PATHOLOGYCAL INVESTIGATION OF AVIAN MYCOPLASMOSIS IN SONALI AT JOYPURHAT DISTRICT

A THESIS

BY

MD. SHAKIL HOSSAIN

Registration No: 1405089

Semester: January- June-2015

Session: 2014-15

MASTER OF SCIENCE (M.S)

IN

PATHOLOGY

Department of Pathology and Parasitology

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Submitted to the

Department of Pathology and Parasitology
Faculty of Veterinary and Animal Science
Hajee Mohammad Danesh Science and Technology University, Dinajpur.

In partial fulfillment of the requirements for the degree of

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Approved as to style and content by

Prof Dr. S.M. Harun-ur- Rashid	**Dr. Md. Golam Azam**
Supervisor	Co- Supervisor

Dr. Md. Haydar Ali

Chairman

Examination committee

Department of Pathology and Parasitology
Hajee Mohammad Danesh Science and Technology University
Dinajpur

June, 2015

Dedicated To My Beloved Parents

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ABSTRACT

The study was designed to investigate the pathological conditions of Avian Mycoplasmosis in the small scale commercial Sonali farms at different region in Joypurhat district. The duration of experiment was 6 month from January to July 2015. The objectives of the study were clinical signs, gross lesion, microscopic lesion, and the prevalence of the diseases. Different organ mainly lung, trachea, heart, & air sac were collected, preserved, and processed for histopathological examination. The total of 500 of the 290 affected sonali birds were examined as mycoplasmosis. Agewise prevalence was 60-67% and season wise prevalence was 55%. The clinical signs were coughing, sneezing, rales, ocular and nasal discharge, dyspnea with typical voices. At necropsy catarrhal exudates in nasal passages, catarrhal and foamy exudates in the trachea, congestion and hemorrhage in the trachea, dark red color appearance in lung. Air sacs were thickened, heart covered with fibrinopurulent, liver was congested and show haemorrhages. Histological lesions in the trachea, mononuclear cellular infiltration in the mucosa and sloughing off mucosal lining were found. Mucosal glandular hyperplasia and lymphoid follicular reaction with connective tissue were found in trachea. Infiltration of mononuclear cell were found in lung parenchyma. Congestion and accumulation of fibrin also found in lung parenchyma.

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ACRONYMOS USED

Abbreviations		Elaborations
+Ve	-	Positive percent
µg	–	Microgram
@	-	At the rate of
-Ve	-	Negative
Ag	-	Antigen
CCU	-	Colour Changing unit
cDNA	-	Complementary, deoxyribonucleic acid
CRD	-	Chronic Respiratory disease
EDTA	-	Ethylene di-amine tetra acetic acid
et.al.	-	And his associates
Fig.	-	Figure
g	-	Gram
HCL	-	Hydrochloric acid
HI	-	Haemagglutination inhibition
Hr	-	Hour
mg	-	Miligram

MG	-	*Mycoplasma gallisepticum*
Ml	-	*Mycoplasma iowae*
Min	-	minute
MM	-	*Mycoplasma meleagridis*
MS	-	*Mycoplasma synoviae*
PPLU	-	Pleuropneumonia Like Organism
PM	-	Post- mortem
sp.	-	Species

CHAPERT-I
INTRODUCTION

The poultry industry present in Bangladesh is a vital sector to improve agricultural growth and the diet of people. This sector is important particularly as it serves as one of the major sources of daily protein and nutritional supply (Raihan & Mahmud, 2008). It is expected that the future demand of poultry products will be enhanced due to high population and income growth, urbanization and increasing high income elasticity of demand. This sector is also significant in generating economic activities involving the women and especially the people from the poorer sections of any society. Poultry represents an asset, which can be liquidated when the need arises. Investing in small-scale poultry is an effective strategy to uphold a pro-poor and comprehensive growth. Invest of US $350 in a poultry litter based biogas plant can earn an income of $15 per month (Saleque, 2013).

In recent years, a noteworthy revolutionary growth was observed in poultry business. According to Halcyan (2011) and Islam *et al.* (2014), there about 43,589 poultry farms were reported in the year 1993-94; it was predicted that this number would have reached about 1,50,000 by the year 2006-07 and approximately above 2,30,000 by 2014. Despite of the rapid growth, poultry industries are facing many constraints likely lacking of efficient marketing system, lack of technical knowledge, lack of adequate laboratory test facilities, inadequate quality and disease control practices, inadequate access to credit facilities, intense foreign competition and high tax on poultry seed import. Raihan & Mahmud (2008) found that access to working capital by smaller stakeholders is also considered as a major hurdle towards the flourish of poultry industries in Bangladesh.

Mycoplasmosis is one of the major problems among avian diseases in emerging poultry industry of Bangladesh. Primarily, this is a disease of chicken and turkeys but also infects many other domestic and wild birds all over the world (Jordan and Amin, 1980; Bradbury et al., 1993). The disease is caused by four commonly recognized pathogens; *Mycoplasma gallisepticum, Mycoplasma synoviae, Mycoplasma meleagridis and Mycoplasma iowae* (Bradbury, 2001). *Mycoplasma gallisepticum* (MG) is a cause of respiratory disease and the most economically important of the avian *Mycoplasma* (Ley and Yoder, 1997). The disease decreases egg production & reduced feed conversion efficiency (Carpenter et al., 1981; Ley and Yoder, 1997). Production losses between 10 and 20% have been reported in

layers (Bradbury, 2001). All ages of chickens and turkeys are susceptible to this disease but young birds are more prone to infection than adults (Nunoya *et al.,* 1995).The disease may be transmitted both by horizontally and vertically and remain in the flock constantly as subclinical form (Bencina *et al.*, 1988a). *Mycoplasma synoviae* spreads more rapidly than *Mycoplasma gallisepticum* is now occurs most frequently as a subclinical infection of the upper respiratory tract. It may also become systemic and causes synovitis resulting in lameness (Denis Carrier, 2003). The organism can be diagnosed by serological tests, culture and nucleic acid based technologies (Nascimento *et al;* 2005).

So, keeping the above view, the study was undertaken with the following objectives:

- To study the clinical findings of Avian Mycoplasmosis at Joypurhat District.
- To study the gross and microscopic lesions of Avian Mycoplasmosis in field outbreak.
- To study the prevalence of diseases in birds.

