PATHO–PREVALENCE OF DUCK CHOLERA IN SELECTIVE UPAZILAS OF BOGURA DISTRICT

MS Thesis

Md. Tomal Mahmud

Master of Science

In

Pathology

Department of Pathology and Parasitology

Hajee Mohammad Danesh Science and Technology University, Dinajpur

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A Thesis

Submitted to
Hajee Mohammad Danesh Science and Technology
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In Partial Fulfillment of the Requirement for the Degree of
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By

Md. Tomal Mahmud
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DEPARTMENT OF PATHOLOGY AND PARASITOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY
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Approved as to style and content by

Prof. Dr. S. M. Harun-ur-Rashid	**Prof. Dr. Md. Nazrul Islam**
(Research Supervisor)	(Research Co-Supervisor)

Prof. Dr. S. M. Harun-ur-Rashid
Chairman,
Examination Committee
Department of Pathology and Parasitology

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY

UNIVERSITY, DINAJPUR

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ABSTRACT

A pathological study was conducted on Duck cholera occurring in selective Upazila of Bogura district of Bangladesh during the period from July 2017 to December 2017 to know the prevalence of Duck cholera, gross and histopathological change of different organs and to identify the effect of age and sex for developing the disease. A total of 207 ducks either sick or dead were examined at different Upazilla Veterinary hospital of Bogura district out of which 23 ducks were found to be positive on duck cholera. The diagnosis of Duck cholera was performed on the basis of history of the affected flock, clinical signs, post mortem examinations, pathological findings, age and sex of affected birds. The major clinical manifestations in the ducks suffering from natural Duck cholera were found as depression and ruffled feather, lameness, greenish diarrhea, Feathers around vent soiled with greenish diarrhea and swollen hock joint with thickening in the skin of footpad. In this study, the overall prevalence of Duck cholera in Bogura district was 11.11% which is followed by 13.95% in Sariakandi, 13.51% in Sonatola, 11.76% in Dhonut, 9.68% in Bogura sadar, 9.09% in Gabtoli and 6.90% in Sherpur upazila. According to the age group of ducks, It was significantly (p>0.01) higher among >2–8 weeks of age (18.52%) which is followed by among >8-20 weeks (10.13%), between 0-2 weeks of age (0%) and among >20 weeks of age (0%). Present study found the prevalence of Duck cholera between sex group and it was significantly ($p<0.05$) higher in female (14.08%) than in male (4.62%) ducks. Main gross findings in affected ducks were haemorrhage on liver, enlargement of liver, pin point haemorrhage on heart, extravassation of heart blood, Straw color pericardial fluid in heart and Enlarged, haemorrhagic and congestion of Spleen. Histopathological findings were dilated central vein, more acidophilic cytoplasm of the hepatocytes, reactive cells infiltration, dilated sinusoids, mild to moderate level of hepatocytic destruction, bacterial colony in liver parenchyma. Present study suggests that Duck cholera is prevalent among the duck farm of Bogura district. So effective control measures should be taken to minimize this problem.

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CHIEPTER I

INTRODUCTION

Among the poultry species, Ducks are playing vital role in supplying complete protein to the villagers of Bangladesh by producing meat and eggs. Next to chickens, ducks are main poultry species which are treated as standby money generator of village women in the coastal and haor area of Bangladesh. Now a day beside chicken farming small and medium scale duck farming are getting priority and popularity. But there are a lot of constraints in the development of duck industry in Bangladesh. Because infectious diseases are considered as one of the most important causes of economic loss in this sector in Bangladesh (Singh *et al*., 2014).

Some infectious diseases such as duck Plague, duck cholera, salmonellosis, colibacillosis, duck viral hepatitis etc. are more common. Presently these diseases become a great threat for duck survival in Bangladesh. Among these diseases Duck Cholera is one of the most important and devastating endemic bacterial disease in Bangladesh (Baki *et al*., 1993) which is caused by *Pasteurella multocida*. It produced 1.8-21% mortalities and 15-20% decreases in egg production, in addition to the costs of vaccination and treatment of diseased birds and finally culling of the infected flock (Wilkie *et al*., 2012).

The transmission of disease among birds believed to occur from bird-to-bird contact and by ingestion of bacteria or aerosol transmission within a contaminated environment. Discharge of pasteurellae from dead or diseased birds is considered an important source of wetland contamination and transmission to susceptible birds. Despite its occurrence in domestic fowl on most continents, avian cholera seems best described as having a limited distribution and significance for most wild bird populations around the world (Botzler, 1991).

The disease is characterized by its acute nature and causing high and rapid deaths. Acute illness is common; infection can result in mortality within 6 to 12 hrs after exposure, although one to two days is more typical (Friend, 1999). Clinical signs of fowl cholera in ducks include nasal and ocular discharges, greenish diarrhea, ataxia, tremors of head and neck followed by coma (Fouad and Hebat Allah, 2008 and Eldin and Reda, 2016). Sub-acute form of duck cholera was characterized by lameness, corneal turbidity and depression (Takahashi *et al*., 1996). The prevalence of duck cholera in healthy Duck flocks is as high as 63%, and mortality may reach 50% which causing a great economic loss and discouraging of duck rearing (Baki *et al*., 1993) to the duck farmers.

Necropsy findings due to acute fowl cholera in ducks were characterized by extensive congestion, enlarge and necrotic foci on spleen and liver, petechial hemorrhage in cardiac muscle, necrotic parenchymatous hepatitis, congestion and hemorrhages in the intestinal mucosa (Fouad and Hebat Allah, 2008; Mohan and Pradeep Kumar, 2008 and Mohamed *et al*., 2012), fibrenous pericarditis, perihepatitis, airsaculitis and meningitis (Rhoades and Rimler, 1988). The histopathological signs of Duck cholera were hemorrhage, congestion and lymphoid cell infiltration in liver, heart and spleen (Shilpa *et al*., 2006).

Bogura district is one of the most important Duck rearing area of Bangladesh. The Duck cholera is considered a major constraint and economically important diseases of ducks of different age and breeds in Bangladesh especially in Bogura district. Previously very few studies were conducted in Bangladesh regarding clinico-pathological investigation of *P. multocida* of duck origin. Therefore, the present study was undertaken with the following aims and objectives-

- To investigate the clinico-pathology of Duck cholera in ducks of the affected flocks
- To study the gross and histopathological changes of organs developed due to Duck cholera from infected/death ducks
- To determine the prevalence of Duck cholera in Bogura district

CHIEPTER II

REVIEW OF LITERATURE

Available literature for the determination of Pathology of Duck cholera in commercial duck farm is reviewed in this part of the thesis after a brief overview on the history, etiology, epidemiology, pathogenesis, clinical signs, gross lesions, microscopic lesions, immunity, control and treatment of Duck cholera.

2.1 Duck cholera

Duck cholera, Fowl cholera, avian cholera, avian pasteurellosis or avian hemorrhagic septicemia is considered to be one of the most contagious and economically important bacterial diseases of poultry caused by *Pasteurella multocida* and remains an important havoc for the poultry production in Bangladesh (Singh *et al*., 2014). It is a commonly occurring avian disease that infects all types of birds in Bangladesh. It usually occurs as an acute septicemia associated with high morbidity and mortality. Fowl cholera causes mortality about 25 to 35% in chickens and ducks of Bangladesh (Ievy *et al*., 2013).

2.2 Synonyms

Duck cholera, Fowl cholera, avian pasteurellosis, avian hemorrhagic septicemia, chicken cholera.

2.3 History of duck cholera

The disease was first recorded in the 18th century. An avian cholera-like disease was reported in domestic birds in Italy as early as 1600. However, Fowl cholera was first described among domestic birds by veterinarians in France in the late 1700s (Gray, 1913), and the infectious nature of the disease was not recognized until the 1850s (Hutyra *et al.,* 1949). Epizootics occurred through the 1800s in domestic birds across several European' countries, including France, Bohemia, Austria, Russia, Italy, and Hungary, as well as the East Indies (Gray, 1913) believed the

disease was introduced to Germany between 1897 and 1899 when geese and other domestic fowl were imported from Russia, Poland, Silesia, and Italy. However, this theory conflicts with other accounts in which avian cholera was reported earlier among ducks, including domestic ducks and various hybrids, swans, and geese in Germany (Wilson, 1995a). Interestingly Wilson (1995a) also noted that Spaitows and other species were not affected during these early epizootics. The causative agent, "*Pasteurella,*" was named in honor of Louis Pasteur (Rosen, 1971) who attenuated the bacterium and produced the first vaccine in the late l800s (Rimler and Glisson, 1997).

Avian cholera was present in South Africa and probably Australia and New Zealand in the early 1900s (Gray, 1913). The disease had a high prevalence in Great Britain in 1900, and Gray (1913) speculated that it had been present there for about 40 years. Avian cholera declined in importance in northern, western, and central Europe in the early 1900s to the point of having little significance; however, it still was considered important in eastern and southern Europe. Avian cholera was reported among domestic birds in the United States between 1880 and 1882 (Gray, 1913). Cases also were recorded in the United States in 1898 and in Canada in 1899.

Later, avian cholera was reported in wild Ruffed Grouse and geese, ducks, turkeys, pheasants, pigeons, quail, and a large number of other wild birds in the United States were found to be susceptible (Shillinger and Morley, 1942).

The first known epizootics in wild North American waterfowl occurred in 1943-1944 among ducks in Texas as well as ducks, American Coots *(Fulica americana),* Tundra Swans *(Cygnus columbianus),* gulls, shorebirds, and other species in northern California (Rosen and Bischoff *et al.*, 1949). The epizootics in both Texas (Gordus, 1993a) and California (Rosen and Bischoff *et al.*, 1949, 1950) were associated with nearby mortality in domestic fowl and suspected disposal of dead birds into the environment.

