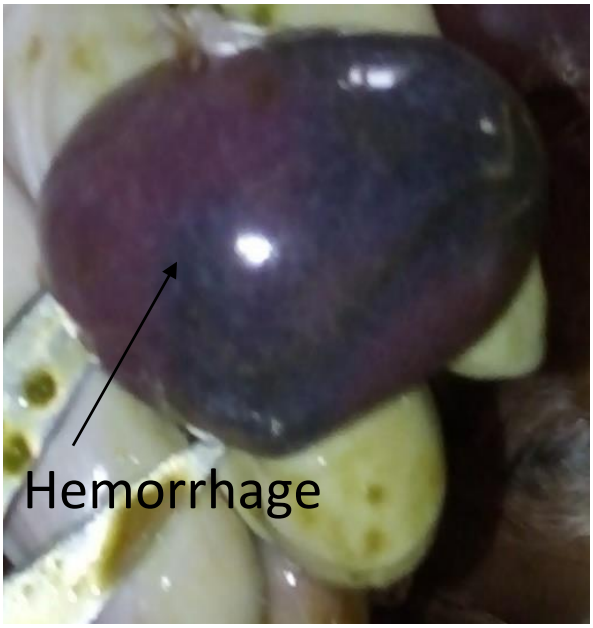

Figure 9: Splenomegaly with hemorrhage

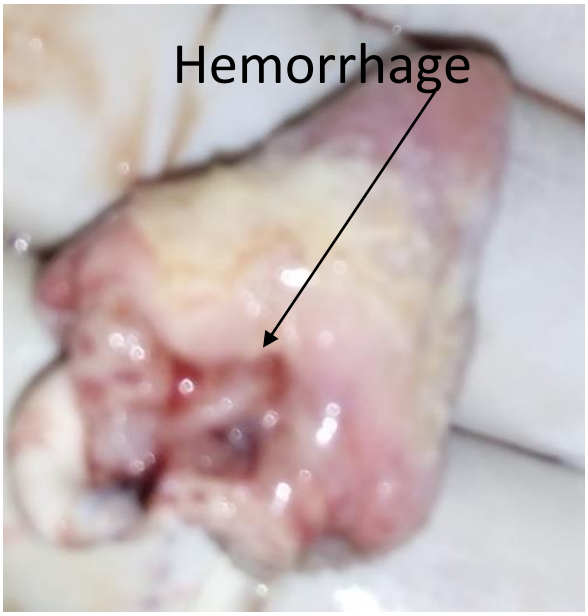

Figure 10: Hemorrhage in base of the heart

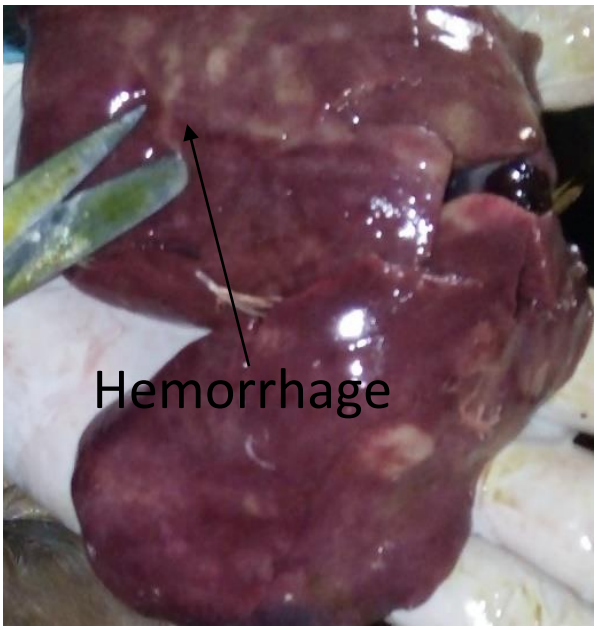

Figure 11: Hemorrhage in liver

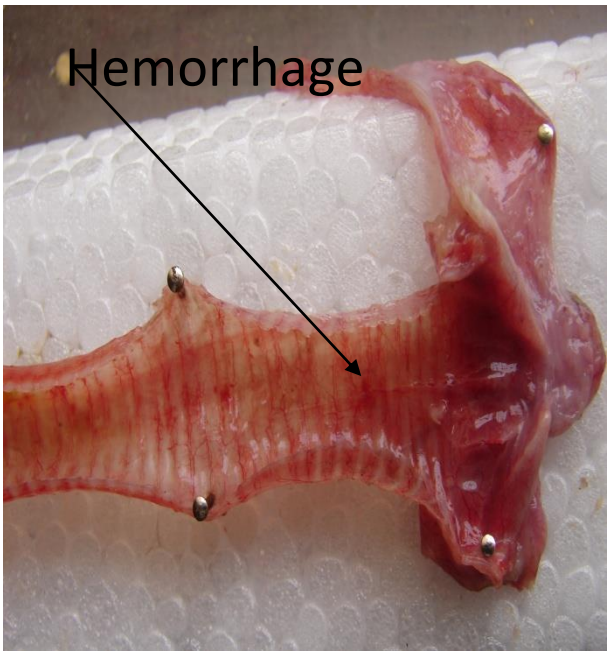

Figure 12: Hemorrhage in trachea

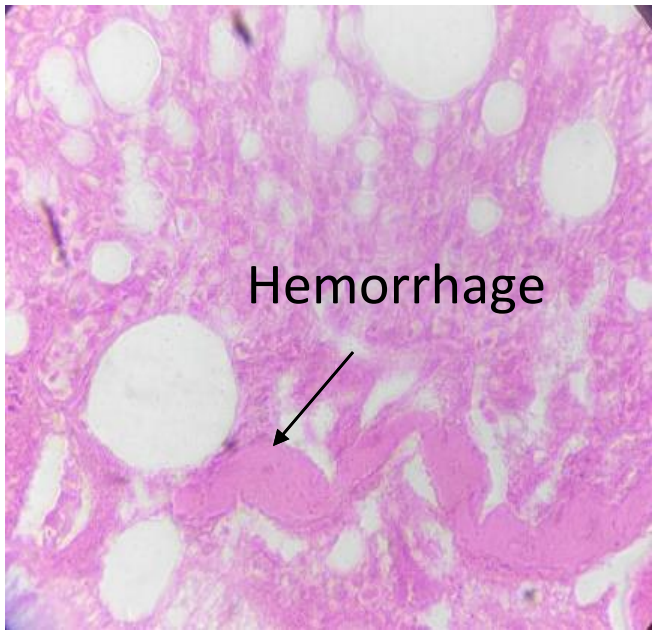

Figure 13: Hemorrhage in liver

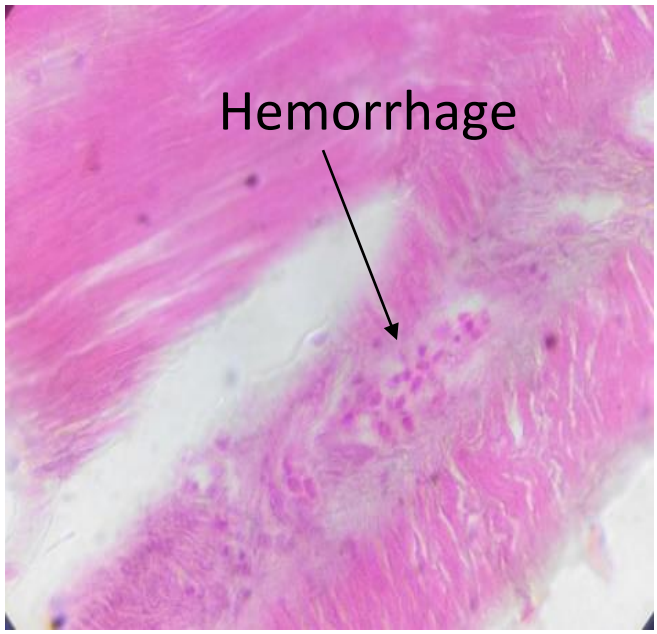

Figure 16: Hemorrhage in proventriculus

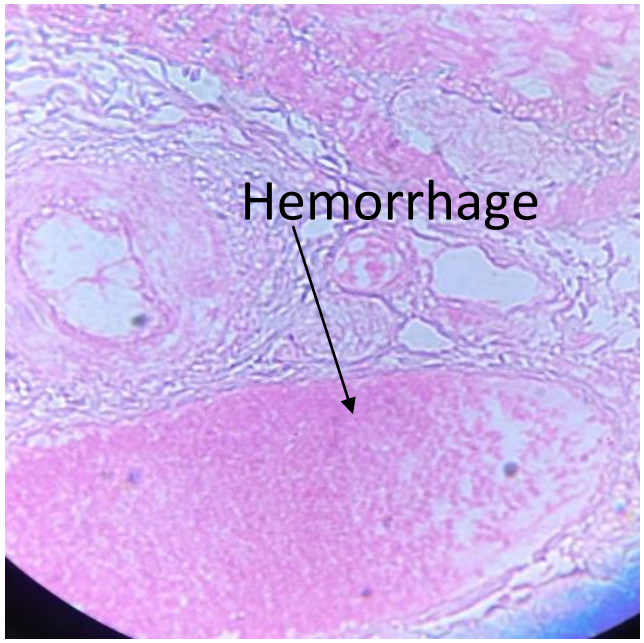

Figure 15: Hemorrhage in lung

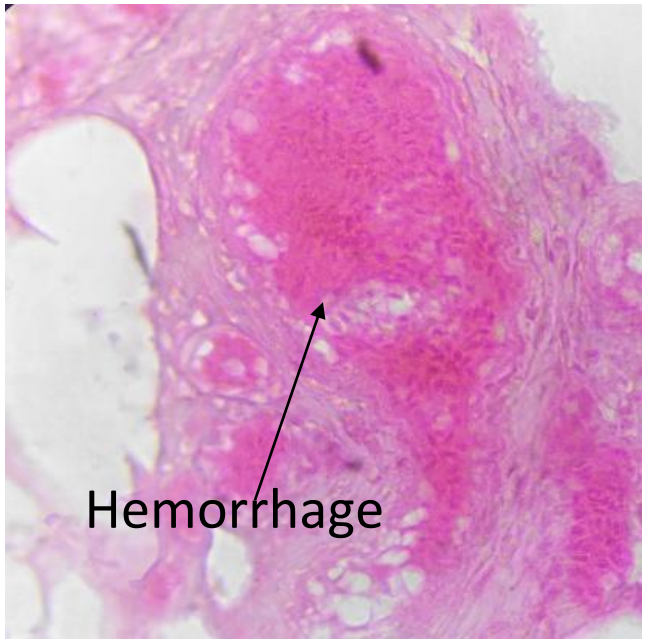

Figure 14: Hemorrhage in cecal tonsil

Chapter IV: Discussion

Newcastle disease (ND) is a threat for poultry industry which causes high mortality and morbidity. ND is said to occur in poultry population throughout the year in most of the country. ND virus infects wide variety of host but in this study ND was present for commercial layers. In this study, in Ramu upazila veterinary hospital the proportionate prevalence of ND was 52% in chicken which is matched with previous study (Asadullah *et al.