CHAPTER-II

REVIEW OF LITERATURE

2.1 AVIAN MYCOPLASMOSIS

The microorganisms of the class Mollicutes (Mycoplasma) were first identified in 1898 as the etiologic agent of the bovine contagiouspleuropneumonia (BCPP) and thereafter, all similar agents were namedpleuropneumonia-like (PPLO-like) organisms (Davis et al., 1973). Avian mycoplasmosis was primarily described in turkeys in1926, and in chickens in 1936 (Charlton et al., 1996). Delaplane & Stuart (1943) referred to it as chronic respiratory disease (CRD) of poultry. Markham & Wong (1952) associated the etiologic agent of CRD to the pathogen responsible for the infectious sinusitis of turkeys. It was then considered as a member of the PPLO group and later named as *Mycoplasma gallisepticum* (MG) (Yoder Jr., 1991b). Infectious synovitis caused by *Mycoplasma synoviae* (MS) was described thereafter (Olson *et al.,* 1956; Kleven *et al.,* 1991; Kleven, 1997). The first reports of MS infection with arthritic involvement date from the decades of 50 and 60 in broiler flocks, but it was only in the 70´s that the respiratory disease caused by MS was described (Rosales, 1991).The first evidence of air saculitis in day-old poults by mycoplasmas other than MG was obtained by Adler *et al*. (1958), who named this new mycoplasma Nstrain. It was later called *Mycoplasma meleagridis* (MM), a mycoplasma that infects turkeys and other birds, but not chickens (Yamamoto, 1991).Mycoplasma iowae (MI), considered an emergingpathogen and a mycoplasma of natural occurrence in turkeys, has also been reported in chickens and otherbirds. It was first diagnosed as the Iowa 695 strain (Yoder Jr. & Hofstad, 1962) and characterized later (Jordan *et al.,* 1982). MI and other *mycoplasmas, including M. iners, M. gallinarum, M. pullorum, M. gallopavonis, M. gallinaceum, M. columbinasale, M. columbinum, M. columborale, M. lipofaciens, M. glycophilum, M. cloacale, M. anseris,* and *Acholeplasma laidlawii,* are *notpathogens* of major concern to the poultry industry because of very low, or even lack of, pathogenicity (Nascimento, 2000). The same applies to *M. immitans,* which cross reacts serologically with MG, but has not yet been isolated from poultry (Fiorentin, 2004). Therefore; this review will focus on MG and MS infections and, to a less extent, to MM infection.

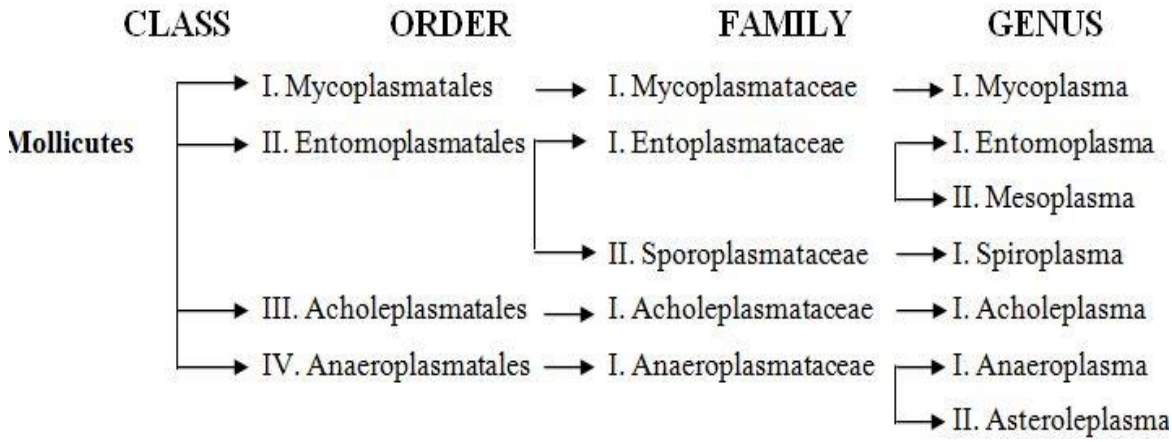
2.1.1 Biology and Taxonomy of the *Mycoplasmas*

Mycoplasmas are very small prokaryotic organisms devoid of cell walls bounded by a plasma membrane (Kleven, 1997). *Mycoplasma gallisepticum* (MG) belongs to the class Mollicutes (*mollis*, soft; *cutis*, skin), order *Mycoplasmatales*, family Mycoplasmataceae and genus *Mycoplasma* (OIE, 2004).

Families subdivision is based on habitat, sterol requirement for growth, genome size, and oxygen tolerance, further genera differentiation takes into account the mechanism used by the organism to obtain energy such as glucose, arginine, or urea fermentation. *Mycoplasma* species are defined by the above criteria, supplemented by additional biochemical properties, and by various measures of serological relatedness (Rosenbusch, 1994).

Figure 1: Taxonomy of avian *mycoplasmas* modified from Rosenbusch (1994).

CLASS ORDER FAMILY GENUS



The primary basis for inclusion of an organism in the class Mollicutes is the absence of cell wall which can be demonstration by a single trilaminar membrane bounds the organism. Lack of cell wall is also the underlying reason for the "fried egg" colonial morphology that is characteristic of growth on solid media. In addition, the absence of cell wall material and cell wall associated proteins renders *mycoplasmas* resistant to the action of antibiotics that interact with these proteins (Rosenbusch, 1994). There are approximately twenty-five *mycoplasma*s belonging to the genera *Mycoplasma*, *Acholeplasma* and *Ureaplasma* have been isolated from avian species (Whithear, 1996).

Generally, *mycoplasmas* have correspondingly small genomes size with 600 000 to 1 350 000 bp only one-sixth the size of the *Escherichia coli* genome, and their G + C content is very low 23-40 %, so that only a part of the total genome is presumable used for expression of genetic information. As a consequence of this limited genetic potential, *mycoplasmas* usually require intimate association with mammalian cell surfaces and manifest complex nutritional requirements for in vitro growth (Rosenbusch, 1994).

Actually, *mycoplasmas* have long been considered model system for defining the minimal set of genes required for living cells. After the previous five *mycoplasmas* have been sequenced, *Mycoplasma gallisepticum* strain rlow was selected as the first avian *mycoplasmas* for complete genome sequencing. The genome is composed of 996 422 bp with overall G + C content of 31 % (Papazisi *et al*., 20).

2.1.2 HISTORY

As previously mentioned, early etiologic studies started form several different directions. Nelson (67) described a chronic coryza like disease caused by coccobacilli form bodies. Delaplane and stuart (27) described a chronic respiratory diseases associated with tracheitis and air sacculitis due to an egg-passaged agent, presumed to be a virus. In 1948, Delaplane (25) described the lesions produced in chicken embryos. Van Rockel *et al.* (89) described the isolation of the "chronic respiratory diseases agent" in chicken embryos. Fabricant (33) (1951) also described a similar agent isolated a chicken embryo yolk sac from layer replacement chickens, which continued to show a chronic tracheitis and air sacculitis following a "controlled exposure" program for infectious bronchitis immunization. This agent was at first suspected to be an ornithosis like agent.

The common thread binding these was found when Delaplane (25) (1948) showed that the agent causing chicken chronic respiratory disease was capable of inducing sinusitis in inoculated turkeys.

In 1952, Markham and Wong (62) made the landmark discovery that chronic respiratory diseases of chickens and infectious sinusitis of turkeys were caused by a pleuropneumonia like organism (PPLO) that could be cultivated in a cell free culture medium. This was soon confirmed by Van Roekel and Olesiuk (88).

Van Roekel and Olesiuk published extensively on the etiology of chronic respiratory diseases in chickens, including studies on transmission, host susceptibility, route of

infection, response to antibiotics, and immunity. These publications described the work conducted at the University of Massachusetts during the period commencing in 1950 [Van Roekel *et al.* (89) 1952] and continuing for approximately 20 years.

A second landmark discovery was the differentiation by Adler and Yamamoto (1957) of pathogenic and nonpathogenic PPLO. They isolated two distinct strains of PPLO from a turkey with infectious sinusitis. One strain produced sinusitis in turkeys and respiratory lesions in chicken; the other apparently was not pathogenic. In collaboration with Berg and Fabricant, other studies on this general topic were published (4,5).