The first report of avian cholera among wildfowl (wild birds) outside North America occulted during the same time period with the mortality of about 40 wild

Egyptian *(Alopochen aegyptiacus)* and Spurwinged Geese *(Plectropterus gambensis)* on Lake Nakuru, Kenya, in 1940 (Hudson *et al.*, 1959).

In 1941, avian cholera was reported among marine ducks, pelicans (probably *Pelecanus occidentalis),* and gulls in Chile. The disease has also been reported in Great Skuas *(Catharacta skua)* from Antarctica (Parmelee *et al.,* 1979) and Rockhopper Penguins *(Eudyptes crestatus)* from New Zealand (De Lisle *et al.,* 1990). In North America, the disease was present in dometic fowl at least 75 years before it was recognized in wild waterfowl (Heddleston and Rhoades *et al.*, 1978) and there is evidence that the disease was absent from waterfowl prior to 1944.

The late 1970s, high mortality of Common Eiders *(Somateria mollissima)* occurred in the Netherlands in overcrowded winter areas such as blowholes in the ic (Mullie *et a1.*,1980). Further epizootics were reported from breeding colonies in 1984 (Swennen and Smit, 1991). In 1996, epizootics occurred on both wintering and breeding areas for Common Eiders in Denmark (Christensen, 1996; Christensen *et a1.*, 1997). Substantial mortality recurred on breeding areas during 2001 (Pedersen *et al.,* 2003). During these later epizootics, Great Cormorants *(Phalacrocorax carbo),* Eurasian Oystercatchers *(Haematopus ostralegus)*, Herring Gulls *(Larus argentatus),* and Greater Black backed Gulls *(Larus marinus)* were also affected (Pedersen, 2003).

Historically, avian cholera was considered a disease associated with wintering waterfowl. However, epizootics have occurred in all four North American waterfowl flyways, during both spring and fall migration in Canada (Wobeser, 1997), during the nesting season for Common Eiders on the east coast of the United States and Canada (Jorde *et a1.*, 1989) and Lesser Snow Geese in the Arctic (Samuel *et a1.*, 1999a), and during summer molt of Redheads *(Aythya americana)* in Saskatchewan (Canada).

The outbreak of avian cholera in Atlantic Canada between December 2006 and March 2007 is the first known report of an outbreak in a pelagic environment, although other marine bird mortality has been reported on St. Lawrence Island, Alaska (Bodenstein *et al.*, 2015) and among Common Eiders wintering along

Danish sea ice (Christensen *et al*., 1997). In Malaysia in 2004, fowl cholera caused 50% of the mortalities in 400 populations of Muscovy duck in the district of Kelantan (2005, Annual report of Regional Veterinary Laboratory, Kota Bharu-unpublished report).

In 2011 an outbreak of avian cholera killed thousands of eider ducks in Arctic regions of Canada. Scientists are studying the outbreak and its potential to spread to Greenland. In March of 2015, another outbreak of avian cholera killed roughly 2,000 snow geese in northern Idaho while flying their spring migration to Canada (Wikipedia).

2.4 Etiology

2.4.1 Texonomy of *pasteurella multocida*

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gamma Proteobacteria

Order: Pasteurellales

Family: Pasteurellaceae

Genus: ***Pasteurella***

Species: ***Pasteurella multocida, Pasteurella avicida, Pasteurella aviseptica, Pasteurella muricida, Pasteurella muriseptica, Pasteurella gallicida.***

2.4.2 Most common speices

P. multocida is a Gram-negative, non-motile, cocco-bacillus, capsulated, non-spore forming bacterium occurring singly, in pairs or occasionally as chains or filaments

belonging to the Pasteurellaceae family (Ashraf *et al*., 2011; Ievy *et al*., 2013). Bipolar staining characteristic is evident with methylene blue, Wright's stain, Giemsa stain, and Gram stain. For a while, each isolate was named according to the clinical presentation and animal group from which it was recovered, such as *P. avicida* or *P. aviseptica,* and *P. muricida* or *P. muriseptica* (Heddleston, 1972). An identification system to distinguish among ***P. multocida*** strains by serological and biochemical characteristics includes five capsular serotypes (A, B, D, E, and F) based on a hemagglutination method (Rimler and Glisson, 1997), 16 somatic serotypes (1-16) based on characterization of capsular and cell wall antigens and three subspecies based on carbohydrate fermentation patterns (Christensen and Bisgaard, 2000).

The complete genetic sequence of one common avian strain recently was completed and may provide a template for development of genetic methods and comparison among strains. More detailed descriptions of the biochemical and serological characteristics of ***P. multocida*** are reported by (Christensen and Bisgaard, 2000). The molecular biology and methods for genetic analysis of ***P. multocida*** have been reviewed in Hunt *et al.,* (2000) and Blackall and Mifiin (2000).

2.5 Epidemiology

2.5.1 Geographical distribution

Historical and recent reports of avian cholera in wild birds are considerably more limited in global distribution. The disease is more frequent in temperate and warm zones. Since about 1980, most reports of substantial epizootics in wild birds North America, where significant epizootics occur almost annually. In other parts of the world, recurrent epizootics have been more limited. In the late 1970s, high mortality of Common Eiders *(Somateria mollissima)* occurred in the Netherlands in overcrowded winter areas such as blowholes in the ice (Mullie *et al*., 1979, 1980). Further epizootics were reported from breeding colonies in 1984 (Swennen and Smit, 1991). In 1996, epizootics occurred on both wintering and breeding areas for Common Eiders in Denmark (Christensen *et a1.,* 1997). Substantial mortality

recurred on breeding areas during 2001 (Pedersen *et al.,* 2003). During these later epizootics, Great Cormorants *(Phalacrocorax carbo),* Eurasian Oystercatchers *(Haematopus ostralegus)*, Herring Gulls *(Larus argentatus),* and Greater Black backed Gulls *(Larus marinus)* were also affected (Pedersen *et al.,* 2003). Although avian cholera in domestic birds has been reported from many parts of eastern Asia and from wild species in zoological collections or farms in Japan (Sawada *et a1*., 1999), it was not reported in wild birds until 2000, when an epizootic killed 10,000 Baikal Teal *(Anas formosa)* in Korea (Kwon and Kang, 2003).

2.5.2 Susceptible host

All domestic and wild species of birds are susceptible to fowl cholera. Most reported outbreaks involve chickens, turkeys and ducks, and occasionally species such as geese, pigeons, pheasant, quail, sparrows and finches. ***P. multocida*** also infects mammals. It causes diseases such as haemorrhagic septicaemia and shipping fever in cattle, fowl cholera in poultry (Glisson *et al*., 2003), and pneumonia in pigs. More than 190 species of birds, from at least 44 families, have been reported as naturally infected with ***P. multocida***. Bird groups most frequently affected by avian cholera are waterfowl and coots, followed by scavengers (gulls, raptors, and crows), and to a lesser extent other water birds (waders, shorebirds and cranes) and upland species.

2.5.3 Susceptible age of infection

This disease was recorded in duck of more than 4 weeks of age in duck farms. This observation is in conformity with the earlier report of Choudhury *et al.,* (1985) who reported Duck cholera in duck aged between 6 to 12 months old.

Talha *et al.,* (2001) reported Duck cholera in ducks from more than 2 weeks of old with highest incidence in adult (> 20 weeks birds) and Rahman *et al.,* (2004) who reported Duck cholera in ducks more than two weeks of age with significantly ($p < 0.01$) highest occurrence in adult ducks. Islam *et al*., (2009) reported the presence

of Duck cholera (13.40%) was the highest and its presence was insignificantly ($p>0.05$) higher in pullet stage (16.70%) than laying stage (10.80%).

2.5.4 Seasons of infection

Seasonal influence on mortality of Fowl cholera in duck showed (13.40%) and it was the highest and its highest occurrence was observed in rainy (22.20%) season followed by summer (10.30%) and winter (10%) season (Islam *et al*., 2009). Rahman and Adhikary. *(*2016) reported the presence of Duck cholera highest in winter (3.10%) season followed by summer (2.37%) and rainy (1.55%) season.

2.5.5 Source of infection

The primary source of ***P. multocida*** infection can be excretions from the nostrils, mouth and eyes of sick birds or chronic carriers. The secondary sources are contaminated feed, water, crates, equipment and shoes. Wild birds, including sparrows and pigeons and many mammals (especially pigs, cats, and wild rodents) can disseminate ***P. multocida***. The organism can persist for years in the oral cavity of rodents and carnivores (Quan *et al*., 1986). Birds bitten by such animals can become infected and disseminate the disease within the flock. Cannibalism of sick or dead birds is also a significant method of dissemination Rahman *et al.,* (2004).

2.5.6 Trasmission

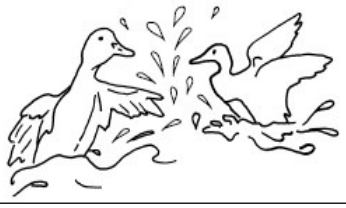
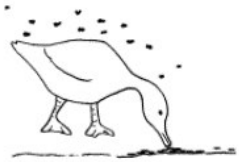
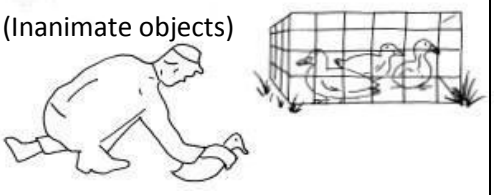
Avian cholera can be transmitted within this contaminated environment in several ways. Ingestion of bacteria in contaminated food and water, including scavenging of diseased carcasses, is an important source of infection for wild birds (Wobeser, 1992). The disease can be transmitted by direct bird-to-bird contact, either between infected and non-infected live birds, or between infected carcasses that serve as "decoys" and non-infected live birds (Fig 2.1). Aerosol transmission is also thought to take place. In wetlands where avian cholera breaks out, the highest concentrations of ***P. multocida*** are found near the water surface rather than deep in the water column (Rosen and Bischoff, 1949; Potter and Baker, 1961), Birds landing,

taking flight, bathing, and otherwise causing disturbance of the water surface cause bacteria laden aerosols (Rosen and Morse, 1959; Blanchard and Syzdek, 1970), which can serve to infect those birds. Other means for transmission of avian cholera have also been reported, each of which may occur for specific situations, but none of which are primary means for disease transmission in wild birds (Table 2.1). *Pasteurella multocida* can enter the mucous membranes of the pharynx upper respiratory tract (Wobeser, 1992) or conjunctiva (Wilkie *et al.,* 2012). Domestic turkeys were very susceptible to ***P.*** *multocida* by inoculation into the palatine air spaces (Donahue and Olson, 1971).