*, 1992). This study was also matched with the study made In Hathazari Upazilla Veterinary Hospital where the frequency of ND was higher in chicken because of a large number of chicken populations in this site (Munmun *et al.*, 2016). In TANUVAS, the frequency of ND is higher in chicken due to large population of chicken and also housing together of them may be another possible cause for the occurrence of ND (Ucan *et al*., 2002). The microscopic lesions and postmortem findings of this present study are matched with the previous study but there were no percentage of each post mortem findings and microscopic lesions (Capua *et al.*, 2002).

Chapter V: Limitations

The study was conducted in a small scale, area and short time period which might not be the representative.

Chapter VI: Conclusion

Newcastle disease is one of the most important animal diseases in the world, both for the number of animals affected every year and for the severe economic impact on the poultry industry. Rapid and reliable detection and confirmation of ND is important to help limit economic losses and contain the disease. Because NDV can cause a wide variety of disease presentations, it is important to enhance the awareness of field personnel as well as utilizing the most efficient and accurate laboratory testing procedures. A thorough understanding of NDV pathology is important in order to recognize the disease in the field and to formulate a list of differential diagnoses. In this present study, Drowsiness, respiratory distress, torticollis, greenish diarrhoea , decline in egg production, decrease in food and water consumptions, soft shell eggs are the main clinical symptoms of ND from the owners complaints. Birds exhibited respiratory signs namely labored breathing, increased rhales, wheezing, and open-mouthed breathing and enteric signs namely watery/tenacious mucus discharge from the nostrils. Routine vaccination should be maintained which may help in control strategies of ND. Therefore, updated information is essential to understand the epidemiology of different diseases in birds to design prevention and control strategies.

Chapter VII: References

Alexander, D. J., Bell, J. G., & Alders, R. G. (2004). A technology review: Newcastle disease, with special emphasis on its effect on village chickens (No. 161). Food & Agriculture Organization.

