CLINICOPATHOLOGICAL STATUS OF DUCK PLAGUE AT DINAJPUR DISTRICT

A Thesis

By

SHABNAM MOSTARI

Registration No. 1305075
Semester: January- June, 2014
Session: 2013-2014

Master of Science (M.S.)
in
Pathology

Department of Pathology and Parasitology

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Submitted to the
Department of Pathology and Parasitology
Hajee Mohammad Danesh Science and Technology University, Dinajpur,
In partial fulfillment of the requirements for the degree of

Master of Science (M.S.)
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June, 2014

ACKNOWLEDGEMENTS

All praises are due to the omnipotent Almighty Allah, the creator and supreme authority of the universe, whose blessings enables the author to complete her research work and preparation of the thesis successfully.

The author highly pleased to expresses her deepest sense of appreciation to her beloved teacher and Research Supervisor *Dr. Md. Nazrul Islam, Associate Professor, Department of Pathology and Parasitology (PPS),* Hajee Mohammad Danesh Science & Technology University (HSTU), Dinajpur, for his sincere interest and valuable suggestions throughout the research work.

The author thanks to her reverend beloved teacher and Research Co-supervisor, *Dr. Md. Haydar Ali, Assistant Professor and Chairman, PPS, HSTU,* Dinajpur, for his advice and guidance throughout this research work.

The author would like to acknowledge her respected teachers Professor Dr. S. M. Harun-Ur-Rashid, Dr. Md. Mominul Islam, Assistant Professor and Dr. Md. Golam Azam, Lecturer, PPS, HSTU, *Dinajpur, for their valuable advices, constant help, continuous encouragement and contribution in completing the thesis.*

The author is grateful to acknowledge the efficient and willing co-operation of Upazila Livestock Officer and Veterinary Surgeon of Sadar, Chirirbandar, Parbotipur, Fulbari, Birampur, and Nawabgonj upazila for their kind collaboration.

The author would also like to thanks her co-workers Dr. Pabitra Kumar, Dr. Abdur Rouf, Dr. Md. Raju Ahmed, Dr. Md. Assaduzzaman Jemy, Dr. Sagar Boshak and Dr.Shakila Reza for their encouraging

attitude, kind help and all-out support in the entire period of the research work.

The author takes the opportunity to express her deepest sense of respect and appreciation to Ministry of National Science and Technology for selected her as a NST fellow and make a significant cooperation and funding for this experiment.

The author

June, 2014

ABSTRACT

Clinicopathological status of duck plague was investigated at different upazila of Dinajpur district during the period from January to June, 2014. The clinical features emphasiging the mortality and prevalence, necropsy for gross morbid lesions, histopathological features were examined. The farm and flock history, managemental aspects, vaccination status, nutrition, etc. were recorded carefully. The data was collected and statistically analysed. Farmer's complaint's about their affected birds were also considered and emphasized. The average mortality rate was recorded as 14.42% and prevalence was 19.81%. The sick birds clinically showed moderate to severe depression, ocular and nasal discharges, ataxia, dypsnia. The affected organs were pathologically characterized as mild to moderate congestion and haemorrhages; misshapen, ruptured cystic ova, and histopathologically characterized as mild to moderate architectural destruction, reactive cell infiltration.

CONTENTS

CONTENTS (Cont...)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (Contd.)

LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
&	and
µm	micrometer
CV	Co-variance
DPV	Duck Plague Virus
DVE	Duck Viral Enteritis
ELISA	Enzyme Linked Immunosorbent Assay
et al.	and other
FAO	Food and Agricultural Organization
Fig.	Figure
Gm	gram
H & E	Hematoxylin and Eosin
LSD	Least standard deviation
Mg	Miligram
ml	Mililiter
No.	Number
^{o}C	Degree celsius
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
VN	Virus Nutralization

CHAPTER I

INTRODUCTION

Household duck keeping has a significant contribution in the economy of rural Bangladesh. There are about 44.12 million ducks in Bangladesh of which 85% reared in rural households under semi-scavenging system (Ajnber and Mia, 2002). Most of the ducks in Bangladesh (90-95%) are of native types (Ahmed, 1986). Ducks are important source of nutritious food, source of income and therefore, their rearing and production has became means of breaking out poverty trap of resource for the poor small holds of low income countries (Pym *et al*., 2002). So, duck farming is an important option of livelihood available to land less farmers because of the fact that duck can exploit common feed resources in natural water bodies, like wet and marshy lands, beefs, haors, rivers, cannels etc. Duck farming is increasing in the recent days in Bangladesh and about 26000 duck farms have already been set up in private sectors (FRYP, 1998).

Household ducks rearing has a significant contribution in the rural economy of Bangladesh. The average annual income in different categories of duck raisers ranged from Tk.79121 to Tk. 382667 (Khanum *et al.,* 2005). Whereas, Huque and Sultana (2003) stated that a farmer with 200 layers with or without hatchery may make an annual profit of Tk. 55353 to Tk. 116722. But there are many constraints for, the development of large scale duck farming either in the rural or urban areas of Bangladesh. Among these, occurrences of diseases are the major hindrance for the development of duck farming in Bangladesh and thereby causing significant economic losses. The cause of mortality of ducks in Bangladesh were due to duck plague (Baki *et al.,* 1986, 1991; Das *et al.,* 1988, 2005).

In Bangladesh duck plague was first reported by Sarker (1980). Subsequently, the virus was isolated (Sarker, 1982, Khan *et al.,* 1990) and Characterized (Dev and sarker, 1982; Islam and Khan, 1995). The pathology of duck plague in Bangladesh was studied on natural infection (Das *et al.,* 1988; Baki *et al.,* 1993)

and experimentel infection (Das *et al.,* 1990; Islam, 1992). Studies were also conducted in Bangladesh on the serological tests and cell mediated immunity in duck plague (Khan *et al.,*1993; Hossain *et al.,* 2005; Islam *et al.,* 2005; Das, 2006; Kayesh, 2007).

Natural infection observed in ages ranging from 7 day old to mature breeder ducks (Saif *et al.,* 2008b). Whereas Shawky and Schat (2002) reported that waterfowls of all ages are susceptible to DVE. Host range of DP comprises 34 species within the order *Anseriformes* (Kaleta *et al.,* 2007) but the gray colour ducks are resistant (Van Dorssen and Kunst, 1955). Mortality in domestic duck range from 5 to 100% (Das *et al.,* 2005; Saif *et al.,* 2008b). The morbidity and mortality in a flock depend on the virulence of the virus and the immunologic status of the birds (Campagnolo *et al.,* 2001). Baki *et al.* (1993) conducted an investigation on the causes of mortality of ducks in Bangladesh. They found that duck plague was the major cause of duck mortality. Clinical signs of duck plague included sudden death, anorexia, listlessness, dropping of wings, inability to stand, swollen face with watery to viscid mucus discharge from eyes and nostrils, profuse watery diarrhoea (whitish to greenish) and death within 2-5 days. High temperature (110-112°F) were recorded which declined to 104-105°F on the onset of diarrhoea. Grossly, hemorrhagic lesions were recorded in crop in some cases. The liver was slightly to moderately enlarged with large number of small focal necrosis. Thick, sticky mucus exudates were found to cover the mucosa of proventiculus, gizzard and, last, part of intestine with underlying hemorrhage in some cases. In ducklings annular hemorrhagic lesions in the intestine were noted. Testes in male were also congested. Spleen in some cases were slightly enlarged and congested. Molecular detection like PCR assay is most useful technique for rapid detection of duck viral enteritis DNA in acute and latent stage of infection (Wu *et al.,* 2011).

To diagnose the exact cause of morbidity and mortality to improve management a thorough knowledge about the proper diagnostic procedure is prerequisite for the prevention and control of the disease. Therefore the present study was undertaken with the following objectives:

- To study the clinical features of the ducks affected with duck plague
- To observe the gross lesions of the duck plague affected bird
- Histopathological study of affected organs collected from the duck plague suspected ducks during necropsy

CHAPTER II

REVIEW OF LITERATURE

Duck rearing is a profitable agro sector in Bangladesh because of low cost feeding in free water bodies of the country. Ducks mortality in Dinajpur district are dramatically increased and reducing the profitability of duck rearing. The available literatures, pertinent to the present study, are reviewed in the following paragraphs. For the convenience of the description, the literatures are presented under several headings and sub-headings.

