Chapter 1: Introduction

In Bangladesh poultry industries play a vital role in poverty alleviation and economic development. Poultry has a major potential as a source of animal protein. Poultry products not only fulfill our nutrient demand but also aid in raising one's socio-economic status.

Profitable poultry farming has become difficult due to various poultry disease conditions which cause economic loss in poultry farming. Various disease conditions such as Newcastle disease (ND), infectious bursal disease (IBD), infectious bronchitis (IB), salmonellosis, colibacillosis, chronic respiratory disease (CRD), fowl cholera, infectious coryza, aspergillosis, coccidiosis, helminthiasis, ascites etc. seriously interrupt profitable poultry farming. The poultry sector in Bangladesh is facing a great challenge due to these harmful diseases (Hossain et al., 2004). According to some studies (Islam et al., 2009, 2012; Al Mamun et al., 2019) occurance of colibacillosis, chronic respiratory disease (CRD), Newcastle disease (ND), infectious bronchitis (IB), aspergillosis, coccidiosis, ascites increases in winter, a vital part of the dry season. Many of those disease conditions involve the respiratory system. Various substances and organisms enter the respiratory system during the inhalation of birds. Ammonia gas, dust, micro organisms, cold stress, etc. affect the normal function of the respiratory system. Among those substances dust in poultry houses are an important one. Dust found in poultry housing is a complex substance originating from non-organic and organic material inputs and the biological shedding of birds including floor bedding, feed, excreta, feather, exfoliated epithelium (dander) and microorganisms (Feddes et al., 1992; Aarnink et al., 1999; Just et al., 2009). Due to their irregular shape, these particles offer a tremendous surface area for binding bacteria (or their components), viral particles, and mold.

Studies have found dust as a source of contamination in case of Marek's Disease Virus (Carrozza et al., 1973; Woz´niakowski et al., 2014), *E. coli* (Oyetunde et al., 1978), Infectious Laryngotracheitis Virus (Yegoraw et al., 2021), *Eimeria* species and *Clostridium perfringens* (Bindari et al., 2021). Several studies have been done on biological and chemical contamination in dust (Clark et al., 1983; Bakutis et al., 2004; Skóra et al., 2016). Studies have also been done on the characteristics of dust components, their emission and effect on production (Wicklen et al., 1988; Takai et

al., 1998; Qi et al., 1992; Homidan et al., 2003; Li et al., 2011; Ahaduzzaman et al., 2021).

Increased concentrations of ammonia gas in poultry housing also affect the respiratory system of birds and make them susceptible to other microorganisms. Many studies have been done on the emission and effect of ammonia gas in broiler chicken (Oyetunde et al., 1978; Wicklen et al., 1989; Homidan et al., 2003).

Ascites is a commonly occurring problem in broiler chicken. Pulmonary hypertension (so-called 'pulmonary hypertension syndrome') may be the most common etiology connected with broiler ascites (Julian, 1993). Pulmonary hypertension may arise from many causes that affect intravascular osmotic pressure in the lungs and heart. Studies have been done on etiology of ascites syndrome and the involvement of the lungs and heart in pulmonary hypertension syndrome (Julian, 1993; Richard, 1999; Decuypere et al., 2000; Balog et al., 2003; Gupta, 2011).

Broiler chickens are commonly reared on floors covered with litter materials. Litter materials are used mainly to prevent direct contact between birds and the floor and to absorb moisture. During the dry season litter materials become dry more often and dust particles easily get suspended in the air. These dust particles are then inhaled by birds and enter to poultry respiratory system where they exert their pathological effect. Though many studies have been done on the emission and characteristics of dust, and their involvement as a source of contamination for microorganisms, there have been few studies on the pathological effect of dust in chickens. Therefore, this study was designed to find out the pathological effect of dust in broiler chickens.

Objectives:

i. To diagnose disease conditions in chicken predisposed to dust.

ii. To find out the pathological effect of dust (gross and microscopic) in chickens.