Other routes of transmission via arthropods or animal bite can also occur, but these sources are unlikely to produce epizootic mortality in wild birds. Among domestic birds, *P multocida* can be carried or transmitted by Mallophaga *(Eomenaconthuss tramineus* and *Menopon gallinae)* (Derylo, 1967, 1969), the soft tick *Argas persicus* (Glukhov and Novikov, 1975). *Pasteurella multocida* also has been recovered from mites *(Dermanyssus* spp.) collected from wild ducks dying from avian cholera and transmission of ***P. multocida*** by tabanid flies has been reported (Krinsky, 1976).

Pasteurellosis can be transmitted to individual birds bitten by mammals, including cats (Korbel, 1990) and raccoons *(Procyon lotor)* (Table 2.1). However, there is no direct evidence that animal or arthropod bites play an important role in infectious transmission of avian cholera among wild birds (Botzler, 1991)

Table 2.1 Potential means for transmission of avian cholera to free-ranging wild birds

Route of transmission	Comments
Bird-to-bird contact 	Secretions from infected birds shedding *P. multocida.* Requires close contact, such as when individuals struggle over aquatic plants that they are feeding upon.
Ingestion 	Probably most common route for transmission. Consumption of diseased carcasses by scavengers and predators. Ingestion of *P. multocida*in food and water from contaminated environments.
	May be important in heavily contaminated environments, such as during major die-offs. Activities that result in splashing of surface waters result in bacterialaden sprays when water becomes contaminated.
Insects 	Biting insects that feed on birds after having fed upon contaminatedcarcasses or contaminated environments (ticks, mites, flies). Insects fed upon by birds (maggots, flies) following ingestion of *P. multocida*by the insect when feeding.
Animal bites 	Not thought to be an important route for infection of wild birds. Nonfatal bites from small mammals, such as raccoon, can result in *P. multocida*infections that become systemic and possibly initiate disease outbreaks. Thought to occur in some domestic turkey flocks, not yet demonstrated in wild birds.
Fomites (Inanimate objects) 	Contaminated cages, equipment, and clothing used in field operations can serve as mechanical transport mechanisms for introducing *P. multocida*. Environmental persistence of *P. multocida*is sufficient for this to be a consideration when personnel and equipment are used to combat an avian cholera outbreak and then are to be redirected for other activities.

Ref. Field Manual of Wildlife Diseases: Birds

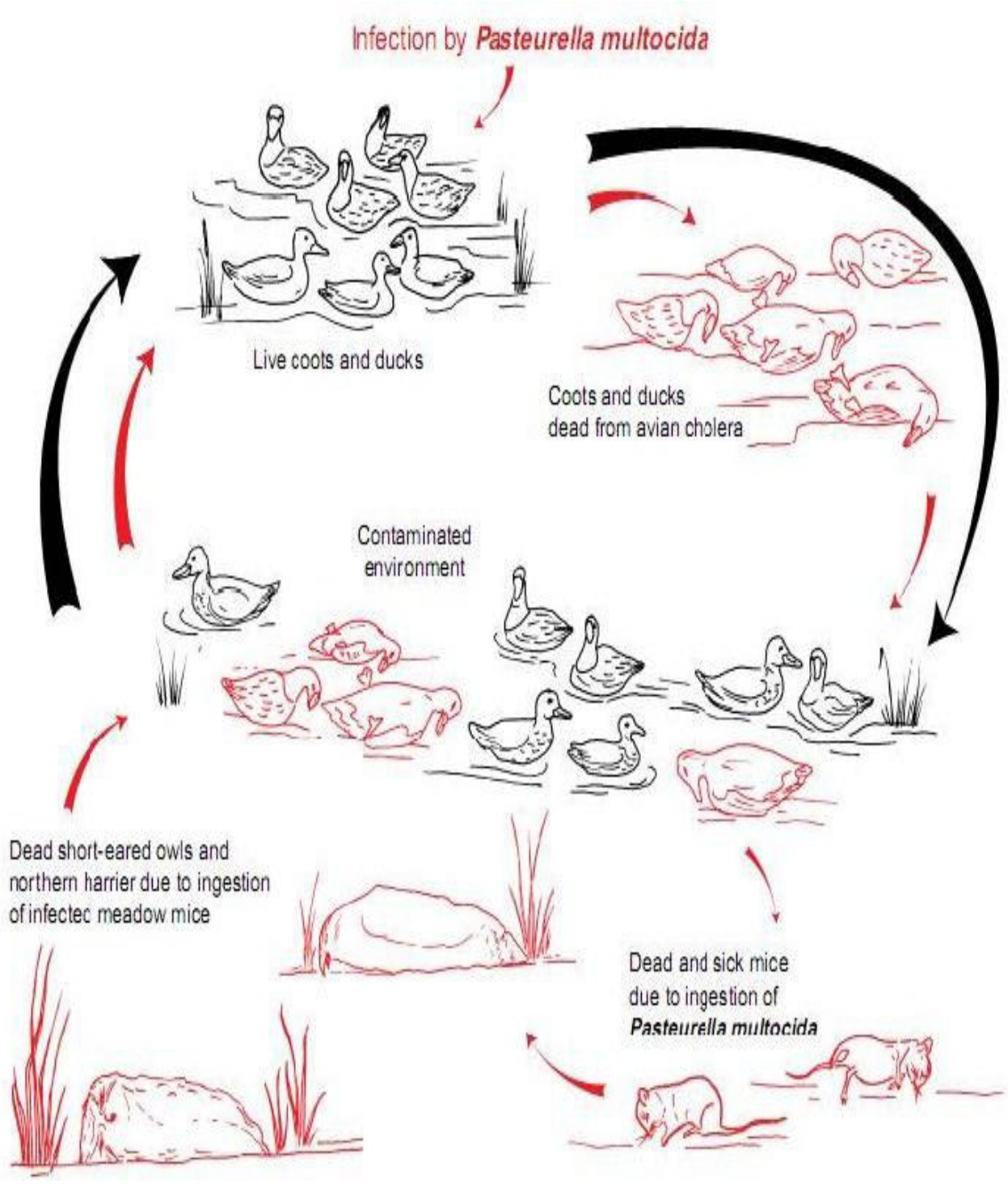


Fig 2.1 Example of an interspecies chain for transmission of avian cholera

Ref. Field Manual of Wildlife Diseases: Birds

2.5.7 Morbidity and mortality

It is usually appears as a septicemic disease associated with high morbidity and mortality (Glisson *et al.,* 2003). It was found that the Astara strain was highly virulent in chickens and mice (Sotoodehnia *et al.,* 2004). Fowl cholera causes mortality about 25 to 35% in chickens and ducks of Bangladesh (Choudhury *et al.,* 1985). Baki *et al.* (1993) reported proportionate mortality rates of 54.55% in chickens and 10.91 % in ducks, caused by Fowl cholera in Bangladesh.

2.5.8 Risk factors

Although ***P. multocida*** infections have been studied in domestic birds for more than two centuries (Rimler and Glisson, 1997), some aspects of the disease in wild birds are yet to be fully clarified, including the importance of host susceptibility, stress factors, and environmental conditions in initiating, maintaining, and terminating avian cholera epizootics. Our knowledge about the roles of these factors in avian cholera epizootics remains insufficient to predict mortality events. In addition, the importance of weather, bird distribution and density patterns, disease carriers, pathogen virulence, and other factors in contributing to avian cholera epizootics has received limited attention.

2.5.9 Environmental factors

Weather conditions including temperature, fog, and precipitation are among the factors that can play an important role in avian cholera epizootics. Cold weather can increase stress levels or crowding of birds on the remaining ice-free wetlands, conditions that appear to precipitate epizootics. Temperatures may also affect host susceptibility to avian cholera. Later disease onset and lower mortality occurred among inoculated domestic turkeys housed at 33-37°C, compared to turkeys held at 22°C or lower (Simensen *et al*., 1980). Two peak periods of avian cholera mortality were noted following several days of very cold temperatures in Nebraska (Windingstad *et al.,* 1998), and cold temperatures were associated with higher mortality after an epizootic began, but snow cover, cold temperature, and number

of geese were not related to the initiation of another epizootic (Windingstad *et al*., 1998). Because *P. multocida* has very poor survival in water at 4° C compared to 20° C (Bredy and Botzler, 1989), these mortality patterns imply that cold weather may influence disease outbreaks primarily through host ecology and susceptibility.