2. Prevalence, incidence and mortality of duck diseases

A survey on the outbreaks of duck diseases in Bangladesh found that, the main causes of mortality was due to duck plague (Bald *et a1*., 1986).

The outbreaks of duck diseases were investigated in different districts of Bangladesh during the period from March 1984 to April 1985. They found that the main causes of mortality were due to duck plague (54.55%) and duck cholera (10.91%). The other diseases causing duck mortality recorded during the survey were gout (18.18 %), (Baki *et a1.,*1993).

In Netrakona the farmers mostly raise indigenous (local) ducks and some of them are Xinding ducks (Khanum *et al.,* 2005). Hamid *et al.* (1988) stated that the Deshi ducks were more resistant to diseases than the exotic breeds. Khanum *et al.* (2005) reported that the mortality rate in Netrakona was 27.1%.

Most of the farmers of hatiya upazila, Noakhali of Bangladesh ranked duck plague as the most important disease, followed by duck cholera, botulism and duck viral hepatitis (Hoque *et al.,* 2010). Recorded the morbidity and mortality rate of duck plague, which ranged up to 100% (Das *et al.,* 1988).

Duck plague virus was observed (duck viral enteritis) outbreaks at two different locations in Pennsylvania in 1991 and 1992. In the first outbreak, four ducks died out of a group of 30 domestic ducks; in the second outbreak, 65 ducks died out of a group of 114 domesticated ducks and 15 domestic geese also died (Davison *et a1.,* 1993).

A preliminary survey made on ducks of 50 villages of Lakhimpur and Dhemaji districts of Assam, India and found that, duck plague, was the common diseases of duck with mortality of 20-25% in ducklings and 15-20% in adult ducks (Mahanta *et a1.,* 2001).

In West Bengal the intestinal tract of 9872 ducks were examined at necropsy in the year 1981-1985. The intestinal infections were diagnosed in 4085 cases, duck plague virus infection was found 1633 (39.97%) in ducks (Bhowmik and Roy, 1987).

Several outbreaks of duck plague was reported in Bangladesh with the mortality rate, 60-70% (Sarker, 1982). Belokobilenko (1964) examined 317 ducks in Southern Kazakh SSR and found that 85.8% ducks were infected with helminthes.

A survey was conducted on the outbreaks of duck diseases in Bangladesh and found that, the main causes of mortality were due to duck plague (54.55%) and duck cholera (10.91 %) (Baki *et al*., 1986).

However, Hoque *et al.,* (2011a) reported that the common diseases of ducks are duck plague (21.1 %) and duck cholera (32.1 %).

Hoque *et al.* (2011b) stated that morbid ducks frequently displayed signs associated with diseases affecting the nervous and digestive systems. Haemorrhagic lesions in various organs and white multiple foci on the liver were frequently observed in dead ducks.

For better understanding of this study the review of literature is cited below under the heading of Duck Plague infectivity as a cause of duck mortality.

2.1 Duck Plague

2.1.1 History

An outbreak of an acute hemorrhagic disease in domestic ducks was first reported by Baudet (1923) in the Netherlands. Subsequently Bos (1942) reexamined the findings of baudet's report and also studied a new outbreak. Since then the disease has been reported in many duck raising countries of the world (Levine and Fabricant, 1950; Mukerji *et al.,* 1963a, b; Jansen and Kunst, 1964). In Bangladesh duck plague was first reported by Sarker (1980). Subsequently the virus was isolated (Sarker, 1982, Khan *et al.,* 1990) and Characterized (Dev and Sarker, 1982; Islam and Khan, 1995). The pathology of duck plague in Bangladesh was studied on natural infection (Das *et al.,* 1988; Baki *et al.,* 1993) and experimental infection (Das *et al.,* 1990; Islam, 1992). Studies were also conducted in Bangladesh on the serological tests and cell mediated immunity in duck plague (Khan *et al.,* 1993; Hossain *et al.,* 2005; Islam *et al.,* 2005; Das, 2006; Kayesh, 2007).

2.1.2 Virus etiology

The etiological agent was DP virus (DPV) with an enveloped, cubic symmetry, double stranded DNA virus, which is the prototype species of the family Herpesviridae in the order *a-herpesvirinae* (Kaleta, 1990). Although DVE is currently classified to the family Herpesviridae, it is not assigned to any subfamily or genus in the family (Li *et al.,* 2006).

2.1.3 Age and host range

Natural infection observed in ages ranging from 7 days old to mature breeder ducks (Saif *et al.,* 2008). Where as Shawky and Schat (2002) reported that waterfowls of all ages are susceptible to DVE. Host range of DP comprises 34

species within the order *Anseriformes* (Kaleta *et al.,* 2007) but the gray call ducks are resistant (Van Dorssen and Kunst, 1955).

2.1.4 Mortality rate

Mortality in domestic duck range from 5 to 100% (Das *et al.,* 2005; Saif *et al.,* 2008b). The morbidity and mortality in a flock depends on the virulence of the virus and the immunologic status of the birds (Campagnolo *et al.,* 2001).

2.1.5 Clinical signs and pathology

The major clinical signs of natural infection of duck plague were sudden death, listlessness, lethargy, drooping wings, swollen face, sticky eyelids, mucus discharge from nostrils, coated eyelids by cheesy exudates, congested and opaque nictitating membrane and greenish watery diarrhea. However, in experimental infections, high, body temperature (110-112°F) that declined to 104-105°F on the onset of diarrhea was observed (Wobeser, 1997). At necropsy, either bronze or greenish yellow enlarged liver with scattered petechiae and grayish white necrotic spots were observed; hemorrhage on the coronary groove of heart and ventricular myocardial surface on incision; necrotic patches on ventricular myocardium; moderately congested kidney, slight congestion of lung; catarrhal enteritis, severe congestion and linear diphtheritic, deposits in the esophagus, cloacaeoophoritis and peritonitis inflammed with ruptured and blood tinged yolk also seen. Histopathologically, severe engorgement of sinusoids, fatty changes, degeneration, necrosis and hemorrhage in liver. Moreover, tubular degeneration and congestion of kidney, focal area of hemorrhages and diphtheritic materials in the mucosa of esophagus; degeneration, necrosis and ulceration of gizzard mucosa, catarrhal to hemorrhagic enteritis; extensive myocardial degeneration and necrosis with infiltration of histiocytes and lymphocyte; degeneration and hemorrhagic necrosis of tracheal mucosa were common. In experimental infection, destroyed intestinal mucosa with increased number of goblet cells, destruction of epithelium, catarrhal inflammation and profuse hemorrhage in all

layers of intestine were found in the sections through annular band (Rajan *et al.,* 1980; Baki *et a1*., 1986).

Postmortem examination was conducted of 30 ducks, which died of duck plague between 1978; 1984.The main lesions were diphtheritic esophagitis and cloacitis, catarrhal proventiculitis and hemorrhagic enteritis. Subcutaneous hemorrhages and hemorrhages in the heart, liver and gonads were also found. Histology of liver showed areas of massive necrosis and hemorrhages, a few of the hepatocytes had esoinophilic infra nuclear inclusion bodies (Chennakesavalu *et al.,* 1987).

The pathology of duck plague was studied in spontaneous and in experimental infections. Grossly, they observed slight enlarged liver with petechial and echymotic hemorrhages and focal necrosis; distended gallbladder. Microscopic changes of liver were parenchymatous degeneration, fatty degeneration, necrosis, congestion of central veins and sinusoids with heterophilic and lymphocytic infiltration. Eosinophilic intranuclear inclusion bodies were found in the degenerated hepatocytes. Infiltration of plasma cells and infra nuclear inclusion bodies were detected in the hepatocytes. Grossly, lung showed congestion of varying degrees with heterophilic infiltrations. Intestine showed catarrhal and hemorrhagic eriteritis grossly and microscopically. Annular bands in intestine were common in ducklings. Diphtheritic membrane or Gaseous plaques formation with hemorrhages and heterophilic infiltrations under the lining surface were seen in esophagus. Microscopically, proventiculus revealed congestion, perivascular edema, glandular cells hyperplasia with fibrosis and mucus in lumen; erosion and ulceration in gizzard. Hemorrhages in cloaca, were frequent. In heart, grossly, there were hemorrhages on the epicardium near the coronary groove, focal necrosis in the ventricles and microscopically, degeneration and necrosis with lymphocytic infiltration were found. Hemorrhage, necrosis and heterophilic infiltration were noted in the pancreas. Kidney showed hemorrhage and tubular degeneration. Degeneration and cellular infiltration with fibrin deposition were the changes in the ovarian follicles. Oviduct showed congestion with catarrhal inflammation. Testesses were congested grossly, degeneration of spermatic cord

and disarrangement of semeniferous tubular cells found microscopically. (Das *et al*., 1988, 1990).