Chapter 2: Review of Literature

Relevant literature on the subject matter of the thesis work has been reviewed in this chapter.

2.1 Poultry sector in Bangladesh

Bangladesh is a densely populated country with a fast-growing economy. According to the World Bank, with an inspiring story of growth and development Bangladesh is on the track to graduate from the UN's Least Developed Countries (LDC) list in 2026 and is anticipated to achieve the status of the upper middle-income nation by 2031 (World Bank, 2023). The livestock sector has a great contribution to socio-economic development in Bangladesh. According to Rahman et al. (2014) livestock is not simply a source of animal protein but also a necessary element of Bangladesh's extensive farming system and a source of employment. The current contribution of livestock to the nation's Gross Domestic Product (GDP) at a constant price is 1.85% with the GDP growth rate of livestock (constant price) at 3.23%. The sector provides direct employment to 20% of the total population and supports 50% of the total population of the country with partial employment. Poultry is one of the most important segments of the livestock sector in Bangladesh. The current population of poultry in Bangladesh is around 3857.04 lakhs (DLS, 2022-2023). Broiler chickens are a significant part of the poultry population. They are frequently reared for meat purposes. The number of broiler chickens in Bangladesh is around 104.4 lakhs (Agriculture Sample Census, 2020). The broiler industry plays a large role in the global economy, particularly in developing nations. Broiler rearing can be extremely important in a nation like Bangladesh, where most of the population lacks access to land, is underprivileged and lacks the education or skills necessary to engage in activities generating revenue.

2.2 Diseases of poultry

The main obstacle facing the poultry industry, among many others, is the death of birds from many lethal infectious and non-infectious diseases (Giasuddin et al., 2002). Poultry diseases are considered the major constraints for developing the poultry industry (Karim, 2003). Several infectious and contagious diseases, including Newcastle disease (ND), infectious bursal disease (IBD), infectious bronchitis (IB),

salmonellosis, colibacillosis, fowl cholera, infectious coryza, chronic respiratory disease (CRD), aspergillosis, coccidiosis, helminthiasis, etc. are currently posing a serious threat to Bangladesh's poultry industry (Hossain et al., 2004). According to Saleque et al. (2003), commercial chickens have afflicted with several illnesses, bacterial, viral, mycoplasmal, parasitic, and non-infectious disorders occurring at rates of 45%, 17%, 12.4%, 4.5 %, and 12.4 %, respectively. Several studies have recorded occurrence of diseases in broiler chickens of Bangladesh as listed in table 2.1 (Al Mamun et al. 2019); table 2.2 (Islam et al., 2012); table 2.3 (Islam et al., 2009).

Table 2.1- Occurrence of diseases in broiler with seasonal variation

Name of the disease	**Summer n=424**	**Rainy n=394**	**Winter n=379**	**Total n=1197**
ND	52 (12.26%)	20 (5.08%)	69 (18.21%)	141 (11.78%)
IBD	152 (35.85%)	85 (21.57%)	114 (30.08%)	351 (29.32%)
IB	31 (7.31%)	18 (4.57%)	62 (16.36%)	111 (09.27%)
Colibacillosis	31 (7.31%)	14 (3.55%)	32 (8.44%)	77 (6.43%)
Salmonellosis	70 (16.51%)	63 (16.99%)	37 (9.76%)	170 (14.29%)
CRD	6 (1.42%)	11 (2.79%)	41 (10.82%)	58 (04.85%)
Coccidiosis	31 (7.31%)	43 (10.91%)	9 (2.37%)	83 (06.93%)