2.6 Pathogenesis

Pehlivanoglu *et al.,* (1999) found no significant difference in mortality among ducks inoculated via intraocular, intranasal, and oral routes. Skin wounds may also serve as a portal of entry. After virulent *P. multocida* enter the body of a susceptible bird, the bacteria multiply rapidly, resulting in septicemia. To accomplish this, the bacteria must evade bacteriocidal properties of serum and the phagocytic defense system. How this occurs in wild birds has not been studied, but in other species resistance to phagocytosis is associated with capsular and outer membrane components of the bacterium (Harmon *et al.,* 1992; Poermadjaja and Frost, 2000). In the acute disease, clinical signs (fever, systemic hypotension, and shock), death, and the predominant lesions of hemorrhage and necrotic foci in the liver are attributed to endotoxin produced by the bacterium. Disseminated intravascular coagulation may be seen, and consumption coagulopathy occurs in turkeys (Friedlander and Olson, 1995).

2.7 Clinical signs

Most birds with avian cholera are found dead with no premonitory signs. Even in large epizootics with extensive mortality, it is uncommon to observe sick birds. Birds have been observed in apparent good health the day prior to death, and the esophagus and proventriculus of dead birds may be filled with recently ingested food, indicating acute mortality. Female Common Eiders often are found dead sitting on their clutch (Swennen and Smit, 1991). In water, birds may die with their head resting on their back. Lethargic birds that can be approached closely may be more common in the late stages of prolonged epizootics among waterfowl (Friend, 1999). Birds with signs suggestive of neurological involvement (uncoordinated

flight, circling while walking or swimming or opisthotonos) have also been reported. Death of birds may be the first sign of Fowl cholera while other signs are depression, diarrhea, ruffled feathers, increased respiratory rate, and cyanosis (Calnek *et al.,* 1997). The common clinical signs were nasal and ocular discharge, darkened head and combs, green diarrhea, ruffled feathers, increased respiratory rate, swollen wattles, high temperatures and lameness which agreed with the findings of (Khan *et al.,* 1997 and Rahman *et al*., 2004).

2.8 Pathology

2.8.1 Gross lesions

In the postmortem examination gross lesions were swollen liver with hemorrhages, focal necrosis on the liver and spleen; increased pericardial and peritoneal fluids, hemorrhages in pericardial band, hyperemia of the upper intestine, petecheal hemorrhage on the coronary band of heart muscle and fat, purulent pneumonia and yolk peritonitis, in chronic Fowl cholera swelling of affected tissues like joints, sternal bursae, and exudates from conjunctivae, hemorrhage at the base of the heart (Calnek *et al.,* 1997; Shivachandra *et al.,* 2005).

In the case of peracute or acute forms of the disease, the post-mortem findings are dominated by general septicaemic lesions including vascular disturbances, as reflected by general passive hyperaemia and congestion thoughout the carcass. Petechial and ecchymotic haemorrhages are often present in the abdominal and coronary fat, and haemorrhages may be observed in the intestinal mucosae and on subserosal surfaces in the thoracic and abdominal cavities (Mohamed *et al*., 2012). The liver and spleen are often swollen and may contain multiple small focal areas of coagulative necrosis or the organs may undergo more generalised necrosis. In the most acute forms of infection, the lung lesions are dominated by haemorrhages, but this is soon followed by necrosis and fibrinous pleuro-pneumonia where affected areas are clearly marked from unaffected tissue (Fouad and Hebat Allah,

2008; Eldin and Reda, 2016). Histologically, the lesions are mainly associated with heterophilic infiltrations (Rimler & Rhoades, 1989; Rimler & Glisson, 1997).

In chronic forms of ***P. multocida*** infections, suppurative lesions may be widely distributed, often involving the respiratory tract, the conjunctiva and adjacent tissues of the head (Rimler & Glisson, 1997).

2.8.2 Microscopic lesions

On histopathological examination of the lung, liver, spleen, heart, kidneys and, mucosa of the intestine, congestion and hemorrhages were the most prominent features, which were relatively more distributed in the kidneys. Reveal typical lesions of septicemia, characterized by generalized passive hyperemia and massive dissemination of bacteria in the liver and spleen, as well as in the blood vascular system throughout the body (Hunter and Wobeser, 1980). One of the additional apparent findings of the present study was the presence of viscid mucosal casts in the lumen of the intestine. Moreover, mild depletion of the lymphocytes of the bursa of Fabricius of some birds was noticed. Congestion and hemorrhage into the parabronchial lumina was also noticed (Glisson *et al.,* 2003).

2.9 Immunity

Some of the first experiments by Pasteur in the field of immunology were conducted using avian cholera, and most subsequent immunological work on this disease has been to protect domestic animals. Today, control of avian cholera in domestic birds in some parts of the world depends largely on vaccination programs in combination with hygiene and other related biosecurity measures. Many live and inactivated vaccines have been developed to control the disease, but both types have limitations. Because live vaccines can also cause disease, most commercial vaccines are produced as killed bacteria (bacterins) (Christensen and Bisgaard, 2000). Although bacterias are inexpensive to produce, they must be injected and may produce short-term (two to three months) immunity only to homologous serotypes. As a result, immunization of wild birds is usually viewed

as impractical. However, immunization may provide a valuable tool for protecting captive populations, populations of special concern or in experimental studies to determine the impact of avian cholera on bird populations (Samuel *et al.,* 1999b). At Arctic breeding areas the prevalence of seropositive geese increased in years with disease epizootics, and approximately 50% of the Lesser Snow Geese infected during breeding ground epizootics survived and seroconverted (Samuel *et al.,* 1999a). In laboratory challenge trials in Mallards with ***P. multocida*** serotype I, Samuel *et al.* (2003a) found that 90% of surviving birds seroconverted, but antibody titers declined to background levels within three to four months following infection.

In Bangladesh, vaccines against fowl cholera/duck cholera are being prepared in Bangladesh Agricultural University (BAU-FCV) by using duck isolate of ***P. multocida*** (PM-38) serotype 1 (X-73) and livestock research institute (LRI-FCV) by using chicken isolate of ***P. multocida*** and available in local market (Samad, 2000). In Bangladesh two types of adjuvanted vaccines are used for the prevention of Fowl cholera in chickens and ducks. Some reports on the immune response and efficacy of locally prepared Fowl cholera vaccines in chickens have been made from Bangladesh (Islam *et al.,* 2004).

2.10 Treatment and control

Antibacterial chemotherapy (sulfonamides and antibiotics) has been used extensively in the treatment of avian cholera in domestic birds (Rimler and Glisson, 1997) and could be used in captive birds. However, against the peracute forms of the disease, drugs may be more valuable as a prophylactic than as a therapeutic agent (Rosen, 1971). Vaccination is commonly used in domestic fowl to reduce the potential for disease epizootics and related mortality.

A vaccination strategy has also been employed for captive Canada Geese (Price, 1985), but most vaccines have a limited duration of 1 year, require individual handling and immunization, and have varying degree of efficacy among species. In

either case, large-scale drug therapy or vaccination of wild populations is likely to be impractical, if not futile. On a limited scale, ponds have also been treated with copper sulfate mixed in hydrochloric acid (Rosen and Bischoff, 1949), small puddles and water holes have been disinfected with a cresylic compound (Gershman *et al*., 1964) and small drinking ponds have been filled (Swennen and Smit, 1991) in attempts to eradicate the disease; however, the efficacy of these techniques is unproven.

All of these treatments and techniques may have beneficial application for individually valuable birds in captive flocks, zoological collections, or endangered species with a high risk of infection. In many cases involving free-ranging birds, the risks associated with capture and handling birds for treatment or vaccination may exceed the risk associated with avian cholera infection. As a general rule, treatment and prevention measures for wild birds are best focused on actions that will benefit the population at risk rather than individual birds. Appropriate actions to control avian cholera epizootics depend on the severity and distribution of disease and the importance of the species or populations involved. Aircraft hazing of Whooping Cranes *(Crus americana)* and creation of artificial feeding sites for Bald Eagles have been used to move these species away from major epizootic areas (Friend, 1999). More typically, the control strategy for wetlands with ongoing disease epizootics involves regular wetland surveillance, carcass removal, and disposal of carcasses.

Under extreme conditions, wetland disinfection, depopulation, or treatment measures may be warranted. Depopulation appears to be feasible only under a limited set of conditions involving a discrete and localized epizootic that presents a high risk to other susceptible species, when complete eradication of infected (Pursglove *et al*., 1976) describe a successful multi-agency depopulation of American Coots at Back Bay, Virginia.

CHAPTER III

MATERIALS AND METHODS

3.1 Study area

Duck cholera outbreaks in the small scale commercial duck farms were investigated at selective Upazila in Bogura district of Bangladesh shown in Fig 3.1 (http://www.daily-sun.com/post/171068/Woman-drug-peddler-gets-life-in Bogra) and the laboratory examinations were conducted at the Department of Pathology and Parasitology under Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur.