Clinical signs of duck plague were history of sudden death, anorexia, listlessness, dropping of wings, inability to stand, swollen face with watery to viscid mucus discharge from eyes and nostrils, profuse watery diarrhoea (whitish to greenish) and death within 2-5 days. Black melanin like pigment was also seen in the serous membrane and even on the muscles. Ovary and oviduct were congested and hemorrhagic. The mature follicles were found to rupture resulting peritonitis, which was common in layers. In ducklings annular hemorrhagic lesions in the intestine were noted. Testes in male were also congested. Spleen in some cases were slightly enlarged and congested. (Baki *et a1*., 1993).

The lesions were described of duck plague. In their observation, the gross lesions of duck plague were hepatomegaly with petechial hemorrhages on the abdominal fat, epicardial surface of heart and multi focal to coalescing area of fibrinonecrotic materials over the mucosal surface of the trachea, esophagus, intestine and cloaca. Histopathologically, in liver random multi focal area of necrosis and eosinophilic intranuclear inclusion bodies in the hepatocytes were found. (Davison *et al.,* 1993).

2.1.6 Diagnosis

Isolation and identification of DVE

Although a presumptive diagnosis can be made on the basis of clinical signs (Burgess and Yuill, 1981), gross and histopathological lesions (Shawky *et al.,* 2000), electron microscopy (Yuan *et al.,* 2005), isolation and identification of DEV confirms the diagnosis even in the absence of typical lesions. Sample recommended for virus isolation are liver, spleen, bursa, kidneys, peripheral blood lymphocytes (PBL) and cloacal swabs (Saif *et al.,* 2008b). Primary virus isolation should be made by inoculation of susceptible one day old white pekin

ducklings or chorioallantoic membran and (CAM) of 9 to 14 day old embryonated duck eggs (Leibovitz, 1989).

Other diagnostic procedures currently used to identify DVE include Different serological test such as virus neutralization (VN), antigen-capture ELISA test, immunohistochemistry, avidin-biotin-peroxidase complex method of immunoperoxidase staining, Immunochromatographic strip (ICS) test (Islam *et al.,* 1993; Islam and Khan, 1995), Conventional PCR assay is used for detection of DVE DNA in tissue samples and in cell culture (Pritchard *et al.,* 1999; Hansen *et al.,* 2000). Recently a quantitative real time PCR assay has been developed that may be useful for rapid diagnosis and detection of duck enteritis virus DNA in acute and latent stage of infection (Qi *et al.,* 2009; Wu *et al.,* 2011). Wang *et al.* (2011) studied on complete genome sequence of virulent duck enteritis virus (DEV) strain 2085 and comparison with genome sequences of virulent and attenuated DEV strains.

CHAPTER III

MATERIALS AND METHODS

The experiment was carried out in the Department of Pathology and Parasitology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur during the period from January, 2014 to June, 2014. Six upazilas (sadar, Chirirbandar, Parbotipur, Fulbari, Birampur and Nawabgonj) of Dinajpur district were investigated to find out sick and dead ducks. Representative samples (liver, trachea, lungs and heart) were collected from the sick and dead duck from the natural cases infection of domestic ducks at different upazilas of Dinajpur District.

3.1 MATERIALS

3.1.1 SAMPLES

At first I visited six upazilas (Sadar, Chirirbandar, Parbotipur, Fulbari, Birampur and Nawabgonj) of Dinajpur district and collected different data from Upazila Livestock Office. Sources of the population in this study were native ducks raised domestically by farmers from different upazila at Dinajpur district. From the selected area, all the dead as well as sick ducks were collected for furthers examination. The organs or tissue like liver, lung, heart were submitted to the laboratory of the Department of Pathology and Parasitology, Hajee Mohammad Danesh Science and Technology University, Dinajpur for the final diagnosis.

3.1.2 INSTRUMENT AND APPLIANCES

Equipment and appliances for necropsy:

- Ducks (Liver, Lung, Heart)
- Scissors
- Forceps

- Gloves
- Musk
- Scalpel
- Knife
- A pair of shears
- 10% formalin

Equipment and appliances for histopathology:

- Samples (Liver, lung, heart)
- 10% formalin
- Chloroform
- Paraffin
- Alcohol
- Tape water
- Xylene
- Hematoxylin and Eosin stain
- Distilled water
- Clean slides
- Cover slips
- Mounting media (DPX)
- Microscope

3.1.3 CLEANING AND STERILIZATION OF REQUIRED GLASSWARE

Test tubes, glass tubes, glass slides, cover slips, beakers, pipettes, reagent bottles, glass bottle, spirit lamp, measuring cylinders etc. were used in this study. The conical flask, measuring cylinder, beakers, glass slides, cover slip, for slide preparation for histopathological study. New and previously used glassware were collected and dipped in 2% sodium hypochlorite solution and left there until cleaned. After overnight soaking in a household dishwashing detergent solution, the glassware were cleaned by brushing and washed thoroughly in running tap

water and rinsed three times in distilled water. The cleaned glass wares were then dried on a bench at room temperature or in an oven at 50-70^{0}C.

3.1.4 CHEMICAL AND REAGENTS USED

10% formalin, Xylene, Hematoxylin and Eosin stain, PBS, Distilled water etc were used for necropsy and histopathology of collected samples.

3.1.4.1 PREPARATION OF HARRIS' HEMATOXYLIN SOLUTION

Hematoxylin crystals	5.0g
Alcohol (100%)	50.0 ml
Ammonium or potassium alum	100 g
Distilled water	1000.0 ml
Mercuric oxide (red)	2.5 g

Hematoxylin was dissolved in alcohol and alum in water by heat. The two solutions were thoroughly mixed and boiled as rapidly as possible. After removing from heat, mercuric oxide was added to the solution slowly. The solution was reheated to a simmer until it became dark purple, and then the vessel was removed from heat and immediately plunged into a basin of cold water until it became cool. 2-4ml glacial acetic acid was added per 100 ml of solution to increase the precision of the nuclear stain. Before use, the prepared solution was filtered.

3.1.4.2 Preparation of Eosin Solution

1% stock alcoholic eosin

Eosin Y, water soluble	1 g
Distilled water	20 ml
95% alcohol	80 ml

Eosin was dissolved in water and then 80 ml of 95% alcohol was added.

Working eosin solution

Eosin stock solution	1part
Alcohol, 80%	3 parts

0.5ml of glacial acetic acid was added to 100 ml of working eosin solution just before use.