Table 2.2- Seasonal occurrence of diseases in broilers

Name of the disease	**Summer n=31**	**Winter n=35**	**Rainy n=39**	**Total n=105**
ND	1 (3.2%)	0	0	1 (1.0%)
IBD	8 (25.8%)	8 (22.9%)	10 (25.6%)	26 (24.8%)
Colibacillosis	8 (25.8%)	13 (37.1%)	7 (17.9%)	28 (26.7%)
Salmonellosis	1 (3.2%)	0	1 (2.6%)	2 (1.9%)
CRD	0	1 (2.9%)	0	1 (1.0%)
Coccidiosis	27 (87.1%)	35 (100%)	38 (97.4%)	100 (95.2%)
Ascites	2 (6.5%)	5 (14.3%)	5 (12.8%)	12 (11.4%)

Table 2.3- Seasonal occurrence of diseases in chicken

Name of the disease	Summer n=90	Winter n=71	Rainy n=90	Total n=251
ND	6 (6.7%)	4 (5.6%)	2 (2.2%)	12 (4.8%)
IBD	20 (22.2%)	15 (21.1%)	20 (22.2%)	55 (21.9%)
Colibacillosis	12 (13.3%)	18 (25.4%)	13 (14.4%)	43 (17.1%)
Salmonellosis	6 (6.7%)	2 (2.8%)	9 (10.0%)	17 (6.8%)
CRD	2 (2.2%)	5 (7.0%)	2 (2.2%)	9 (3.6%)
Coccidiosis	74 (82.2%)	69 (97.2%)	78 (86.7%)	221 (88.0%)
Ascites	2 (2.2%)	5 (7.0%)	5 (5.6%)	12 (4.8%)

The presence of ammonia, dust in chicken houses, overcrowding, poor hygienic management, excessive shed temperatures, etc. are some factors that make the birds more vulnerable (Barnes and Gross, 1997). Compared to the other two seasons in Bangladesh, these factors are more prominent during the winter season.

2.3 Ascites in Poultry

An increase in the amount of lymph, typically present in the peritoneal cavities is known as ascites. Increased intravascular pressure in the liver's portal system and the capillaries of the organs in the abdominal cavity contribute to the accumulation of fluid in the peritoneal cavities. This portal hypertension results from right ventricular valvular insufficiency brought on by right ventricular hypertrophy (RVH), a reaction to pulmonary hypertension (PH) (Julian, 1993). According to Gupta (2011), ascites can be triggered by a variety of reasons, which include as follows:

i. **High and low altitude:** Increased prevalence at high altitudes is associated with increased oxygen demand to support rapid growth and the incapacity of the heart and lungs to supply enough oxygen to the tissues to maintain the nutritional and growth rate potential. Ascites are seen at low altitudes, where it is connected to respiratory diseases and inadequate ventilation (Teuscher et al., 1971; Huchzermeyer, 1986; Olkowski and Classen, 1998).
ii. **House environment:** Ascites is more common because of dust and ammonia production in the poultry house. Dust particles can transmit disease-causing

microorganisms that can irritate or infect the lungs of birds and limit the exchange of oxygen between them and the environment (McGovern et al., 1998).

iii. **Cold:** A key element is the cold. When the environment is cold, T3 concentration tends to rise, which is necessary for the production of additional metabolic heat to keep the body warm. Pulmonary hypertension and right ventricular failure are the results of the subsequent increase in metabolic rate, which causes the blood pressure to rise as the heart tries to maintain the oxygen supply to the organs and muscles (Julian et al., 1989; Acar et al., 1995).

iv. **Heat:** The occurrence of ascites is also enhanced by heat as there is an increased need for oxygen (Tattori et al., 1995).

v. **Age:** Ascites is more likely to develop in broilers between the ages of three and seven weeks (Kamindjolo et al., 1978; Banday and Maqbool, 1994).

vi. **Sex:** Compared to females, male birds are more susceptible to ascites (Coello et al., 1985; Wideman et al., 1997).

vii. **Breed:** Compared to slow-growing breeds, fast-growing breeds need more oxygen to maintain their rapid rate of growth, and because they have smaller lung volumes than their bodies, they are more likely to develop hypoxia and ascites syndrome (Julian, 1989).