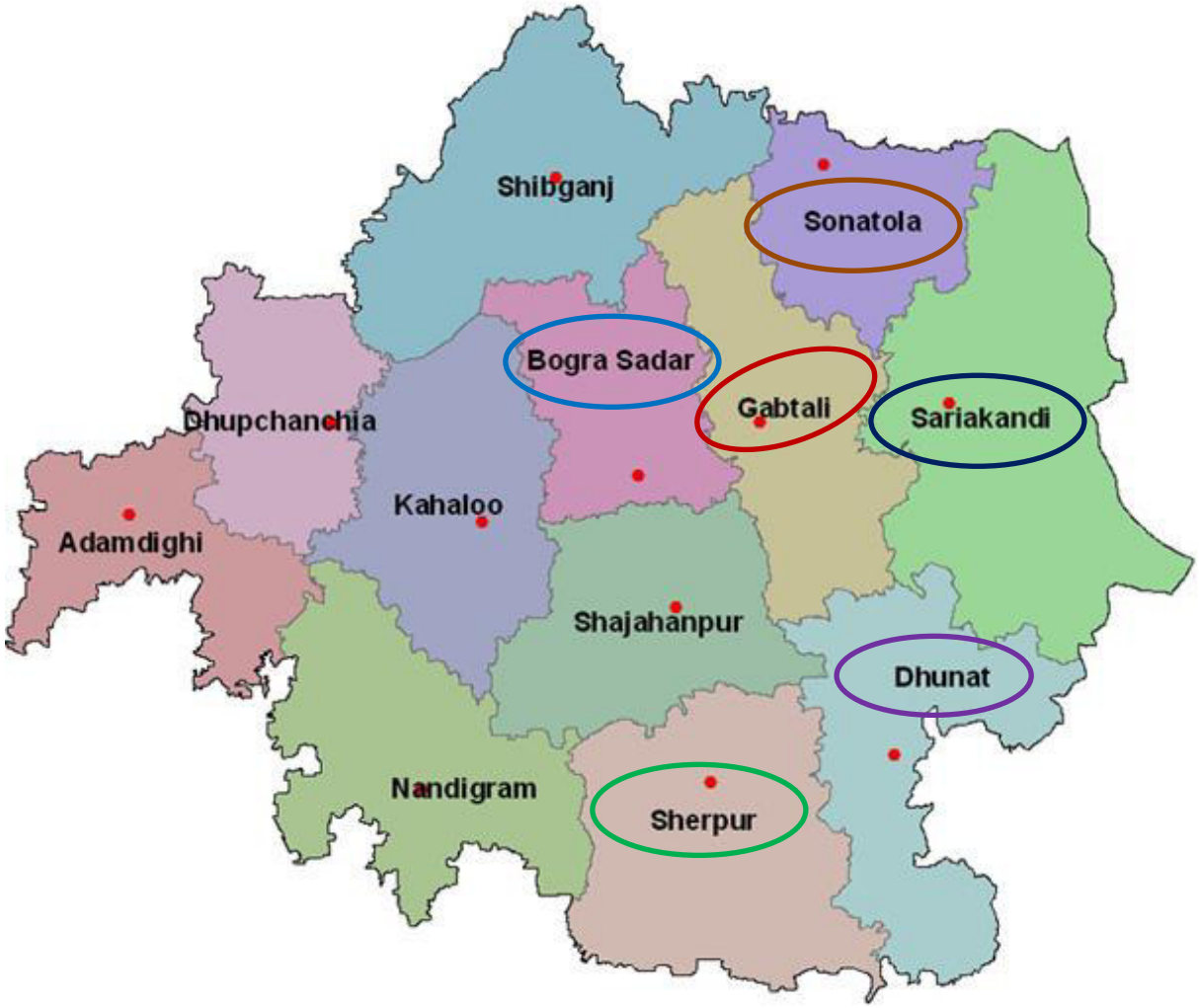


Fig 3.1 Map of Bogura district

3.2 Clinical investigation

A total of 207 diseased and dead ducks were examined from different farms during study period. The numbers of birds in the farms were variable ranging from 50 to 300 and they were reared on scavenging and semi scavenging system. A detail flock history in relation to the incidence of disease including housing system, location of poultry farms, sources of birds, age, sex and population of the birds per flock, rearing system, feeding and watering system, biosecurity of the farms, previous history on Duck cholera outbreaks, intervals between the batches, rearing of one more batches in the same farm at the same time, morbidity and mortality etc. were also considered and recorded. Diagnosis of the disease was made considering the history of the flock, age of the affected birds, clinical signs and postmortem lesions. For the convenience of the study, ducks were divided into male and female also four age groups 0-2 wks, >2-8 wks, >8-20 wks and >20 wks. The birds affected with duck cholera were submitted to the Pathology laboratory for the diagnosis and treatment that considered as the principal experimental birds and some affected birds were also collected during the physical visit of farms.

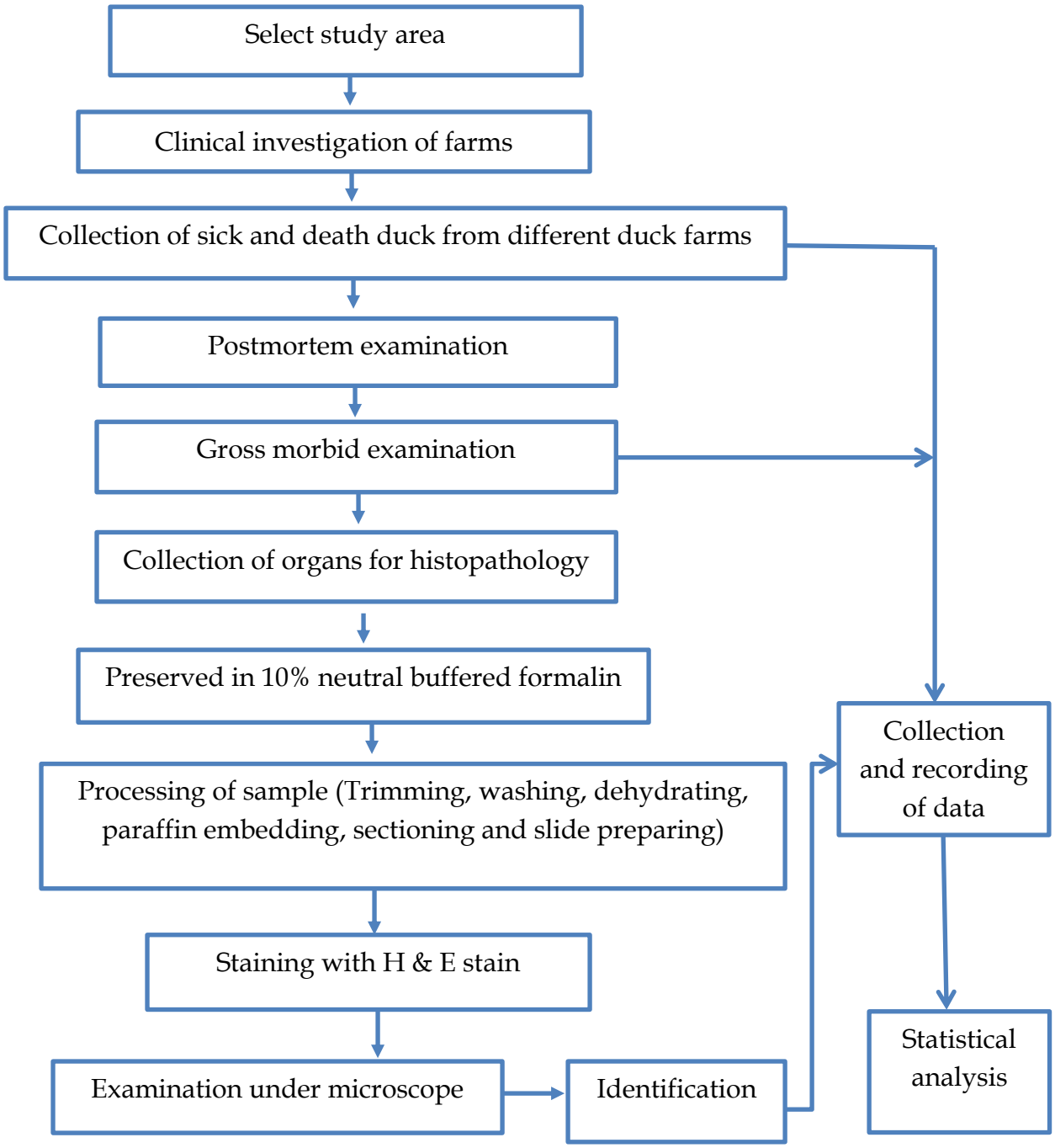
3.3 Research period

The duration of experiment was 6 months from July, 2017 to December, 2017.

3.4 Sampling occasion

There was no scheduled sampling occasion. Birds affected with duck cholera were examined and only the duck cholera affected birds were collected and submitted to the laboratory for detailed necropsy and histopathological examination.

3.5 Study layout



3.6 Cleaning and sterilization of required glassware

Beakers, pipettes, reagent bottles, glass bottle, test tubes, glass tubes, glass slides, cover slips, 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 and staining of organisms after smear and pipettes, reagent bottle, glass tubes for different biochemical tests. 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.

3.7 Clinical findings

The age of the duck and general health condition was recorded. During the physical visit of the affected flocks, the clinical signs were recorded and the farmer's complaints about the affected birds were also considered and noted. Prevalence may be defined as the proportion of a population with a disease or a particular condition at a specific point in time (point prevalence) or over a specified period of time (period prevalence) (Encyclopedia Britannica). Prevalence refers to the total number of individuals in a population who have a disease or health condition at a specific period of time, usually expressed as a percentage of the population. Prevalence is a statistical concept referring to the number of cases of a disease that are present in a particular population at a given time (Medical Dictionary).

3.8 Necropsy findings

Postmortem examination was done as described by Bermudez & Stewart-Brown (2003). The sacrificed birds were opened aseptically and postmortem examination was done on the suspected dead and diseased ducks which taken from different upazila in Bogura district. During postmortem, gross morbid changes were observed and recorded carefully by systemic dissection. After that 10% neutral buffered formalin was used for the preservation of collected samples for farther histopathological study.

3.8.1 Equipment and appliances for necropsy

- Sample (ducks)
- Scissors (3)
- Gloves
- Musk
- Chisel
- Forceps (4)
- Scalpel
- 10% formalin

3.8.2 Procedures

- The duck was wet in a detergent solution thoroughly so that it helped to reduce the chances of feathers floating around the area while the examination was done.
- A newspaper was unfolded on post mortem table then the bird was laid on it. The paper served to absorb most blood and fluid, and provided a convenient wrapper for the carcass after examination.
- The bird was positioned in such way so that the legs and feet were facing the examiner.