3.2 Methods

3.2.1 Experimental Layout

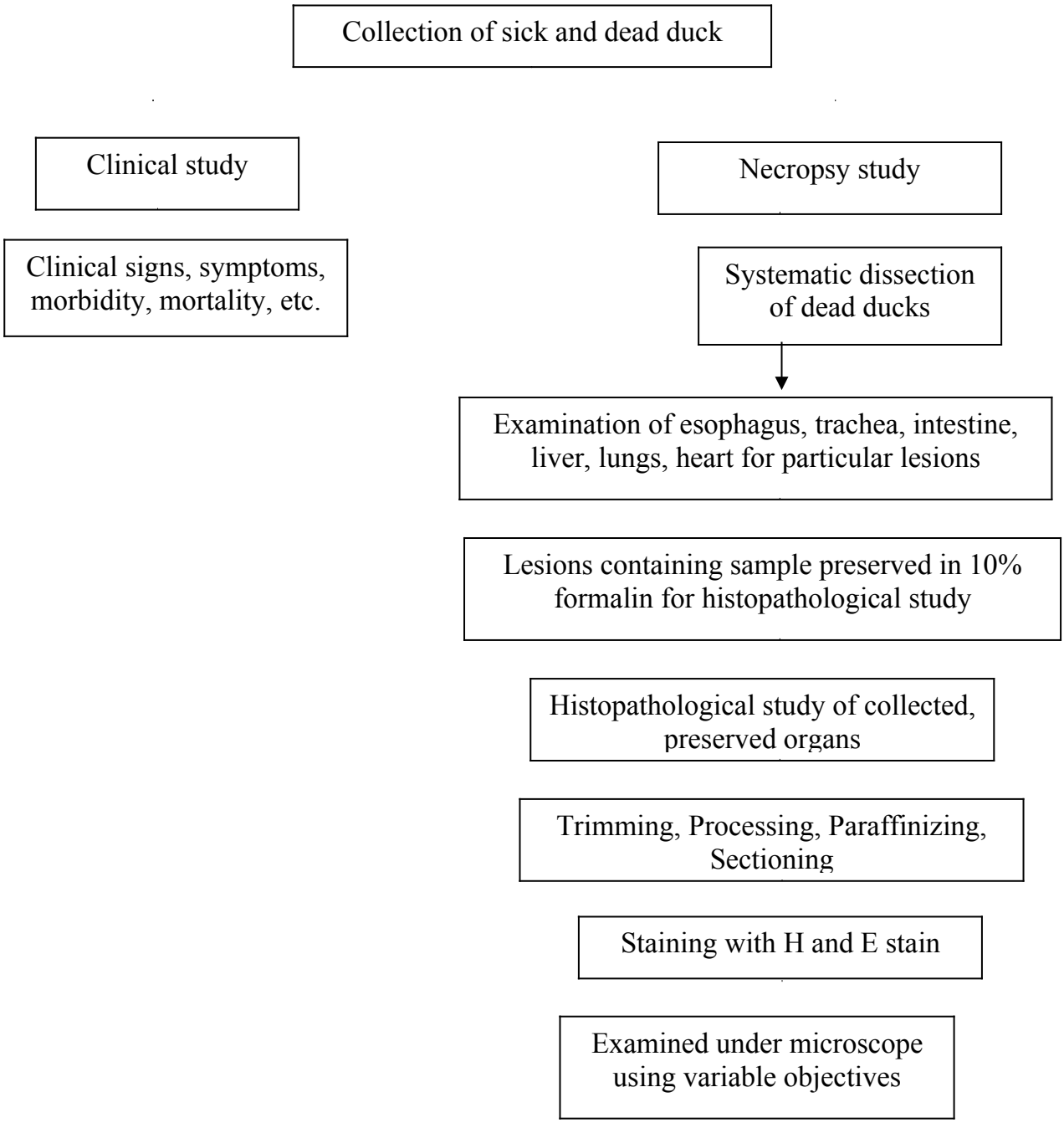

Fig. 1 Schematic illustration of the experimental layout

3.2.2 Field investigation of outbreaks and collection of samples

At the time of field investigation a details of history, age, incidence, morbidity rate, mortality rate, vaccination status was recorded. Clinical signs were observed and postmortem examinations were done on dead and clinically affected ducks. Samples were collected from six upazilas (Dinajpur sadar, Chirirbandar, Parbotipur, Fulbari, Birampur and Nawabgonj upazillas). Different organ like liver, lungs, heart were collected during necropsy for further study. All the diagnostic works were carried under the Laboratory of Department of Pathology & Parasitology, Hajee Mohammad Danesh Science and Technology University (HSTU). Clinical diagnosis and in some cases necropsy examinations were carried out at the place of sampling where as histopathology of all samples were done in the laboratory.

3.2.3 Clinical Examination

The general health condition of the ducks were recorded. The clinical signs were observed from the visual examination. The clinical signs were recorded during the physical visit to the selected area. Farmer's complaints about the affected ducks were considered in some cases.

3.2.4 Necropsy Examination of Suspected Ducks

The necropsy was done on the affected ducks taken from selected area. At necropsy, gross changes were observed and recorded carefully by systemic dissection. The lesion containing tissues and organs were also collected and preserved in 10% formalin for the histopathology. The routine necropsy examination was carried out as follows-

- At first the duck was laid on its back and each leg, in turn drawn outward away from the body while the skin was incised between the leg and abdomen on each side.

- Then the both legs were then grasped firmly in the area of the femur and bent forward, downward, and outward, until the heads of both femurs were broken free of the acetabular attachment so that both legs lied flat on the table.
- The skin was cut between the two previous incisions at a point midway between keel and vent.
- The cut edge was then forcibly reflected forward, cutting was necessary until the entire ventral aspect of the body including the neck was exposed.
- For exposing of the viscera, knife was used to cut through the abdominal wall transversely midway between the keel and vent, then through the breast muscle on each side.
- Positioning shears were used to cut the rib cage, the coracoid and clavicle on both sides.
- The changes in the internal organs were carefully noted.

3.2.5 Histopathological Study

During necropsy, Liver, lung, heart were collected, preserved in 10% formalin for histopathological studies. Formalin fixed tissue samples were processed for paraffin embedding, sectioned and stained with hematoxylin and eosin according to standard method. Details of tissue processing, sectioning and staining are given below.

3.2.5.1 Processing of Tissues and Sectioning

The tissues were processed and sectioned as followed:

Collection of tissue and Processing: During tissue collection the following point 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 was as less as possible (5mm approximately). The tissues (liver, lung, heart) were collected from the ducks that were examined in Dinajpur area. The representative tissues with its normal periphery were collected.

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

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

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%, and 100% ethanol, one hour in each.

Impregnation: Impregnation was done in melted paraffin at 56- 60°c for 3 hours.

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.

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

3.2.5.2 Routine Hematoxylin and Eosin Staining Procedure

The sectioned tissues were stained as described below-

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 absulate alcohol, three minute in each, 95% alcohol for two minutes, 80% alcohol for two minute, 70% alcohol for two minutes) followed by distilled water for 5 minutes.

The tissues were stained with hematoxylin for fifteen minutes and washed in running tap water for 10-15 minutes.

Then the tissues were differentiated in acid alcohol by 2 to 3 quick dips (1 part HCI and 99 parts 70% alcohol) and washed in tap water for five minutes followed by 2-3 dips in ammonia water until sections were bright blue.

Then the section on the slide were stained with eosin for one minute.

The section was differentiated and dehydrated in alcohol (95% alcohol: three changes, 2-3 dips each, absulate alcohol: three changes 2-3 minute for each cleaned in zylene three changes, five minute in each).

Tissues were mounted with cover slip by using DPX.

The slide were dried at room temperature and examined under a low (10X) and high (40X, 100X) power microscopic field.

3.3 Statistical Methods

3.3.1 Determination of Mortality Rate

Mortality rate is a measure of the number of deaths due to a specific cause in a given population. In this study the mortality rate was calculated by the following statistical formula-

$$\text{Mortality rate}(\%) = \frac{\text{Death occurring during a given time period}}{\text{Duck population during the same time period}} \times 100$$

3.3.2 Determination of Prevalence

Prevalence of a disease is the proportion in a given population which have a particular disease at a specified point in time, or over a specified period of time. In this study the Prevalence was calculated by the following statistical formula-

$$\text{Prevalence}(\%) = \frac{\text{Duck plague infected duck during specific time period}}{\text{Duck population during the same time period}} \times 100$$

CHAPTER IV

RESULT

Different upazila like Sadar, Chirirbandar, Parbotipur, Fulbari, Birampur, and Nawabgonj of Dinajpur district were considered as the study population for this research work. The dead and sick duck were collected and subjected to pathology and Parasitology laboratory of Hajee Mohammad Danesh Science and Technology University (HSTU) to determine the clinical signs, gross and histopathological lesions and the status of mortality, prevalence of Duck Plague in native duck of Dinajpur district. The results of different clinical and pathological examination are as follows.

4.1 Results of Clinical Examination

The general health condition of the duck were recorded. The duck were observed to detect clinical signs by visual examination. The characteristic clinical signs include inappetence, weakness, ataxia with ruffled feather and droopy wings (Figure 2), respiratory distress, watery nasal and ocular discharge (Figure 3) and greenish watery diarrhoea (Figure 4).

Fig. 2 Duck showing ataxia with ruffled feather and droopy wings

Fig. 3 Watery nasal and ocular discharge

Fig. 4 Greenish watery diarrhoea

4.2 Degree of Infectivity

The study revealed the following status of mortality and prevalence of duck plague disease (DP) in native ducks. The overall prevalence at Dinajpur district is 19.81% and whereas 14.55%, 14.81%, 30.00%, 11.60%, 32.05% and 15.82% in Sadar, Chirirbandar, Parbotipur, Fulbari, Birampur, and Nawabgonj upazila respectively. The highest mortality was observed at Birampur upazila (25.64%) and lowest (8.21%) at Fulbari upazila with total mortality rate 14.42%. Table-1 showed the mortality and prevalence of DP at different upazila of Dinajpur district. Graphical presentation of mortality and prevalence of duck plague at different upazila of Dinajpur district is shown in figure 5.