viii. **Light:** Ascites in broilers occur more frequently when there is continuous lighting than when there is intermittent lighting (Gordon, 1997; Buys et al., 1998).

ix. **Nutritional factors:** The incidence of ascites syndrome is increased by pelleted feed and high-calorie diets. Broilers develop ascites when fed a diet high in T3, and sodium, and deficient in phosphorus, as well as vitamin C, vitamin E, and selenium (Dale, 1987; Silva et al., 1988; Ekanayake et al., 2004).

x. **Sodium chloride:** Ascites can result from acute salt poisoning. This is because it causes the erythrocyte deforming ability to decline, which may impair blood flow dynamics and result in pulmonary hypertension and ascites (Julian, 1987; Mirsalimi et al., 1992).

xi. **Diseases:** Ascites can be brought on by *Aspergillus* species (Zafra et al., 2008), *E. coli*, infectious bronchitis virus, and avian leukosis virus

(Huchzermeyer, 1986; Hihara et al., 1998; Yamaguchi et al., 2000; Stedman and Brown, 2002).

xii. **Toxins:** Ascites in broilers can also be caused by hepatotoxin, mycotoxins, and high levels of furazolidone in feed (Bhagat et al., 1990).

2.4 Poultry dust

Large volumes of airborne contaminants are produced by the poultry business, and poultry buildings are a major source of dust or particulate matter, endotoxins, offensive odors, and gases including methane, hydrogen sulfide, carbon dioxide, and ammonia, among other things (Wathes et al., 1997; Seedorf and Hartung, 2000). The majority of the inhalable and respirable particles in poultry buildings are caused by poultry house dust, often known as "poultry dust", which is one of the most well-known airborne contaminants (Wathes, 1998). Feed, bedding and litter particles, feces and urine crystals, skin flakes and feathers, pollen, mites, fungi, spores, bacteria, and viruses are among the substances found in poultry dust. (Koon, 1963; Skóra et al., 2016).

Numerous microorganisms have been found in poultry dust, with concentrations of bacteria reaching $14x10^6$ colony-forming units (CFU/m^3) and fungal spores reaching $2.8x10^4$ CFU/m^3, respectively. This suggests that dust transports infections through the air (Lee et al., 2006). In addition, endotoxins, which are toxins produced by gram-negative bacteria, are present in poultry dust. Asthma and organic dust toxic syndrome are inflammatory reactions that can result in fever, headache, coughing, nasal congestion, allergies, nausea, chest tightness, and phlegm. Many acute and chronic occupational respiratory diseases are caused by endotoxins (Clark and Larsson, 1983).

Numerous variables, including bird type and age, production system and housing style, stocking density, bedding type, litter age, litter management techniques, and environmental variables like temperature, ventilation rate, and relative humidity, affect the amount of dust produced and accumulated in poultry houses. Furthermore, when building's ventilation rates are decreased to their bare minimum during colder months, it is typical for poultry dust levels to rise (Takai et al., 1998). The bedding and litter used in animal production have the potential to produce significant volumes of dust. Compared to pig or cattle buildings, poultry houses have a higher

concentration of dust. In chicken production systems, caged layer systems have much lower dust content than broiler flooring systems (with litter) (Aarnink et al., 1999).

2.5 Effects of poultry dust on poultry

Both the performance indicators and the health of poultry birds can be significantly impacted by high quantities of dust. Mammals easily deposit dust particles from the inhalable and respirable fractions in their lungs when doing regular breathing. A similar behavior occurs with birds. The tiniest particles are deposited in the lungs, but unlike mammals, some of these particles get up in the air sacs that are attached to bones and joints, causing inflammatory reactions and systemic infections (Clemmer et al., 1960).