- Then an incision was given on skin in between the thighs towards the back and through skinning was done to observe liver,kidney and heart of carcass for detection of haepatomegally, necrotic foci on liver, discoloration of liver, haemorrhage on kidney, haemorrhage at the base of heart.
- Body cavity of bird was opened and the liver, heart, spleen, and other unnecessary organs were detached to facilitate the examination.
- Segments of the intestine, liver, kidney and heart were observed carefully for important post mortem lesions.
- Then the organs were opened longitudinally by knife or scissors to observe the colour, consistency and appearance of intestinal contents, liver, heart and mucosal surfaces gradually.

Fig 3.2 Postmortem of duck

3.8.3 Gross lesions

Gross morbid lesions of different organs were registered during the course of necropsy of the birds.

3.9 Histopathological findings

Tissue specimens were collected from liver, heart, spleen, intestine and lungs of diseased and dead ducks after during post-mortem examination. Then these specimens were fixed in 10 % neutral buffered formalin. Then formalin fixed samples were washed, dehydrated, cleared and processed for paraffin embedding, sectioning and staining with haematoxylin and eosin (H & E) according to standard method (Luna, 1968). Details of tissue processing, sectioning and staining were given below.

3.9.1 Equipment and appliances

- Sample (liver, heart, kidney etc.)
- Clean slides
- Cover slips
- Microtome machine
- Chloroform
- Mounting media (DPX)
- Alcohol
- Paraffin
- Tape water
- 10% formalin
- Xylene
- Hematoxylin and Eosin stain
- Distilled water
- Microscope

3.9.2 Processing of tissues and sectioning

- The tissues were properly trimmed to obtain a good cross section of the tissue.
- To remove the fixative, tissues were washed under running tap water for overnight.
- The tissues were dehydrated in ascending grades of alcohol (Fig. 3.4) using 50%, 70%, 80%, 90% alcohol (Fig. 3.3), and three changes in absolute alcohol, for 1hr in each.
- The tissues were cleared in two changes in chloroform, 1.5hr in each.
- The tissues were embedded in molten paraffin wax at 56^0C for two changes, 1.5hr in each.
- Paraffin blocks containing tissue pieces were made using templates and molten paraffin.
- The tissues were sectioned (Fig. 3.5) with a microtome at 4-5mm thickness according to Bancroft and Gamble (2008), which were allowed to spread on warm water bath (42^0C) containing small amount of gelatin and taken on oil and grease free glass slides.
- Then the slides were air dried and kept in cool place until staining.

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

Fig 3.3 Preparing different solution

Fig 3.4 Tissues passed through ascending grades of alcohol

Fig 3.5 Sectioning of tissue by Microtome machine

3.9.3 Staining protocol

- The sectioned tissues were deparaffinized in xylene (three changes; two minutes in each).
- The sectioned tissues were rehydrated through descending grades of alcohol (Fig. 3.6) (3 changes in absolute alcohol, 2 minutes in each change; 95% alcohol for 2 minutes; 80% alcohol for 2 minutes; 70% alcohol for 2 minutes) and distilled water for 10 minutes).
- The tissues were stained with Harris' hematoxylin (Fig. 3.7) for 10 minutes.
- The sections were washed in running tap water for 10 minutes.
- Then the staining was differentiated in acid alcohol (1part HCl and 99 parts 70% alcohol) 2-4 dips.
- The tissue sections were then washed in tap water for 5 minutes and dipped in ammonia water (2-4 times) until sections became bright blue.
- The sections were stained with eosin for 1 minute and then differentiated and dehydrated in alcohol (95% alcohol, 3 changes, 2-4 dips in each change; absolute alcohol 3 changes, 2-3 minutes in each).
- The stained sections were then cleaned by 3 changes in xylene, 5 minutes in each change and finally the sections were mounted with cover slip using DPX.
- The slides were studied (Fig. 3.8) under microscope using various magnifications.

3.9.4 Microscopic lesions

Microscopic lesions of different organs were registered during the course of microscopic examination.

Fig 3.6 Processing of tissue slide for staining

Fig 3.7 Staining of tissue slide

Fig 3.8 Microscopic observation of slide

3.10 Photography

All images related to the present study were taken directly from microscope using different objectives manipulation of zooming system of a digital camera (Canon 1XY, 16.1 Mega pixels, Japan). The images were provided following minute modification for the better illustration of the study.

3.11 Statistical analysis

The data in relation to prevalence of Duck cholera were collected, organized and analyzed statistically following IBM SPSS (Statistical Package for the Social Science) Statistics 20 software.

CHAPTER IV

RESULTS

Pathological investigation of Duck cholera encountered in commercial duck farms at different Upazila in Bogura district was studied and different clinical signs, necropsy findings and histopathological conditions were recorded during the study period.

4.1 Prevalence

A total of 207 ducks either dead or sick were examined during the period from June 2017 to September 2017 from various duck farms of Bogura district. Among the ducks, 31 were from Bogura sadar, 43 Sariakandi, 33 Gabtoli, 34 Dhonut, 37 Sonatola and 29 Sherpur upazila out which 23 ducks were found to be positive among them 3 were Bogura sadar, 6 Sariakandi, 3 Gabtoli, 4 Dhonut, 5 Sonatola and 2 Sherpur upazila. The overall prevalence of duck cholera in Bogura district was 11.11% which is followed by 13.95% in Sariakandi, 13.51% in Sonatola, 11.76% in Dhonut, 9.68% in Bogura sadar, 9.09% in Gabtoli and 6.90% in Sherpur upazila.

Table 4.1 Overall prevalence of Duck cholera in selective Upazila of Bogura district found during the period from July 2017 to December 2017 (n=207)

SL No	Name of Upazila	No. of duck	No of affected	Prevalence (%)	Chi-square (P value)
1.	Bogura Sadar	31	3	9.68	1.305 $(0.934)^{NS}$
2.	Sariakandi	43	6	13.95	
3.	Gabtoli	33	3	9.09	
4.	Dhonut	34	4	11.76	
5.	Sonatola	37	5	13.51	
6.	Sherpur	29	2	6.90	
	Overall	207	23	11.11	

n= Number of duck, NS= Not Significant

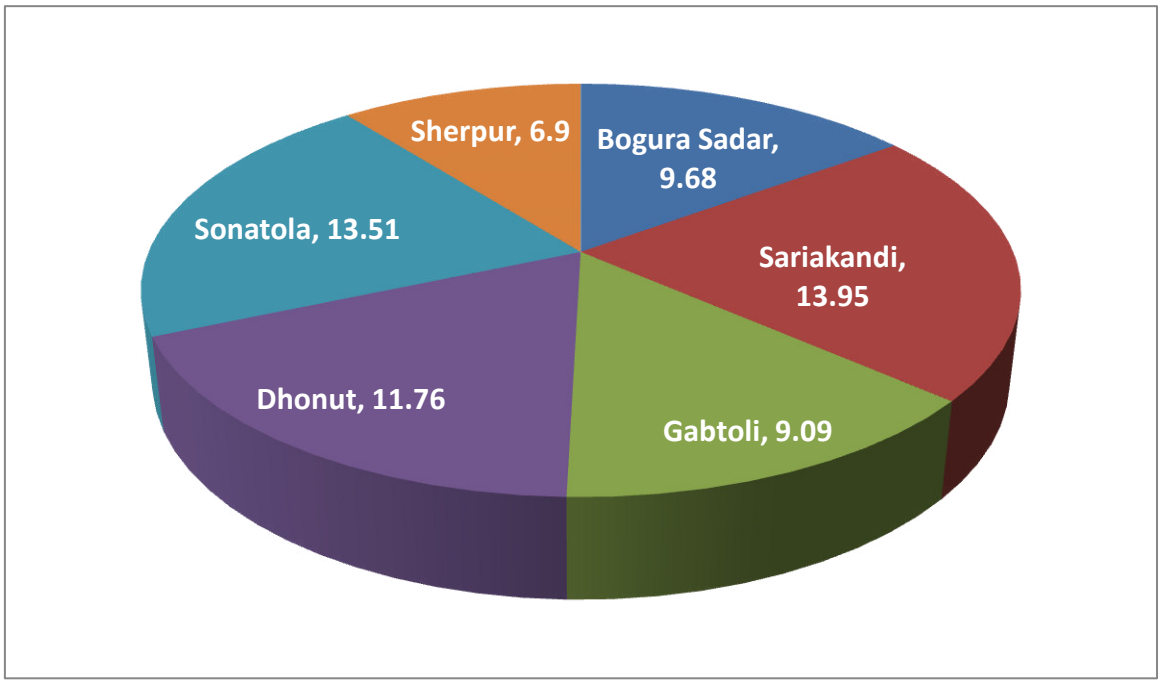


Fig 4.1 Overall prevalence of Duck cholera in selective Upazilas of Bogura district

According to the results obtained, Overall prevalence of Duck cholera in different age group of Bogura district was significantly (P<0.01) higher (18.52%) among >2-8 wks followed by (10.13%) among >8-20 wks, (0%) between 0-2 wks and (0%) among >20 wks.

Table 4.2 Overall prevalence of Duck cholera in different age group in Bogura

Age group	No. of duck	No of affected	Prevalence (%)	Chi-square (P value)
0-2 wks	28	0	0	10.543 (0.015*)
>2-8 wks	81	15	18.52	
>8-20 wks	79	8	10.13	
>20 wks	19	0	0	
Overall	207	23	11.11	

*= Significant

In age group study, the highest prevalence of Duck cholera was found in Sariakandi (21.05%) upazila among >2-8 wks of age which is followed by Sonatola (20.00%), Dhonut (18.75%), Bogura Sadar (18.18%), Shetpur (16.67%) and Gabtoli (14.29%) upozila. Among the age group of >8-20 wks highest prevalence was found in Sariakandi (14.29%) followed by Sonatola (12.50%), Dhonut (8.33%), Shetpur (8.33%), Gabtoli (8.33%) and Bogura Sadar (7.69%) upozila. The prevalence was 0% between 0-2 wks and among >20 wks of age.