Table 1 Mortality and prevalence of duck plague at different upazilla of Dinajpur district

Name of upazila	No. of total duck	No. of infected duck	No. of dead duck	No. of Vaccinated duck	Percentage of Mortality	Percentage of Prevalence
Sadar	2,63,998	38,400	34,560	22,000	13.09	14.55
Chirirbandar	2,20,192	32,600	22,820	38,600	10.36	14..81
Parbatipur	2,55,490	76,647	38,323	56,900	14.99	30.00
Fulbari	1,24,100	14,400	10,080	35,200	8.21	11.60
Birampur	57,245	18,347	14,677	25,400	25.64	32.05
Nawabgonj	78,241	12,380	11,142	28,500	14.24	15.82

Table 2 Mortality and prevalence of different upazilas adjusted by DMRT

Upazila	Mortality (%)	Prevalence (%)
Sadar	13.09 ^{bc}	14.55 ^{bc}
Chirirbandar	10.36 ^{cd}	14.81 ^{bc}
Parbotipur	14.99 ^{b}	30.00 ^{a}
Fulbari	8.210 ^{d}	11.60 ^{c}
Birampur	25.64 ^{a}	32.05 ^{a}
Nawabgonj	14.24 ^{b}	15.82 ^{b}
LSD	**	**
CV %	12.01	10.51
Mean ± SEM	14.42±2.47	19.81±3.6

**0.01% level of significance

LSD = Least Standard Deviation

CV = Co-variance

SEM = Standard Error Mean
DMRT = Duncan Multiple Range Test

Table 2 shows highest mortality and prevalence by ‘a’ symbol and ‘d’ posses lowest mortality and prevalence. The value of co-efficient of variation indicates they are more homogenous and significant.

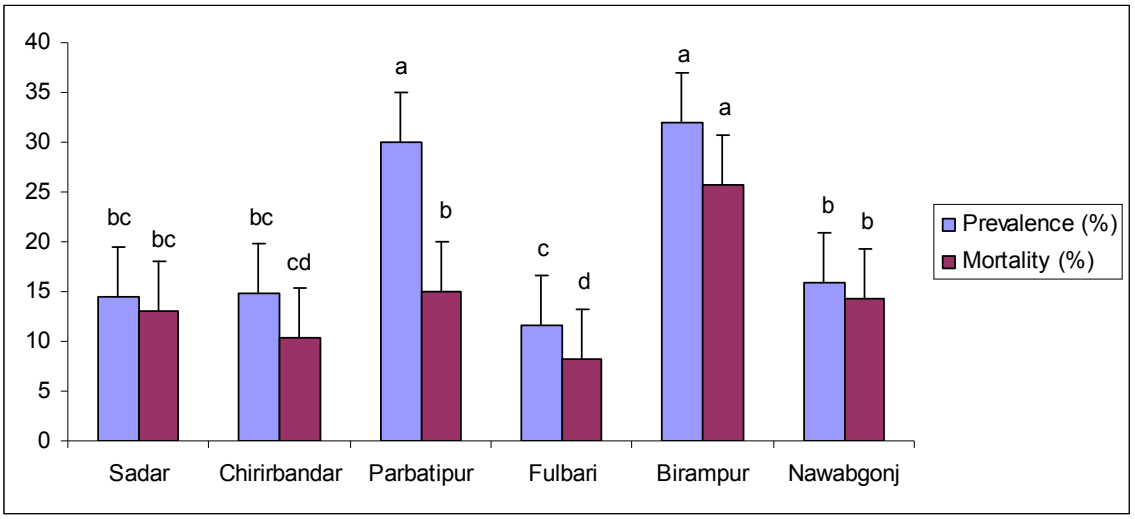

Fig. 5 Prevalence and mortality of duck plague at different upazilla of Dinajpur district. Each bar represents Mean ± SEM value. Without a common lowercase different letter on bars indicate significant differences ($P<0.01$) among different farms.

4.3 Necropsy Findings

At necropsy prominent changes observed in ducks were; multiple tiny haemorrhagic spots and fibrinous exudates with petechiation on internal surface of esophagus (Figure 6,7), the liver was pale copper colour with haemorrhagic spots and white foci (Figure 8,9), congestion in lungs (Figure 10), congestion and minute haemorrhagic spots on heart surface (Figure 11), ruptured follicular contents in peritoneum (Figure 12), misshapen, cystic and ruptured yolk with stalk formation (Figure 13), anaemic oviduct (Figure 14), yellow mucosa of proventiculus (Figure 15), greenish colour of internal surface of gizzard and gizzard content (Figure 16,17), balloned cloacal portion (Figure 18).

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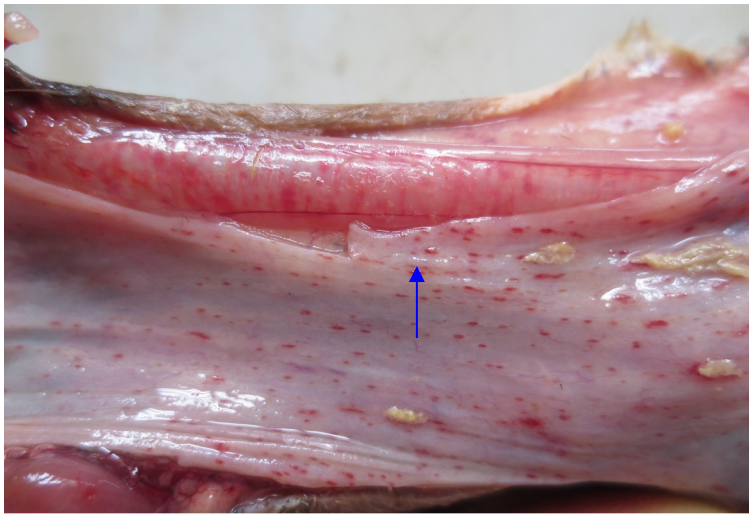


Fig. 6 Multiple tiny haemorrhagic spots on internal surface of esophagus

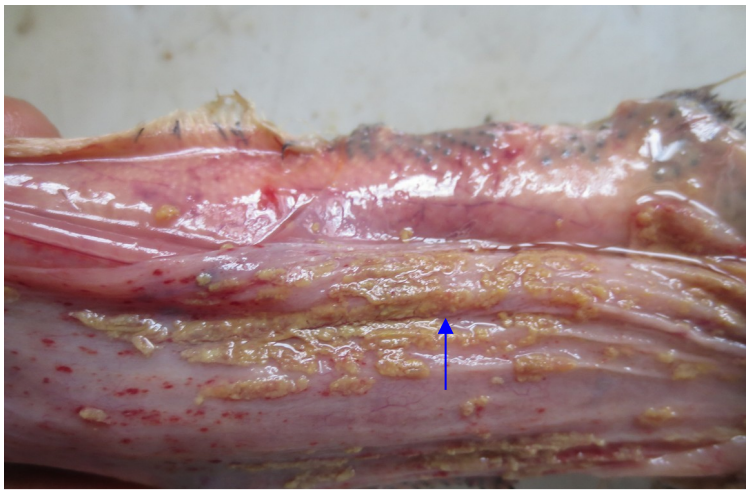


Fig. 7 Fibrinous exudates with petechiation on internal surface of esophagus

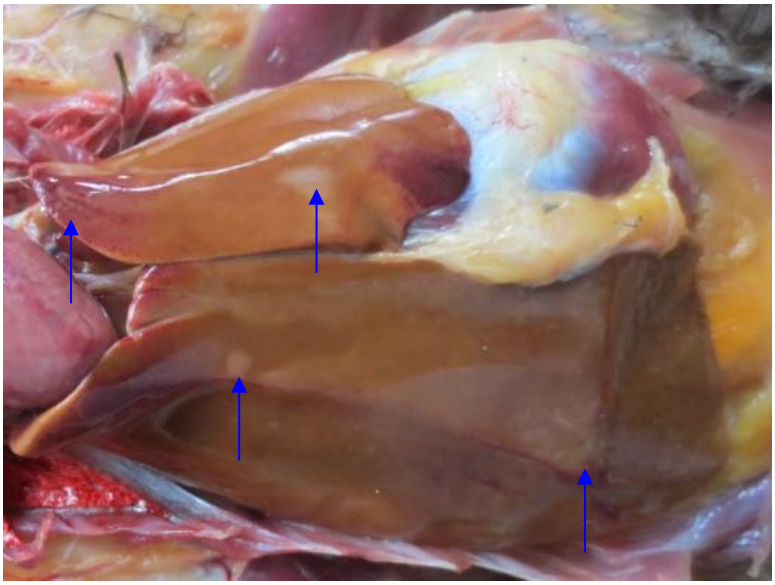


Fig. 8 The liver surface of infected duck was pale copper colour with haemorrhage and white foci