Ali, M. J. (1994). Current status of veterinary biologics production in Bangladesh and their quality control proceeding of BSVER symposium held on July 28, 1994 at NIPSOM auditorium. Mohakhali, Dhaka, Bangladesh.

Asadullah, M. (1991). Village chickens and Newcastle disease in Bangladesh. Newcastle Disease in Village Chickens, Control with Thermostable Oral Vaccines, 161-162.

Bell, J. G., Kane, M., & Lejan, C. (1990). An investigation of the disease status of village poultry in Mauritania. Preventive Veterinary Medicine, 8(4), 291-294.

Capua, I., Dalla, P. M., Mutinelli, F., Marangon, S., & Terregino, C. (2002). Newcastle disease outbreaks in Italy during 2000. Veterinary Record, *150*(18), 565-568.

Capua, I., Scacchia, M., Toscani, T., & Caporale, V. (1993). Unexpected isolation of virulent Newcastle disease virus from commercial embryonated fowls' eggs. Journal of Veterinary Medicine, Series B, 40(1- 10), 609-612.

Dortmans, J. C., Koch, G., Rottier, P. J., & Peeters, B. P. (2011). Virulence of Newcastle disease virus: what is known so far?. Veterinary research, 42(1), 122.

Ezeokoli, C. D., Umoh, J. U., Adesiyun, A. A., & Abdu, P. (1984). Prevalence of Newcastle disease virus antibodies in local and exotic chicken under different management systems in Nigeria. Bulletin of animal health and production in Africa= Bulletin des sante et production animales en Afrique.

George, M. M. (1991). Epidemiology of Newcastle disease in rural Uganda. Cited by Awan et alet al. (1994). The epidemiology of Newcastle disease in rural poultry: a review. Avian Path, 23, 405-423.

George, M. M., & Spradbrow, P. (1991, October). Epidemiology of Newcastle disease and the need to vaccinate local chickens in Uganda. In Newcastle disease in Village chickens-a Proceeding of an International Workshop held in Kuala Lumpur, Malaysia (Vol. 39, pp. 155-158).

Kaleta, E. F., & Baldauf, C. (1988). Newcastle disease in free-living and pet birds. In Newcastle disease (pp. 197-246). Springer, Boston, MA.

Kurath, G., Batts, W. N., Ahne, W., & Winton, J. R. (2004). Complete genome sequence of Fer-de-Lance virus reveals a novel gene in reptilian paramyxoviruses. Journal of virology, 78(4), 2045-2056.

Kutubuddin, K. (1973). Pathological investigation on the causes of mortality of chickens in the BAU poultry farm(Doctoral dissertation, M. Sc. Thesis, Department of Pathology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh).

Mayo, M. (2002). A summary of taxonomic changes recently approved by ICTV. Archives of virology, 147(8), 1655-1656.

Mishra, U., & Spradbrow, P. (1991, October). Present status of poultry in Nepal. In Newcastle Disease in Village Chickens, Control with Thermostable Oral Vaccines, Proceedings of an international workshop of ACIAR, Kuala Lumpur, Malaysia (pp. 163-166).

Munmun, T., Islam, K. M. F., Jalal, S., Das, T., & Tofazzal, R. (2016). Investigation of Proportionate Prevalence of Newcastle Disease in Chicken, Pigeon and Duck at Selected Veterinary Hospitals in Bangladesh and India. *J Dairy Vet Anim Res*, *4*(2), 00118.

Njagi, L. W., Nyaga, P. N., Mbuthia, P. G., Bebora, L. C., Michieka, J. N., Kibe, J. K., & Minga, U. M. (2010). Prevalence of Newcastle disease virus in village indigenous chickens in varied agro-ecological zones in Kenya. Livestock Research for Rural Development, 22(95).

Nyaga, J. M., Nyaga, P. N., & Kariuki, D. P. (1985). Epidemiology of Newcastle disease in Kenya. Bulletin of animal health and production in Africa= Bulletin des sante et production animales en Afrique.

Paul, D. C., Haque, M. F., Abedin, M. Z., & Akter, M. S. (1990, October). Participation of women in poultry husbandry in rural Bangladesh. In 10th AFSRE symposium held at Mat Chining University, USA.

Ucan, S. U., & Cataloluk, O. (2002). Housing quails and chickens together is the possible cause of Newcastle disease's spread: An overlook measure taken to prevent the disease. *Turk. J. Vet. Anim. Sci*, *26*, 419-420.