Table 4.3 Age wise prevalence of Duck cholera at selective Upazila in Bogura district

SL No	Name of Upazila	0-2 wks		>2-8 wks		>8-20 wks		>20 wks	
		No. of duck	Affected (%)	No. of duck	Affected (%)	No. of duck	Affected (%)	No. of duck	Affected (%)
1.	Bogura	4	0	11	2 (18.18)	13	1 (7.69)	3	0
2.	Sariakandi	5	0	19	4 (21.05)	14	2 (14.29)	5	0
3.	Gabtoli	3	0	14	2 (14.29)	12	1 (8.33)	4	0
4.	Dhonut	4	0	16	3 (18.75)	12	1 (8.33)	2	0
5.	Sonatola	4	0	15	3 (20.00)	16	2 (12.50)	2	0
6.	Sherpur	8	0	6	1 (16.67)	12	1 (8.33)	3	0

*= Significant

Prevalence of Duck cholera also studied between sex group and it was found that the prevalence was significantly ($p<0.05$) higher in female (14.08%) than in male (4.62%) ducks.

Table 4.4 Sex wise prevalence of Duck cholera in Bogura district

Sex	Total duck	No of affected	Prevalence (%)	Chi-square (P value)
Male	65	3	4.62	4.048 (0.044*)
Female	142	20	14.08	
Overall	207	23	11.11	

4.2 Clinical findings

The present clinical examination identified different type of clinical signs caused by *Pasteurella multocida.* During clinical examination following clinical signs were depression and ruffled feather (Fig 4.2), Swollen hock joint with thickening in the skin of footpad (Fig 4.3), Greenish diarrhea (Fig 4.4), Lameness (Fig 4.5) and Feathers around vent soiled with greenish diarrhea (Fig 4.6) are considered to be a most important clinical sign in the examined ducks.

Fig 4.2 Depression and ruffled feather

Fig 4.3 Swollen hock joint (thick arrow) with thickening in the skin of footpad (thin arrow)

Fig 4.4 Greenish diarrhea

Fig 4.5 Lameness

Fig 4.6 Feathers around vent soiled with greenish diarrhea

4.3 Necropsy findings

Necropsy findings in liver and heart of the ducks were detected such as pin point haemorrhage on heart (Fig. 4.7), extravassation of heart blood (Fig. 4.8), haemorrhage on liver (Fig. 4.9), enlargement of liver (Fig. 4.10), Straw color pericardial fluid in heart (Fig. 4.11) and Enlarged, haemorrhagic and congestion of Spleen (Fig 4.12).

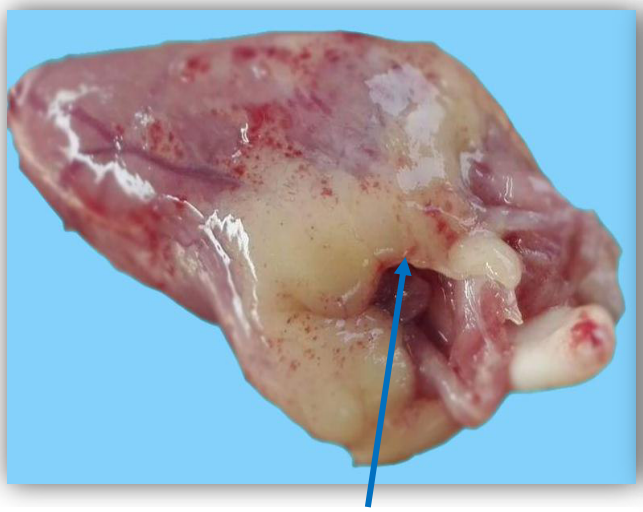


Fig 4.7 Pin point hemorrhage on Heart

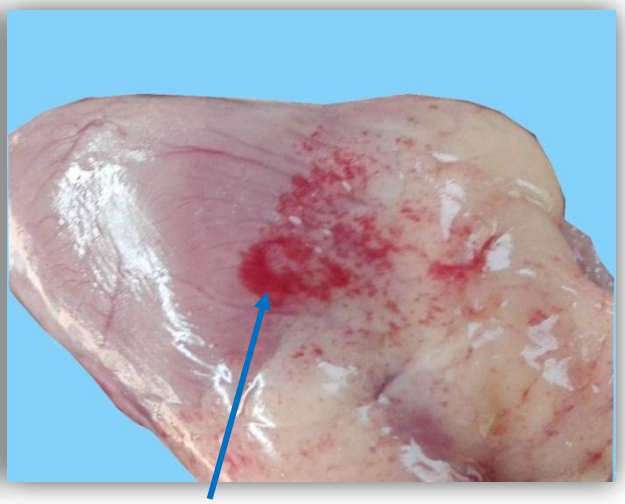


Fig 4.8 Extravasation of on heart blood

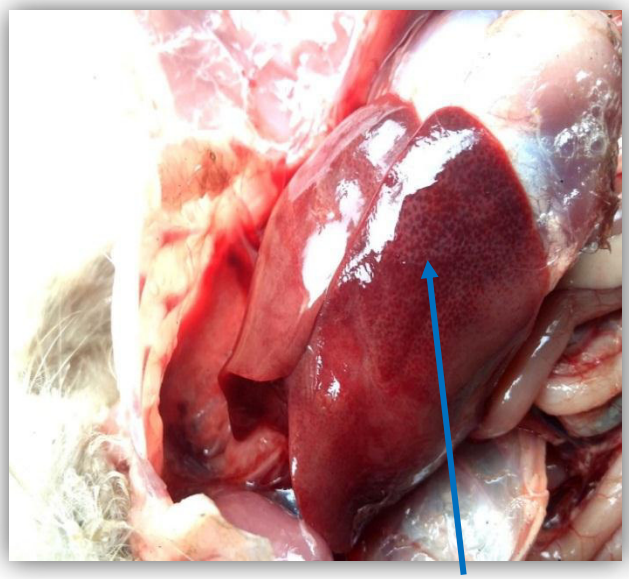


Fig 4.9 Hemorrhage on liver

Fig 4.10 Enlargement of liver

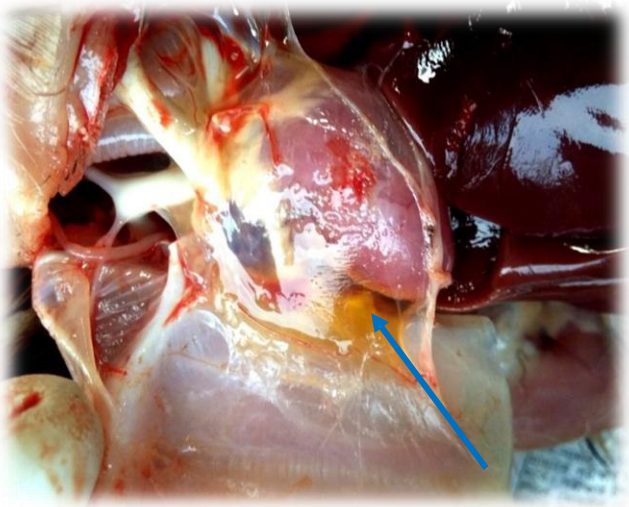


Fig 4.11 Straw color pericardial fluid in heart

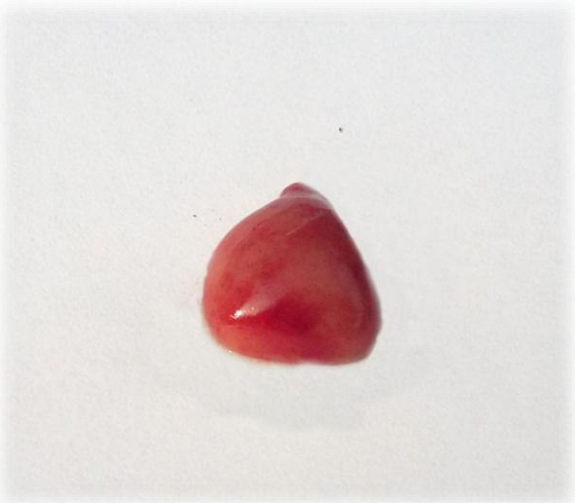


Fig 4.12 Enlarged, hemorrhage and congestion of Spleen

4.4 HISTOPATHOLOGICAL FINDINGS

In this study, the histpathological features of liver represents the destruction of hepatocytes and hepatic cord (Fig. 4.14), necrotic foci on liver and reactive cell infiltration in liver. Liver was highly affected and characterized as dilated central vein (Fig. 4.14), more acidophilic cytoplasm of the hepatocytes (Fig. 4.13, 4.15), reactive cells infiltration (Fig. 4.13, 4.14), dilated sinusoids (Fig. 4.13) mild to moderate level of hepatocytic destruction (Fig. 4.13, 4.15), bacterial colony in liver parenchyma (Fig. 4.15).