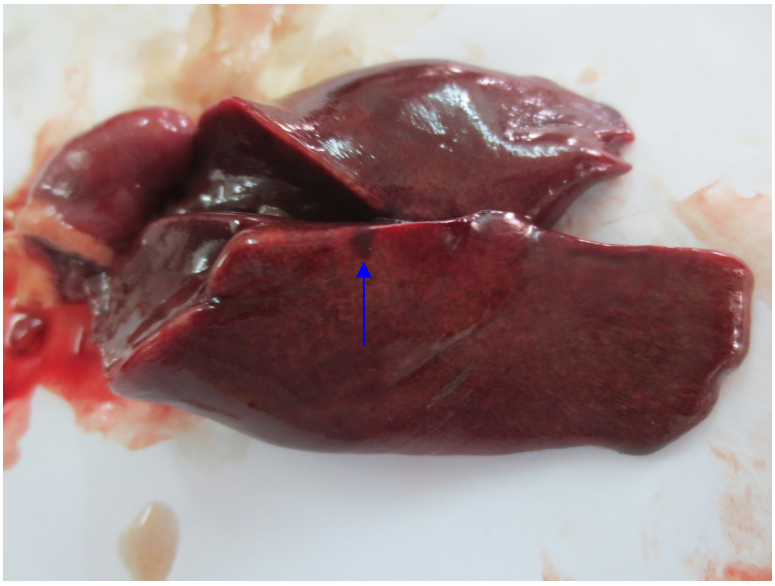


Fig. 9 Congestion and haemorrhagic spots in liver

Fig. 10 Congested lungs

Fig. 11 Congestion and minute haemorrhagic spots on heart muscles

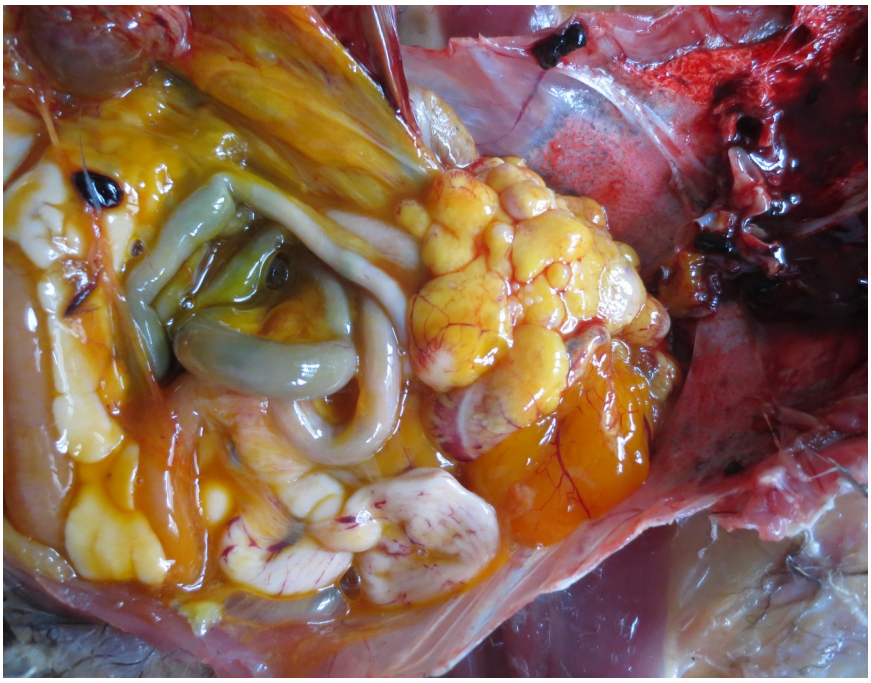


Fig. 12 Ruptured follicular contents in peritoneum

Fig. 13 Misshapen, cystic and ruptured yolk with stalk formation

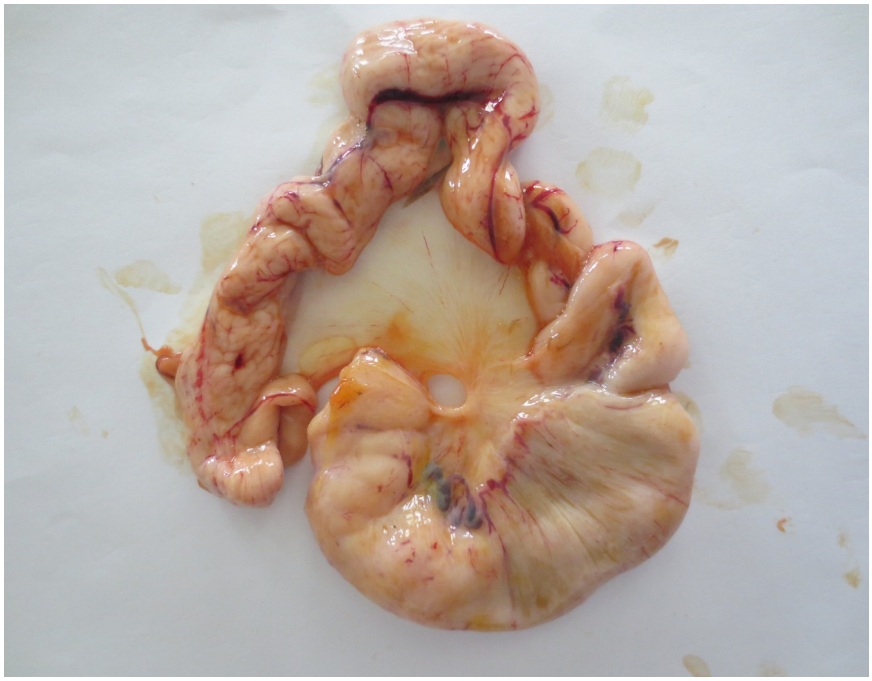


Fig.14 Anaemic oviduct

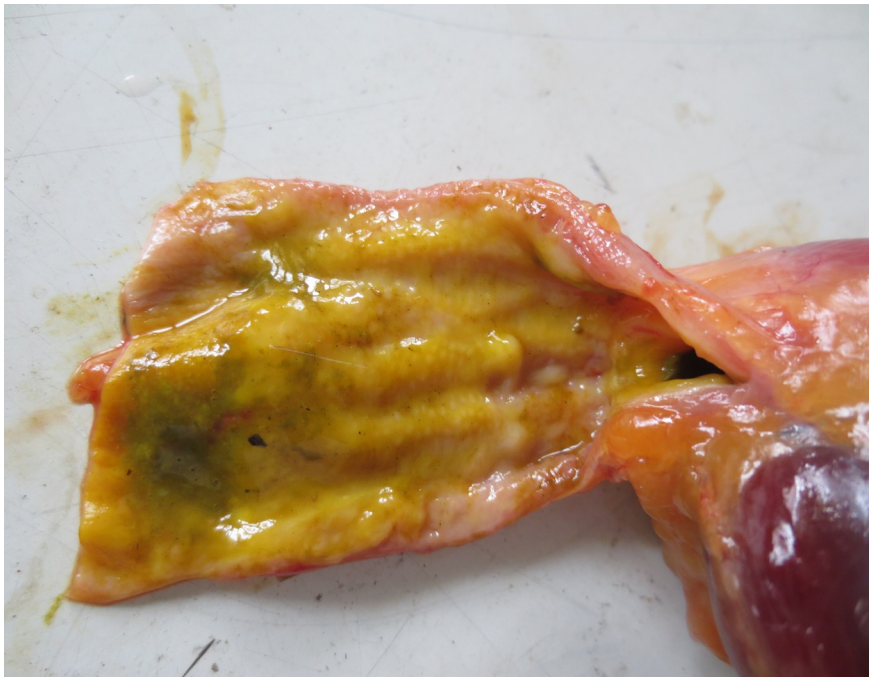


Fig. 15 Yellow mucosa of proventiculus

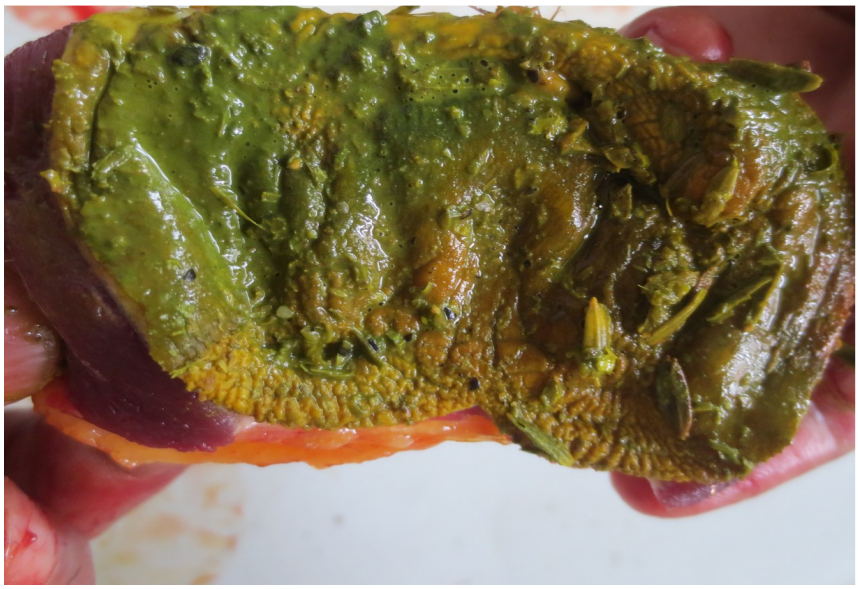


Fig. 16 Greenish colour of internal surface of gizzard

Fig. 17 Greenish gizzard contents