At this point, it was realized that the problem of diagnosis was complicated by the frequent occurrence of pathogenic PPLO (MG) as part of a flora often mixed with non pathogenic species of PPLO. This work was extended during 1958, when Yamamoto and Adler (95, 96) reported their studies on antigenic analysis, comparative pathogenicity, and cultural, biochemical, and morphological characteristics of avian PPLO. For this work, a total of eight strains (isolates) were used. Among these isolates was the S6 strain, originally isolated by D.V. Zander in 1954 from the brain of a turkey showing nervous signs. Zander (105) recorded that re-inoculation of the S6 strain into turkeys caused nervous symptoms and locomotor disturbances. Yamamoto and Alder showed that that the strains of PPLO of avian origin available to them could be separated into five groups according to morphological, physiological, and antigenic characteristics.

The term pleuropneumonia-like organism predominated in the published literature during most of this period. Not until 1960 did authors begin to refer to the organism as *mycoplasma*. This change resulted from the paper by Edward and Kenarek (1960), who proposed the name *Mycoplasma gallisepticum* for the PPLO that caused chronic respiratory diseases in chickens and infectious sinusitis in turkeys.

The pleuropneumonia group of organisms was first included in the Bergey Manual of Determinative Bacteriology in 1948. It was unfortunate that the avian *mycoplasmas* were not described in the Bergey Manual until the 7th edition (1957). The type strain described at that time was *Mycoplasma gallinarum* as reported by Edward and Freundt (1956). That organism was a non pathogenic isolates recovered from the upper respiratory tract of a fowl and later classified as a B serotype of Kleckner (1960) or the K18B group of Fabricant. The typical pathogenic species of the avian *mycoplasmas* was named *Mycoplasma gallisepticum* by Edward and Kanarek and was listed in the Bergey Manual

8th edition (1974). That isolates is the avian serotype S6 of Alder *et al.* and Fabricant (1960).

The diversity of avian PPLO was demonstrated by Fabricant (34), who used hyperimmune antisera to classify 170 cultures. This isolates fell into six different groups plus group of unclassified cultures. Eighty-one-nearly half of the isolates-were identified as the S6 pathogenic type, Kleckner (79) (1960) characterized a total of eight serotypes. Yoder and Hofstad (103) described the Iowa 695 serotype (now designed M. iowae), Roberts (79) (1954) described two more serotypes from poultry named M. anatis from ducks. Grumbles et al. (46) (1964) characterized several of the avian serotypes using tissue culture systems. Nineteen serotypes ("A" through "S") have now been combined and reduced to some 12 serotypes, all of which have received genus and species designations.

The ultrastructure of MG was explored by different research groups. However, the report by Domermuth *et al.* presented a logical description of all components of the organism. Ribosomes were clearly evident within some sections.

A controversial series of reports concerned the apparent reversion of some avian mycoplasma cultures to vegetative bacteria. This suggested that some of the PPLO (later named mycoplasma) were actually L forms bacteria. However, it now appears that the majority of the bacteria from so called reverted cultures were gram positive bacteria which were present in some broth cultures of some truth *mycoplasma,* but were almost inhibited from growth by the presences of high level of thallium acetate and penicillin. The death knell of this concept was not rung until many years later when DNA homology techniques proved there was no relationship between these PPLO and their suspected revertant forms.

The gross pathology and histopathology of *mycoplasma* infections of poultry were studied by Jungherr *et al.* (1953), Olesiuk and Van Roekel (1960), Rhodes *et al.* (1965), and Barber (1962). Hematological studies were reported by Fedde and Pomeroy and Chute (1960) described the pathology of *mycoplasma* infections in chicken embryos.

2.2 EPIDEMIOLOGY AVIAN MYCOPLASMOSIS

2.2.1 ETIOLOGY AND PATHOGENICITY

Mycoplasma is a trivial designation to the prokaryotes belonging to the class Mollicutes (mollis=soft and cutes=skin), i.e., bacteria that lack cell wall, which make them resistant to antimicrobials that act on this cell structure, such as penicillin. They have been considered extracellular agents, but scientists now a day's admit that some of them are obligatory intracellular parasites, whereas all other *mycoplasmas* are facultative intracellular organisms (Razin *et al*., 1998). More detailed information on *mycoplasma* taxonomy and pathogenicity can be found elsewhere (Yamamoto, 1990; Razin *et al*., 1998; Nascimento, 2000).

In order to survive within the host organism, induce disease and evade the host immune system, *mycoplasmas* use some pathogenicity tools and mechanisms. These include adherence to host target cells, mediation of apoptosis, innocent bystander damage to host cell due to intimate membrane contact, molecular (antigen) mimicry that may lead to tolerance, and mitotic effect for B and/or T lymphocytes, which could lead to suppressed T-cell function and/or production of cytotoxic T cell, besides *mycoplasma* by-products, such as hydrogen peroxide and superoxide radicals. Moreover, *mycoplasma* ability to stimulate macrophages, monocytes, T-helper cells and NK cells, results in the production of substances, such as tumor necrosing factor (∞), interleukin (IL-1, 2, 6) and interferon. These mechanisms may explain the transient suppression of humoral and cellular immune responses during *mycoplasma* infection in birds, the immune tolerance and auto immune diseases, as well as the massive lymphoid cell infiltration in the respiratory tract and joint tissues of infected fowls (Razin & Tully, 1995; Yamamoto, 1990; Razin *et al*., 1998).

Besides these mechanisms that may be used by MG, MS and MM, latency is common to avian *mycoplasmas*. Thus, these pathogens induce disease after the host is affected by other disease-causing agents such as bacteria and viruses and/or after an episode of host weakness (Yoder Jr, 1991a; Whitford *et al*., 1994). The latent status, i.e., when the mycoplasma is not recognized by the host immune system, may be explained by its intracellular location due to environmental pressure, as can be exemplified by the presence of antimicrobials in host tissues for the treatment of MG, MS or MM infection of birds (Razin *et al*., 1998). Recent experimental evidences that MS causes immune depression by

affecting the chicken cellular and humoral immune system have been found when complete hemogram was analyzed in chicks submitted to four conditions: negative control, Newcastle disease-vaccinated (ND-vaccinated), MS-infected, and MS infection plus ND vaccination (Nascimento *et al.*, 2003; Silva, 2003).

Moreover, mycoplasmas are more susceptible to mutations than other bacteria (Woese *et al*., 1985), and this can be explained by their defective DNA repair system, as demonstrated in the case of MG (Ghosh *et al*., 1977). The frequent changes on surface antigens (antigenic variations) allow *mycoplasmas* to evade the host immune system, and facilitate their survival when adhered to the host respiratory tract, as noticed for MG (Markhan *et al*., 1994). Cytadherence and/or cytadhesin membrane surface proteins that undergo changes are represented by pMGAs (hemagglutinins), MGC1, MGC2 and PvpA for MG, and MSPA and MSPB for MS (Razin *et al*., 1998; Bencina, 2002).

2.2.2 TRANSMISSION AND HOSTS

Mycoplasmas may be transmitted horizontally, through infectious aerosols coughed and sneezed by infected birds and through contaminated feed, water, contact personal and communicant animals, mainly birds. Transmission occurs vertically from parents to their offspring, through contamination of laid eggs (transovarian transmission), as previously mentioned (Charlton *et al*., 1996). Mycoplasma is commonly transmitted within species and/or between closely related species, that is, they are host-specific, with rare exceptions (Nascimento, 2000). MG and MS infections occur mostly in chickens and turkeys. However, they have been frequently isolated from quails (*Coturnix coturnix*) as reported previously (Nascimento & Nascimento, 1986; Nascimento *et al*., 1997; Nascimento *et al*., 1998), and from several avian species (Stipkovitis & Kempf, 1996; Lobão *et al.*, 2003). MM can infect other avian species, but reports on isolation from Japanese quails, peacocks and pigeons have not been confirmed (Yamamoto, 1991; Stipkovitis & Kempf, 1996).