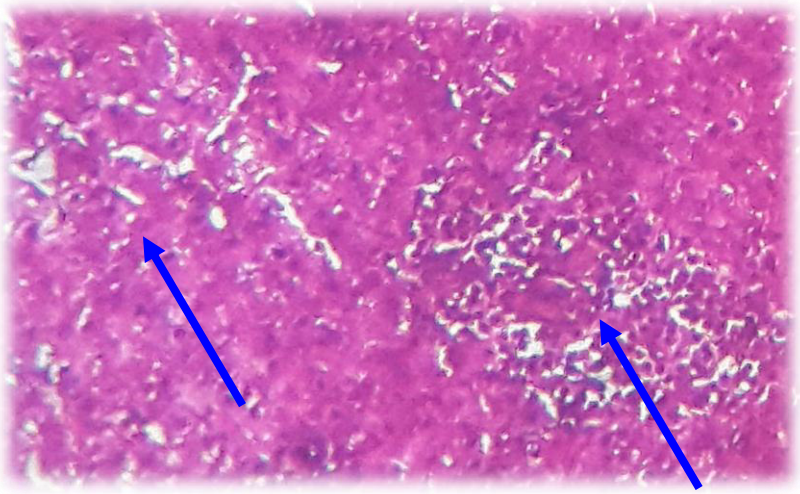


Fig 4.13 Dilated sinusoids, more acidophilic cytoplasm and reactive cell in filtration

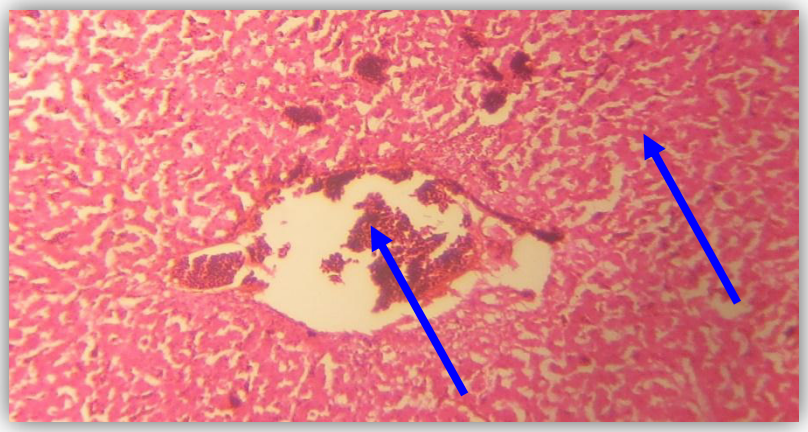


Fig 4.14 Dilated central vein and reactive cell in filtration

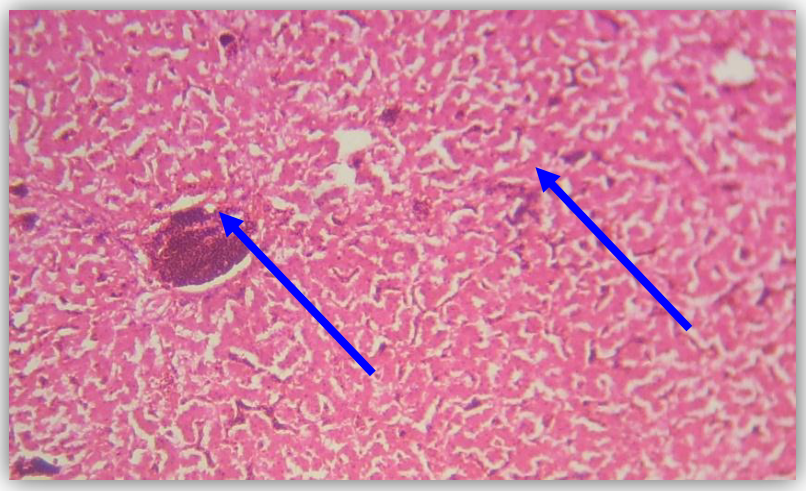


Fig 4.15 Bacterial colony present and acidophilic cytoplasm of the hepatocytes

CHAPTER V

DISCUSSION

This study was undertaken to investigate the clinico-pathological condition of Duck cholera from the outbreak at commercial duck farms at different Upazila in Bogura district.

5.1 Prevalence

The study was conducted in different small scale commercial Duck farms in different Upazila at Bogura district. A total of 207 ducks either dead or sick were examined out of which 23 (11.11%) ducks were found to be positive for Duck cholera. The overall prevalence of Duck cholera was found 11.11% in Bogura district. There was an insignificant relationship found among the prevalence of Duck cholera in different Upazilas of Bogura District. The prevalence was maximum in Sariakandi Upazila (13.95%) which is statistically followed by Sonatola (13.51%) and Dhonut (11.76%). Medium prevalence was found in Bogura sadar (9.68%) and Gabtoli Upazila (9.09%), On the other hand, the minimum prevalence of Duck cholera was (6.90%) which is founded in Sherpur Upazila (Table 4.1). This observation is similar to those reported in other authors where the prevalence of Duck cholera was recorded 7.03% by Rahman and Adhikary, 2016; 11.42% by Panna *et a*l., 2015 and 13.4% by Islam *et al.*, 2009. The statement is dissimilar to the report by Das *et al*., 2005 who reported the prevalence of Duck cholera was 2.4% in his study.

According to the age group of ducks, the highest prevalence of Duck cholera was recorded as 18.52% among the age group of >2-8 weeks which is followed by 10.13% among >8-20 weeks of age, 0% between 0-2 weeks of age and among >20 weeks of age respectively. Age of the duck had a significant relationship on prevalence and mortality of the disease. It was significantly (p>0.01) higher among >2–8 weeks of age (18.52%) which is followed by among >8-20 weeks (10.13%), between 0-2 weeks of age (0%) and among >20 weeks of age (0%). This statement is

similar to the report by Islam *et al*., (2009) who reported that the prevalence of Duck cholera was higher in pullet stage (16.70%) than laying stage (10.80%). This observation is dissimilar to the report of Choudhury *et al.,* (1985) who reported that prevalence of Duck cholera in duck highest in aged between 6 to 12 months old. Variation may be due to the session, geographical location, nutritional status and poor management system of the study area.

Present study found the prevalence between sex group and it was significantly ($P<0.05$) higher in female (14.08%) than in male (4.62%) ducks. This finding did not match with Biswas *et al*., (2005) who found the highest occurrence of the disease in male (6.81%) than in female (6.69%) ducks. This variation may be due to the session, geographical location, nutritional status and poor management system of the study area.

5.2 Clinical findings

Clinical manifestation of ducks naturally infected with Duck cholera was studied. In the present study, the common clinical manifestations in the ducks suffering from natural Duck cholera were found as depression and ruffled feather (Fig. 4.4), lameness (Fig. 4.7), greenish diarrhea (Fig. 4.6), Feathers around vent soiled with greenish diarrhea (Fig. 4.8), Swollen hock joint with thickening in the skin of footpad and other signs were fever, anorexia, mucous discharge from mouth and increased respiratory rate. These findings are also similar by Bhattacharjee *et al.,* 1996; Rimler and Glisson *et al*., 1997; Bhattacharjee *et al.,* 1996; Mohan and Pradeep Kumar, 2008 and Eldin and Reda, 2016.

5.3 Postmortem findings

A total number of 23 dead and sick birds suspected to be infected with Duck cholera were collected from commercial duck farm at different Upazila in Bogura district and subjected to postmortem examination. Gross pathological changes of the various organs of the affected ducks were studied. At necropsy, the major pathological lesions were haemorrhage on liver (Fig. 4.11), enlargement of liver

(Fig. 4.12), pin point haemorrhage on heart (Fig. 4.9), extravassation of heart blood (Fig. 4.10), Straw color pericardial fluid in heart (Fig. 4.13) and Enlarged, haemorrhagic and congestion of Spleen (Fig 4.14). These gross lesions are also reported by Glisson *et al.,* 2003; Fouad and Hebat Allah, 2008; and Eldin and Reda, 2016.

5.4 Histopathological findings

In this study, the histpathological features of liver represents the destruction of hepatocytes and hepatic cord, necrotic foci on liver and reactive cell infiltration in liver, Liver was highly affected and characterized as dilated central vein, more acidophilic cytoplasm of the hepatocytes, reactive cells infiltration, dilated sinusoids, mild to moderate level of hepatocytic destruction, bacterial colony in liver parenchyma. This observation is similar to those reported Heddleston, K. L., 1972; Rhoades, K. R, 1964; Glisson *et al.,* 2003 and Eldin and Reda, 2016.

CHAPTER VI

SUMMARY AND CONCLUSION

In the present investigation the diseases were tentatively diagnosed on the basis of history of the affected flock, clinical sign, post mortem examinations, pathological findings, age and sex of affected birds, 'but not confirmed by other laboratory tests. A total of 207 ducks either sick or dead were examined at different Upazilla Veterinary hospital of Bogura district during the period from July 2017 to December 2017 out of which 23 (11.11%) ducks were found to be positive on Duck cholera. The major clinical manifestations in the ducks suffering from natural Duck cholera were found as depression and ruffled feather, lameness, greenish diarrhea, Feathers around vent soiled with greenish diarrhea and swollen hock joint with thickening in the skin of footpad. Main gross findings in affected ducks were haemorrhage on liver, enlargement of liver, pin point haemorrhage on heart, extravassation of heart blood, straw color pericardial fluid in heart and enlarged, haemorrhagic and congestion of Spleen. Histopathological findings were dilated central vein, more acidophilic cytoplasm of the hepatocytes, reactive cells infiltration, dilated sinusoids, mild to moderate level of hepatocytic destruction, bacterial colony in liver parenchyma.

From the above facts and findings, it could be concluded that –

- Outbreaks of Duck cholera in the commercial Duck farms is higher in Bogura district
- The farmers are not aware of Duck cholera and they are not usually use vaccine routinely and not maintain biosecurity strictly.

Moreover, the data depicts the prevalent of Duck cholera of greater Bogura district of Bangladesh, not the whole country. Proper immunization of the flock may save the birds from unwanted mortality. Improved management practice e.g. hygiene and biosecurity should implement to keep away of disease agents. Nevertheless, these data would enrich the knowledge of Veterinarians and farmers regarding the Duck cholera prevalent in greater Bogura district and in Bangladesh as well.

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