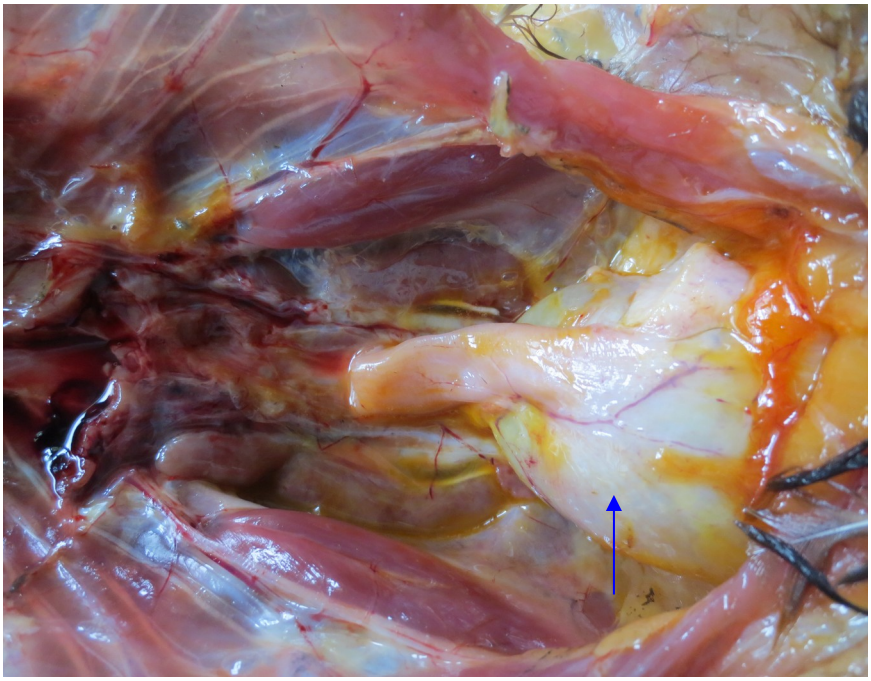
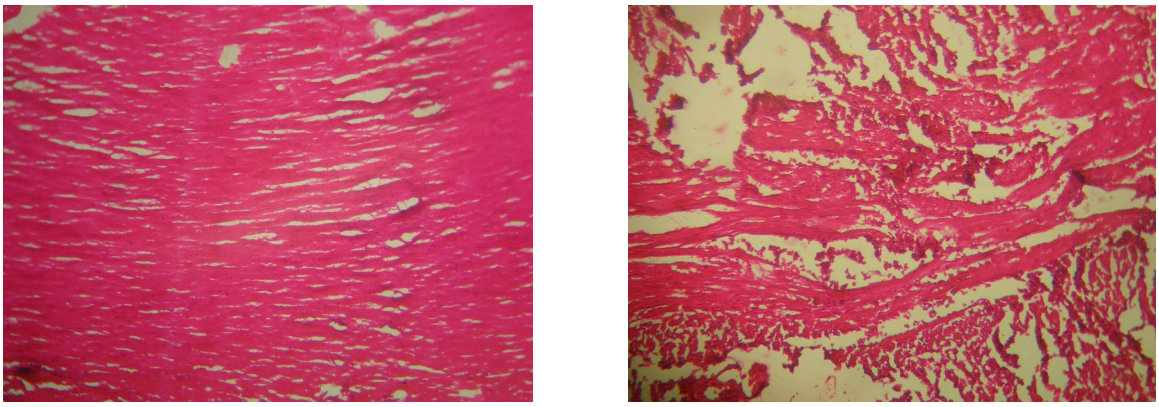


Fig. 18 Balloned cloacal portion

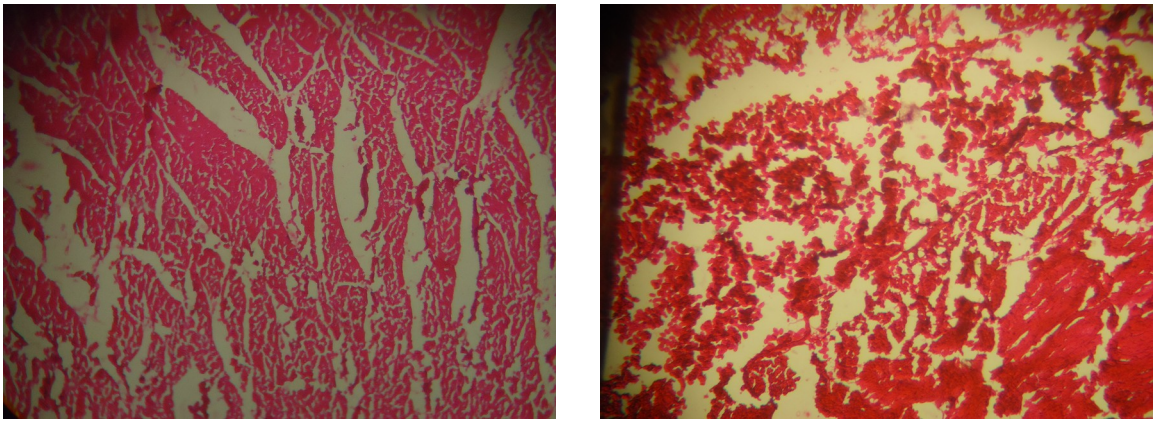
4.4 Results of Histopathological Examination

After staining with H and E stain of the processed heart, there was destruction of muscle fibers, hyperacidophilic sarcoplasm with reactive cells infiltration (Figure 19,20). Thickened interalveolar spaces, alveoli filled with tissue debris and reactive cell infiltration also found in lungs (Figure 21). Breaking of hepatic cords, more acidophilic cytoplasm with fewer leukocytic infiltration were found in liver (Figure 22).