2.2.3 INCUBATION PERIOD

The incubation period varies from 6-21 days. However, development of clinical signs can be highly variable depending on *M. gallisepticum* strain virulence (disease-producing power), complicating infections, and environmental and other stress factors. Thus, many variable factors influence the onset and extent of clinical disease. Therefore, meaningful

incubation periods cannot be stated. Layer birds usually develop clinical infections at the onset of egg production (Vegard, 2012).

2.2.4 PREVALENCE OF AVIAN MYCOPLASMA

Prevalence of Mycoplasma vary from rearing system. In Bangladesh Vola district MG infection was higher (62.5%) in backyard chickens as compared to those being reared in commercial farming system (53.61%), MG prevalence was highest in pullet (60.63%) followed by adults (55.83%) and chicken reared in winter showed higher prevalence of MG (60.42%) as compared to those reared in summer (51.25%) (Biswas PK *et al.*, 2003). In Batna of Eastern Algeria prevalence of MG was (60.33%) (Heleili *et al.*, 2011).

2.2.5 SYMPTOMS

The classical diseases caused by avian *mycoplasmas* are: CRD, an upper respiratory disease primarily seen in chickens and infectious sinusitis of turkeys, caused by MG; and infectious synovitis, caused by MS, and air sacculitis caused by MG, MS and MM. However, chronic and asymptomatic infections are the most common and of a major concern, due to the losses they cause (Yoder Jr, 1991a).

The clinical manifestations of MG are coughing, sneezing, snicks, rales, ocular and nasal discharge, decrease in feed consumption and egg production, increased mortality, poor hatchability, and, primarily in turkeys, swelling of the in fraorbital sinus (es). In chickens, turkeys and other birds, a milder form of some of these symptoms can be seen in MS infections, besides lameness, pale comb and head, swollen hocks and foot pad. Acutely affected birds may show green feces, but respiratory infection caused by MS is usually asymptomatic. Most of the symptoms of MM infection are mild or inapparent, and are characterized by impaired hatchability and embryo piping, increased embryo mortality, poor weight gain, and, occasionally, the same symptoms are seen in chickens affected by MS (Charlton *et al*., 1996).

2.2.6 GROSS LESIONS

Gross lesions in birds with mycoplasmosis include catarrhal inflammation of sinuses, trachea, and bronchi. Air sacs are often thickened and opaque, and may contain mucous or caseous exudate, besides hyperplastic lymphoid follicles on the walls. At slaughter,

carcass condemnation may result from the presence of air sacculitis, fibrinous perihepatitis and adhesive pericarditis; interstitial pneumonia and salpingitis, which are often seen in chickens and turkeys (Yamamoto, 1991; Charlton *et al*., 1996).

2.2.7 HISTOPATHOLOGY

The observed histological alterations are mononuclear cell infiltration, mucosal glandular hyperplasia and lymphoid follicular reaction, with tendency to affect also the connective tissue. In the lungs, it can be observed interstitial pneumonia, lymphoid follicular reactions and, less frequently, granulomas (Yoder Jr., 1991b; Ficken, 1996; Lay & Yoder Jr. 1997; Rodrigues *et al.*, 2001). Air sacs are affected mostly due to the physiology of the avian respiratory system, in which part of the inspired air goes first through the bronchi to this serosa and afterwards to the lungs (Nascimento, 2000).

2.2.8 ECONOMIC IMPORTANCE

Losses attributed to mycoplasmosis, mainly MG infection, are due to decrease in egg production and egg quality, poor hatchability (high rate of embryonic mortality and culling of day-old birds), poor feed efficiency, increase in mortality and carcass condemnations, besides medication costs (Mohammed, *et al*., 1987; Yoder Jr, 1991b; Lay & Yoder Jr, 1997). According to previous studies, a MG-infected chicken lays 15.7 eggs less than a healthy one, contributing to a loss of 127 million eggs in the USA in 1984, which corresponded to an annual loss of 125 million dollars (Mohammed *et al*., 1987). In Brazil, using slaughter data from the Federal Inspection Service, there was a loss of 34 thousand tons of broilers in the end of the production cycle due to respiratory diseases, which corresponded to 30 million dollars in 1994 (Projeto, 1994). Particularly for *Mycoplasma synoviae*, losses have been attributed to transient immune depression, increase of 1 to 4% in the mortality rate of broilers in the final phase of production (Shapiro, 1994), decrease of 5 to 10% in egg production and 5 to 7% in hatchability (Mohammed *et al*., 1987; Stipkovits & Kempf, 1996). Moreover, MG infection alone is considered one of the diseases that cause more losses to the poultry industry (Yoder Jr, 1991b; Charlton *el al.*, 1996; Lay & Yoder Jr, 1997).

In Brazil, the prevalence of *Mycoplasma synoviae,* in chicken flocks is increasing since the 80's, overcoming that of MG in breeding flocks (Balen & Fiorentin, 1990). Although

Mycoplasma synoviae,is mostly involved in asymptomatic infections and sometimes considered harmless to chickens, it is pathogenic for birds (Stipkovits & Kempft, 1996). Besides, *Mycoplasma synoviae,* has been proven to affect the humoral response of chicks vaccinated with a La Sota strain of Newcastle disease virus (ND). Hemagglutination inhibition protection values for Newcastle (ND HI) (GMT $\geq$ 4.0, titer $\geq$ 1:16) were detected in non-MS-infected birds up to 45 days after a single ND vaccination, but not in birds that were MS-infected and ND-vaccinated. Protection of MS-infected broilers was induced only after a second dose of vaccine (Nascimento *et al*., 2003; Silva, 2003).

CHAPTER-III

MATERIALS AND METHODS

The study was carried out in the Department of Pathology and Parasitology, Hajee Mohammed Danesh Science and Technology University, Dinajpur, for the pathological investigation of Avian Mycoplasmosis in poultry.

3.1 EXPERIMENTAL CHICKENS

The chickens of different commercial poultry farms were considered as experimental chickens. *Mycoplasma* outbreaks in the small scale commercial poultry farm were investigated at Joypurhat district of Bangladesh and the laboratory examinations were conducted in the Department of Pathology and Parasitology under Hajee Mohammed Danesh Science and Technology University (HSTU), Dinajpur.

A total of 500 samples (diseased and dead birds) were collected from 50 farms. The number of birds in the farms was variable ranging from 1500 to 5000 and they were reared on litter. A detail flock history in relation to the incidence of disease including housing system, location of poultry farms, sources of birds, age and population of the birds per flock, rearing system, litter material, feeding and watering system, bio-security of the farms, previous history on mycoplasma outbreaks were recorded. The birds affected with Mycoplasma were submitted to the Pathology laboratory for the diagnosis and treatment were the principal experimental chickens and some affected chickens were also collected physically.

3.2 RESEARCH AREA

Chickens (Sick and dead) were collected from different small scale commercial poultry farms at Joypurhat district and examined in the laboratory belonging to the Department of Pathology and Parasitology under Hajee Mohammed Danesh Science and Technology University (HSTU), Dinajpur.

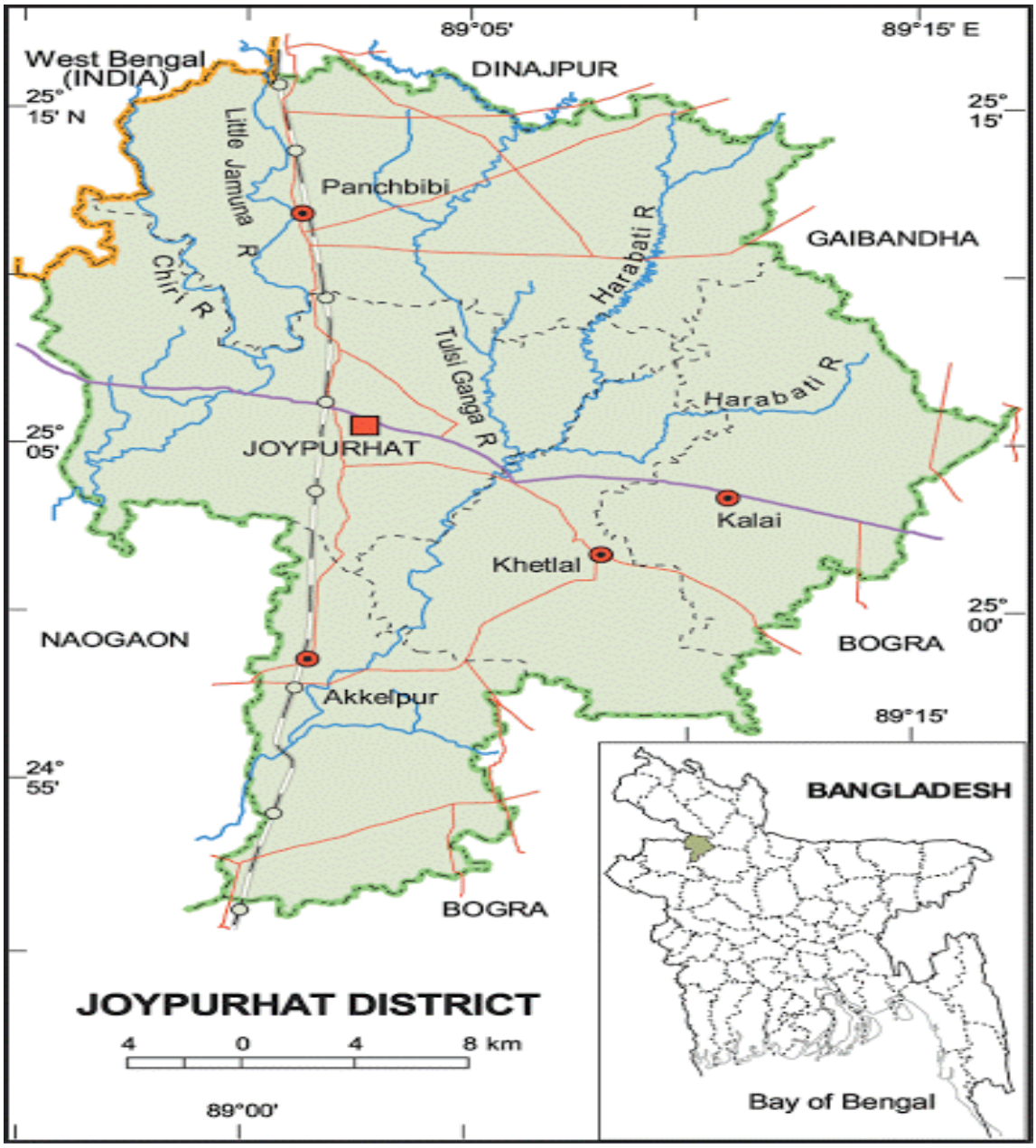


Figure: Working Areas are shown in the map by special circle

3.3 RESEARCH PERIOD

The duration of experiment was 6 months from January, 2015 to June 2015.

3.4 SAMPLING OCCATION

There was no scheduled sampling occasion. Birds affected with Mycoplasma were collected and examined when submitted to the laboratory only as well as the collection physically when informed.

3.5 THE MAJOR WORKS OF THE PRESENT STUDY

- Clinical examination of affected birds.
- Post mortem examination of dead birds to detect gross pathological changes.
- Histopathological examination of lung and trachea.

3.6 EXPERIMENTAL LAYOUT

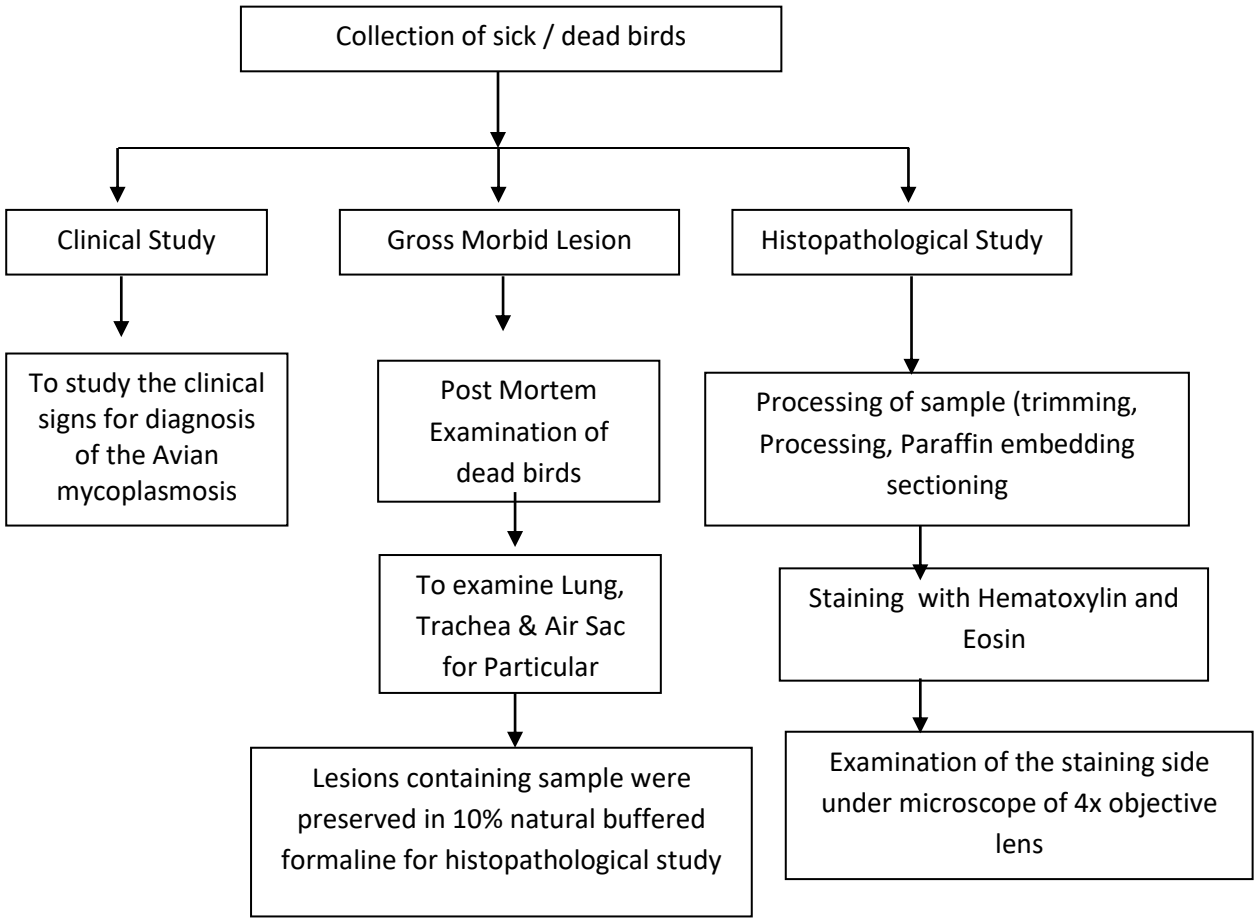


Figure: Flow diagram of the experimental design

3.7 CLINICAL STUDY

The general health condition and age of the chicken were recorded. The chickens were observed to detect clinical signs. The clinical signs were observed from the visual examination. The clinical signs were recorded during the physical visit of the affected flocks and farmers complaints about the affected birds were also considered.