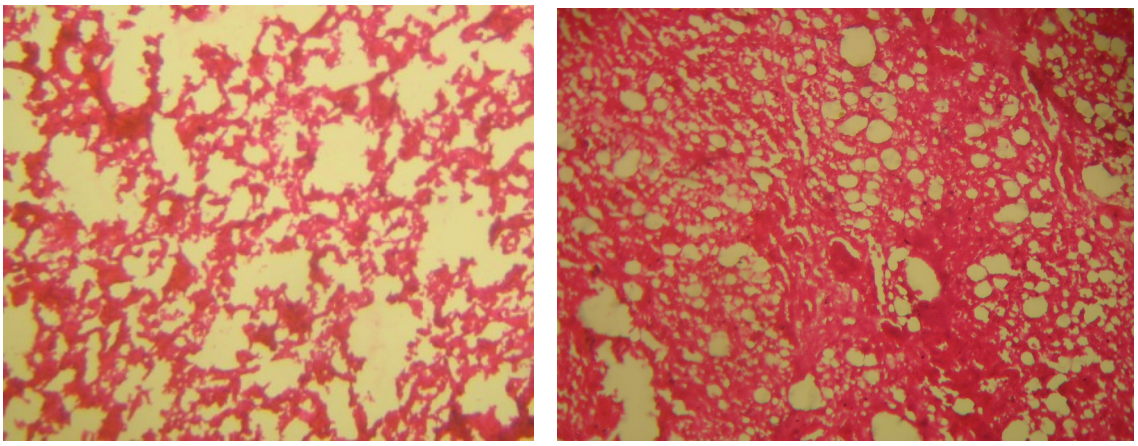
A B

Fig. 19 A. Cardiac muscle (Longitudinal section) : Normal
B. Hyperacidophilic sarcoplasm with reactive cells infiltration in muscles



A B

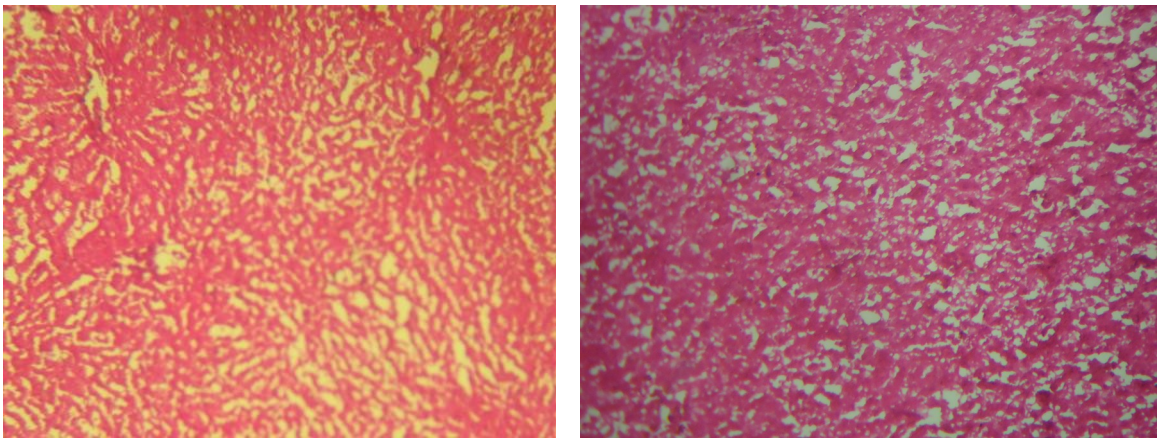
Fig. 20 A. Cross section of heart muscle: Normal
B. Destruction of muscle fibres with reactive cells infiltration in cardiac muscle



A **B**

FIg. 21 A. Normal lung section

B. Thickened interalveolar spaces, alveoli filled with tissue debris and reactive cells infiltration



A **B**

Fig. 22 A. Normal liver section

B. Breaking of hepatic cords, more acidophilic cytoplasm with fewer leukocytic infiltration

CHAPTER V

DISCUSSION

This study was undertaken to investigate the pathological condition of duck plague at different upazillas of Dinajpur district. The study was conducted in the Department of Pathology and Parasitology, HSTU, Dinajpur, during January 2014 to June 2014. At least 3 ducks per affected flock were collected from Dinajpur district and diagnosis of diseases was based on history, clinical sign, gross and microscopic lesions as had been diagnosed by Burgess and Yuill, (1981); Shawky *et al.,* (2002).

On the basis of visual examination, the characteristic clinical signs include inappetence, weakness, ataxia with ruffled feather and droopy wings, respiratory distress, watery nasal and ocular discharge, and greenish watery diarrhoea which correspond with the findings of Brand and Docherty (1984); Wobeser (1997). Major clinical signs of duck plague found by Baki *et al.,* (1993) were sudden death, anorexia, listlessness, droopy wings, inability to stand, swollen face with watery to viscid mucus discharge from eyes and nostrils, profuse greenish watery diarrhoea. Polydypsia was recorded during the physical visit of the farms and this clinical manifestation is due to the fibroblastic exudates in the esophageal mucosa.

The present study showed that overall prevalence at Dinajpur district were 19.81% whereas 14.55%, 14.81%, 30.00%, 11.60%, 32.05% and 15.82% in Sadar, Chirirbandar, Parbotipur, Fulbari, Birampur, and Nawabgonj upazila respectively (Table 1). The highest mortality was observed at Birampur upazila (25.64%) and lowest (8.21%) at Fulbari upazila with total mortality rate 14.42%. These results vary with the reports of Das *et al*., (2005); Saif *et al.,* (2008). Khanum *et al.,* (2005) reported that the mortality rate in Netrakona was 27.1%. This result variation may be due to the geo-climatic condition, biological barriers, immunization status, social awareness and mostly on the health status of the birds.

In this observation, the gross pathological lesions were multiple tiny haemorrhagic spots and fibrinous exudates with petechiation on internal surface of esophagus, the liver was pale copper colour with haemorrhagic spots and white foci, congestion in lungs, congestion and minute haemorrhagic spots on heart surface, ruptured follicular contents in peritoneum with misshapen, cystic and ruptured yolk with stalk formation, yellow mucosa of proventiculus and greenish colour of internal surface of gizzard and gizzard content, congestion and haemorrhages in vascular channels of mesentary. These findings support the observation of Chennakesavalu *et al.,* (1987); Mahmud (2012); Brand and Docherty (1984). This changes may occure because of the organism causes damage to endothelial cells of small blood vessels, lymphoid tissue, and some epithelia. That leads leakage of blood into tissue and cause vascular damage and tissue necrosis.

Histopathological study revealed the finding as destruction of cardiac muscle fibers, hyperacidophilic sarcoplasm with reactive cells infiltration. In lung there was thickened interalveolar spaces, alveoli filled with tissue debris and reactive cell infiltration. Breaking of hepatic cords, more acidophilic cytoplasm with fewer leukocytic infiltration also found in liver. These lesions were in agreement with those described by Das *et al.,* (1988 and 1990); Davison *et al.,* (1993) who reported that microscopically in heart there were degeneration and necrosis with reactive cell infiltration, in liver multi focal area of necrosis and degeneration of hepatocytes were found. This lesions developed due to the organisms cause vascular damage that leads to tissue necrosis.

Characteristic flock history, clinical manifestations recorded during the physical visits, farmer's complaint's about their affected flocks, remarkable gross morbid lesions observed during the course of necropsy examination as well as cardinal histomorphological features of the affected organs collected during postmortem examination would certainly help to diagnose the disease in the investigated area.

CHAPTER VI

SUMMARY AND CONCLUSION

Duck rearing is a profitable agro sector in Bangladesh because of low cost feeding in free water bodies of the country. As these water sources supply natural aquatic feeds, duck rearing has become a means of reducing rural poverty in those areas especially. But the major constrains of duck rearing in Bangladesh are the outbreaks of various diseases and increase rate of mortality. Farmers do not know about the prevention and control strategies of these diseases. Moreover, mis-management and dependency only on natural feed sources, stresses the ducks for various infectious and non-infectious diseases aggravate the situation.

For the prevention and control of the diseases, a thorough knowledge about the occurrence of diseases, their epidemiology including morbidity and mortality pattern, pathogenesis and pathology of the diseases are essential. The fact is that the prevalence of diseases in a specific area depends on various factors like geo-climatic condition, biological barriers, immunization status, social awareness and mostly on the health status of the birds.

The present pathological investigation and prevalence study on to the disease of ducks in Dinajpur district of Bangladesh was conducted in the Department of Pathology and Parasitology, HSTU, Dinajpur, during the period from January 2014 to June 2014. A total of five ducks were collected from Dinajpur district and diagnosis of diseases was based on history, clinical signs, gross and microscopic lesions. Investigation of causes of duck mortality is key to prevent the outbreak of diseases of duck. Present work was undertaken to uncover clues of duck mortality. The field investigation, history, clinical signs and postmortem findings were of the affected ducks of Dinajpur area recorded. From these findings, duck plague was suspected. Laboratory investigation was conducted by histopathological examination of duck plague. From all these findings it may be concluded that duck plague was an important cause of duck morbidity and mortality. Since duck

plague appeared as an important disease hindering the duck industry in Dinajpur area, regular vaccination, treatment and bio-security measures used to follow farmers under veterinarian supervision. Further investigation is needed to uncover clues about few more diseases of ducks leading to duck morbidity and mortality with wider ecology and large number of sample. The study indicates that there are great potentials for an improvement of duck production in rural Bangladesh. This research will be very helpful in formulating strategies for the betterment of duck farming. From this research interest point of view

- Molicular characterization of duck plague was necessary for further study.

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