3.8 NECROPSY FINDINGS OF SUSPECTED CHICKENS

The necropsy was done on the selected chicken taken from different village of Joypurhat. At necpsy, gross tissue changes were observed and recorded carefully by systemic dissection. The samples were also collected in 10% neutral buffered formalin for the histopathological study.

3.9 EQUIPMENT AND APPLIANCES FOR NECROPSY

1. Bird
2. Scissors
3. Forceps
4. Gloves
5. Musk
6. Bone cutting saw
7. Scalpel
8. Chisel
9. 10% neutral buffered formalin.

3.9.1 PROCEDURE

1. At first the chicken was wet in a detergent solution thoroughly to lessen the chances of feathers floating around the area while the examination.
2. The bird was laid on a pad of newspaper on post mortem table. The paper served to absorb most blood and fluid, and provided a convenient wrapper for the carcass after examination.
3. 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 paleness condition of carcass for detection of anaemia. Body cavity of bird was opened.

4. Lung, Trachea, Air Sac were observed carefully for importants post mortem lesions. Then the parts opened longitudinally by knife or scissors to observe the colour, consistency and appearance of intestinal contents.

3.10 CLEANING AND STERILIZATION OF REQUIRED GLASSWARE

Test tubes, glass tubes, glass slides, coverslips, 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 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°C.

3.11 GROSS LESION

Gross morbid lesions of different organs were observed after necropsy examination of birds.

3.12 HISTOPATHOLOGICAL EXAMINATION

Formalin-fixed samples of the lungs and trachea from the sacrificed bird collected by necropsy were processed for paraffin embedding, sectioned and stained with Haematoxylin and Eosin according to standard method (Luna, 1968) for histopathological study. Details of tissue processing, sectioning and staining are given below.

3.12.1 EQUIPMENT AND APPLIANCES

- Sample (lungs)
- 10% Neutral buffered formalin
- Chloroform
- Paraffin

- Alcohol
- Tape Water
- Xylene
- Hematoxylin and Eosin Stain
- Distilled water
- Clean Slides
- Cover slips
- Mounting media (dpx)
- Microscope

3.12.2 PROCESSING OF TISSUES AND SECTIONING

- The formalin fixed tissue samples were trimmed at 1.5×1 cm. in size with scalpel handle and blade and fixed for 48-72 hours in fresh neutral buffered formalin.
- The tissues were washed under running tap water for overnight to remove the fixative.
- The tissues were dehydrated in ascending grades of alcohol using 50%, 70%, 80%, 95% alcohol, 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, 1.5 hr in each.
- Paraffin blocks containing tissue pieces were made using templates and molten paraffin.
- The tissues were sectioned with a microtome at 5mm thickness, 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. The slides were air dried and kept in cool place until staining.

3.12.3 HEMATOXYLIN AND EOSIN STAINING PROCEDURE

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

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.

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.

3.12.4 STAINING PROTOCOL

- Deparaffinization of the sectioned tissues was done by 3 changes in xylene (3 mins in each),
- Rehydration of the sectioned tissues was done through descending grades of alcohol (3 changes in absolute alcohol, 3 mins in each; 95% alcohol for 2 mins; 80% alcohol for 2 mins; 70% alcohol for 2 mins) and distilled water for 5 mins,
- The tissues were stained with Harris' hematoxylin for 10 mins,
- The sections were washed in running tap water for 10 mins,
- 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 mins and dipped in ammonia water (2-4 times) until sections became bright blue,
- The sections were stained with eosin for 1 min and then differentiated and dehydrated in alcohol (95% alcohol, 3 changes, 2-4 dips in each; absolute alcohol 3 changes, 2-3 mins in each),
- The stained sections were then cleaned by 3 changes in xylene, 5 mins in each and finally the sections were mounted with cover slip using DPX,
- The slide were dried at room temperature and examined under a low (10X) and high (40X) power objects.

CHAPTER-IV

RESULTS

Pathological investigation of Avian Mycoplasmosis encountered in small scale commercial poultry farms at Joypurhat district was studied and different clinical, necropsy and microscopic conditions were recorded during the study period.

4.1 CLINICAL EXAMINATION

The clinical signs of the birds affected with Mycoplama varied from farm to farm. The signs were nasal secretion (Fig. 2), water discharge from eye (Fig. 3), Swellen of eyelid (Fig. 4), difficult breading, (Fig. 5) tracheal rales, coughing, gasping, less intake of feed. Whitish to greenish feces also found.

4.2 STATUS OF PREVALENCE OF THE DISEASE

The study revealed the following actual status of prevalence of Avian Mycoplasmosis in Sonali. Table-1 showed the prevalence of Mycoplasmosis at different region of Joypurhat district. A total of 500 birds were examined during the study period from which 290 birds were found infected with mycoplasmosis. The prevalence of mycoplasmosis of young birds were 62% and adult birds 58% were recorded.

Table-1: Prevalence of Avian Mycoplasmosis at different commercial Sonali farms in Joypurhat District. (Age wise)

Location of the farm (Upazila)	No. of farm visited	Age	No. of necropsy done	Mycoplasma encountered	Percentage %	Mortality	Percentase %
Sadar	8	55 days	80	51	63.75	4	5
Panchbibi	5	62 weeks	50	27	54	2	4
Akkelpur	5	50 weeks	50	29	58	3	6
Khetlal	4	21 days	40	26	65	2	5
Kalai	4	85 days	40	25	62.5	1	2.5
Total	26		260	158	60.76	12	4

Table-2: Prevalence of Avian Mycoplasmosis at different commercial Sonali farms in Joypurhat District. (Season wise)

Location of the farm	No. of farm Visited	Season	No. of necropsy done	Mycoplasma encountered	Percentase %
Sadar	6	Summer (April-July)	60	28	48.33
Panchbibi	5	Summer (April-July)	50	27	54
Akkelpur	5	Summer (April-July)	50	26	52
Khetlal	4	Winter (November-February)	40	25	62.5
Kalai	4	Winter (November-February)	40	26	65
Total	24		240	132	55

Fig. 1 Affected chick Shows Mycoplasmosis (21 days)

Fig. 2 Nasal Discharge (50 weeks, winter season)

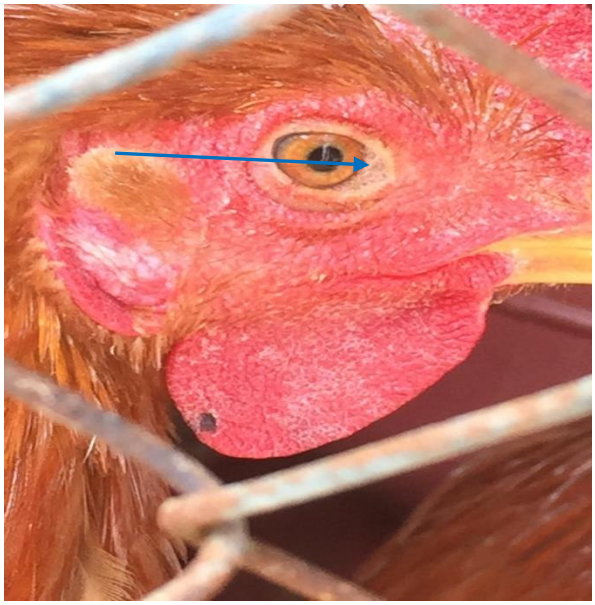


Fig. 3 The gross examination of 62 weeks age of birds shows water discharge from eye

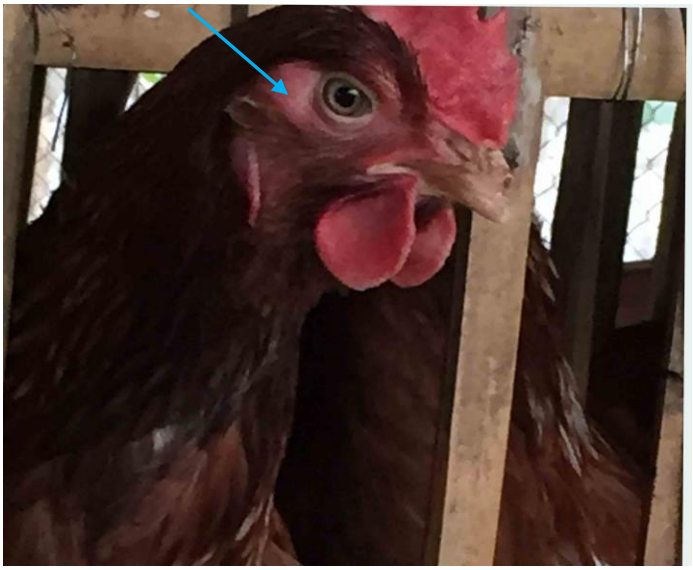


Fig. 4 The gross examination of 48 weeks age of birds shows swollen eyelid

Fig. 5 The gross examination of 85 days age of birds shows difficult breathing

4.3 NECROPSY EXAMINATION

Postmortem findings included catarrhal exudates in the nasal passages and trachea. Serious involvement of trachea, lungs, air sacs, heart and catarrhal exudates in nasal passages, catarrhal and foamy exudates in the trachea were reported in *Mycoplasma gallisepticum* infected flock. In some cases, hemorrhages were also present in trachea (Fig. 6). Lungs were severely consolidated with fibrin deposition and severely dark spotted by severe hemorrhage in lung (Fig. 7). Heart covered with fibrin purulent (Fig. 8) and Air sac become thickened (Fig. 9).

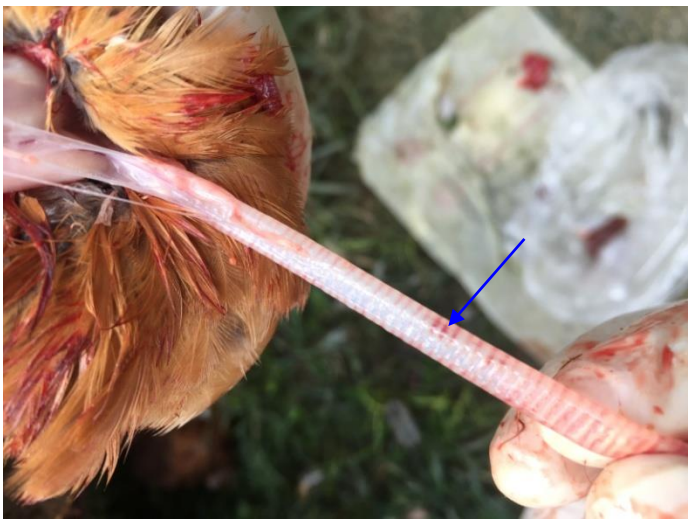


Figure -6: The gross examination of 21 days age of birds shows Hemorrhage in trachea

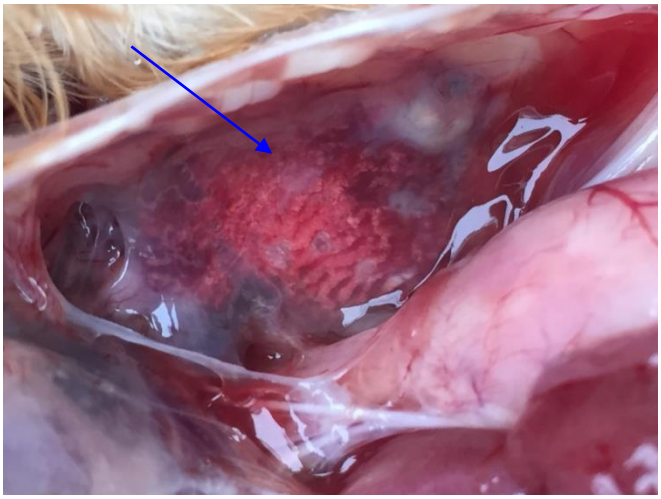


Figure -7: The gross examination of 21weeks age of birds shows Hemorrhage in lung

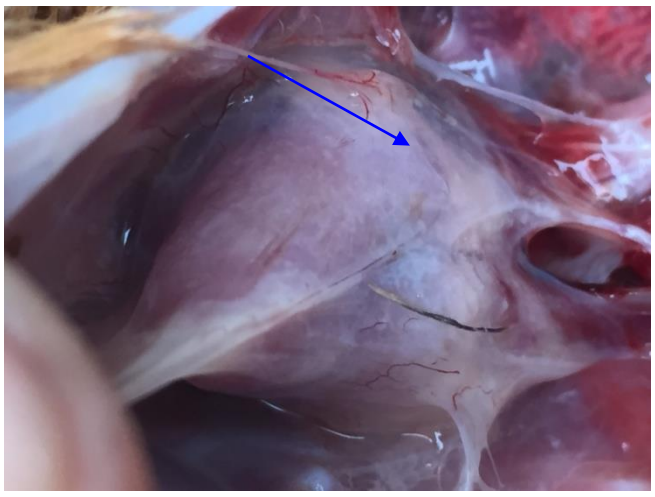


Figure -8: The gross examination of 40 weeks age of birds shows Fibropurulent Heart

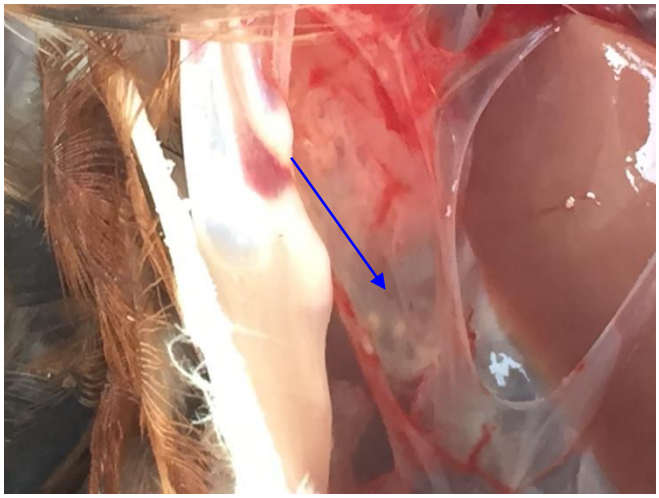


Figure -9: The gross examination of 80 days age of birds shows Thickened Air Sac

4.4 HISTOPATHOLOGICAL STUDY

In Trachea, mononuclear cellular infiltration in the mucosa and sloughing off mucosal lining were found (Fig. 10). Mucosal glandular hyperplasia and lymphoid follicular reaction with connective tissue were found in trachea. Infiltration of mononuclear cell were found in lung parenchyma (Fig. 11). Severe congestion and accumulation of fibrin also found in lung parenchyma (Fig. 12).

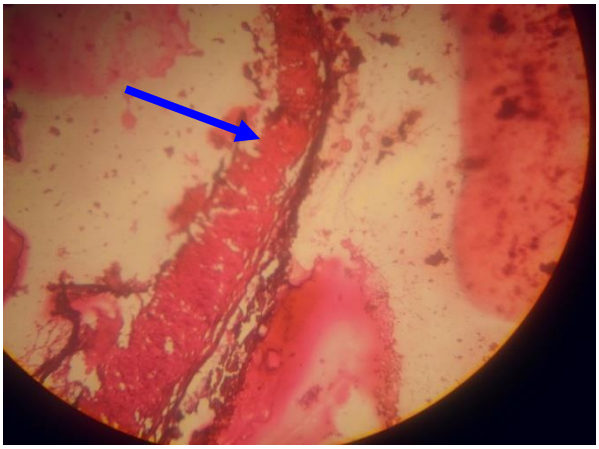


Figure- 10: The section of trachea showing mononuclear cellular infiltration in trachea

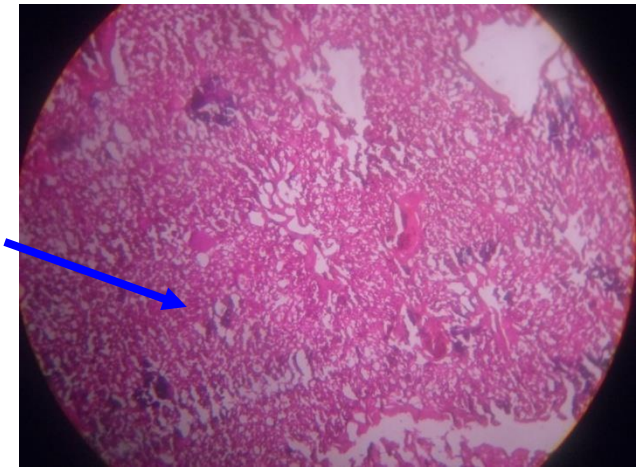


Figure-11: The section of Lung showing lymphoid follicular reactions in lung parenchyma

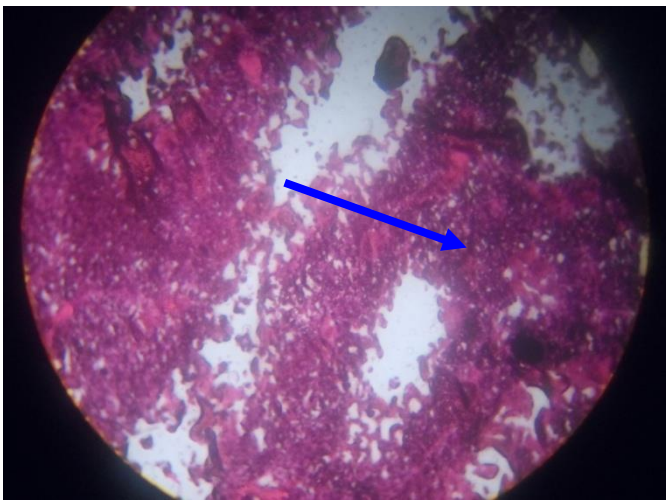


Figure-12: The section of lung showing severe congesting in the lung

CHAPTER-V

DISCUSSION

This study was undertaken to investigate the pathological condition of Mycoplasmosis at small scale commercial sonali farm in different upazilla of Joypurhat district from July to December, 2015.

A total of 500 of the 290 affected Sonali birds were examined as Mycoplasmosis and observed clinical signs were coughing, sneezing, tracheal rales, ocular and nasal discharge, decrease feed consumption and egg production which corespond with the findings of (Charlton *et al.*, 1996).

(Table 1) Age wise Prevalence of Mycoplasmosis at different commercial sonali farms in Joypurhat District are showing- total 26 farms visited in different thana. Total 260 diseased and dead birds were examined out of which 158 birds were found to be positive for Mycoplasmosis. The Prevalence of Mycoplasmosis of pullet were (63.75%,Sadar), (65%, Khetlal), (62.5%, Kalai) and of Adult were (54%, Panchbibi), (58%, Akkelpur) which were similar findings reported by (Biswas P. K. *et al.,* 2003) stated that Pullet (60.63%) and Adult (55.83%) prevalence of Vola in Bangladesh. Mortality rate was (5%) which was similar findings reported by (Shapiro, 1994) stated that (1-4%). The Prevalence of Mycoplasmosis was (60.76%) which was similar findings reported by (Heleili *et al*, 2011) stated that (60.33%) prevalence in Eastern Algeria.

(Table 2) Season wise Prevalence of Mycoplasmosis at different commercial sonali farms in Joypurhat District are showing- total 24 farms visited in different thana. Total 240 diseased and dead birds were examined out of which 132 birds were found to be positive for Mycoplasmosis. The prevalence of mycoplasmosis during summer season were (48.33%, Sadar), (54%, Panchbibi), (52%, Akkelpur) and during winter season (62.5%, Khetlal), (65%, Kalai) which were similar findings reported by (Biswas P K et al,2oo3) stated that during summer (51.25%) and winter (60.42%) prevalence of Vola district in Bangladesh.

Histological lesions in the trachea, mononuclear cellular infiltration in the mucosa and sloughing off mucosal lining were found. Mucosal glandular hyperplasia and lymphoid follicular reaction with connective tissue were found in trachea. Infiltration of

mononuclear cell were found in lung parenchyma. Congestion and accumulation of fibrin also found in lung parenchyma. Similar result were also reported by (Yoder Jr., 1991b; Ficken, 1996; Lay & Yoder Jr. 1997; Rodrigues *et al*., 2001).

CHAPTER-VI

CONCLUSIONS

Avian Mycoplasmosis is consider to be one of the most important disease in Sonali birds throughout the country. The present study was conducted mainly to explore a pathological investigation on Mycoplasmosis based on clinical, parasitilogical, gross and histopathological lesion. A total number of 50 farms were visited, 500 diseased and dead birds were examined out of which 290 birds were found to be positive for mycoplasmosis.

The clinical signs of the affected birds were recorded as nasal secretions, tracheal rales, gasping, coughing, whitish to greenish diarrhea, less intake of feed, decrease egg production & decrease body weight.

The investigation of Avian Mycoplasmosis grossly in the naturally infected birds were catarrhal exudates in nasal passages, catarrhal and foamy exudates in the trachea, bronchi, air sacs, perihepatitis, air sacculitis, severe haemorrhage in lungs.

Histopathological changes in trachea, mononuclear cellular infiltration in the mucosa and sloughing off mucosal lining were found. Pneumonia was characterized by severe congestion and accumulation of fibrin in the lung parenchyma.

On the basis of this study it is assumed that although Mycoplasmosis is a serious problem at poultry industry in Bangladesh, it possible to control under routine preventive and control measure which is prime essential for substantial improvement in poultry production.

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