When compared to broilers raised in poultry houses with greater dust concentrations, broiler production traits like average body weight, body weight gain, and feed efficiency were improved in poultry houses with lower dust concentrations (Almuhanna et al., 2011). According to a study, the body weights of birds dramatically rose when the concentration of dust in broiler houses was reduced. According to this study's findings (Willis et al., 1987), at seven weeks old, birds raised in dust-controlled rooms weighed 165gm more than their counterparts raised in non-dust-controlled environments. Microbes that produce pathogen-associated molecular patterns (PAMPs) like lipopolysaccharides (LPS), toxins, lipoteichoic acid, and β-glucans play a significant role in the formation of poultry dust. These molecules interact with antigen-presenting cell receptors to cause the production of inflammatory cytokines, which triggers inflammatory reactions. Compared to the birds in the control group, broilers treated with dust components (LPS, β-glucans) gained considerably less body weight after the challenge (Parmentier et al., 2008).

The exposure to dust has an impact on avian mortality rates as well. The relationship between the amount of airborne dust and the mortality rate was examined in a study involving 56,000 laying hens. According to the study, the regression coefficients between mortality rate and total dust and $PM_{5.0}$ percent were +2.2 for total dust and +9.15 for $PM_{5.0}$ fraction, respectively. The mortality rate increased by nine points for every one-point rise in the concentration of dust ($PM_{5.0}$) (Guarino et al., 1999).

Bird's respiratory systems undergo macro and microscopic changes when exposed to high levels of dust. Broiler hens exposed to dust for four weeks developed considerable coagulative necrosis with fibrinocellular exudate in their air sacs, and their lungs exhibited severe congestion and necrotic foci. Comparatively to the birds in the control group, the tracheal mucosa displayed localized squamous metaplasia with hypertrophy (Oyetunde et al., 1978). In a related study, 400 turkeys were given 12 weeks of exposure to two different quantities of poultry dust. The incidence of airsacculitis was strongly impacted by dust concentration; it was twice as high in the high dust levels treatment compared to the low dust levels treatment. When examined under a microscope, the tracheal epithelium of turkeys raised in environments with high levels of dust showed loss of cilia, hyperplasia of the cells that produce mucus, as well as focal lung consolidation and cellular exudate in the atrial lumen. These results provide proof that dust causes damage to bird's respiratory systems (Wolfe et al., 1968).

Bird's ability to live healthy lives can be impacted by air quality. As previously stated, exposure to high levels of dust is linked to inflammation, decreased pathogen resistance, and decreased respiratory tract clearance. Reduced oxygen intake and respiratory function are caused by all of the above conditions. In broilers that grow quickly, a decreased gas exchange may cause hypoxia to develop quickly. Low blood oxygen levels raise pulmonary artery blood pressure, which causes the right heart chambers to enlarge and dilate. Birds experience extreme discomfort and anguish as a result of the development of ascites, which is caused by an increase in pulmonary pressure (Broom, 1988; Jensen et al., 2000).

2.6 Poultry bedding or litter material

Feathers, skin scraps, dirt, feed, bedding material, and microorganisms are all components of poultry litter. The performance, health, and welfare of animals are directly impacted by bedding, which is a material or substrate that serves to absorb moisture and provides the animal with a comfortable surface for resting, protecting them from the cold floor (Grimes et al., 2002; Malone et al., 2008). Bedding is a crucial component of animal production.

2.7 Different types of litter materials

The best bedding material must be very absorbent, non-toxic to animals, clean, simple to dry, widely accessible, and reasonably priced. Inorganic elements like sand and clay can be used to make poultry bedding, as can organic materials like wood and crop leftovers (Hafeez et al., 2009). Different geographical areas have different bedding material pricing and availability. Sawdust, rice husk, sugarcane pulp, sugarcane bagasse, wheat straw, soybean straw, chopped straw, paper mill byproducts, sand, peanut hulls, wood shavings, maize cobs, oat hulls, dried leaves, and coffee husk are the typical types of litter used in poultry houses around the world (Veltmann et al., 1984; Benabdeljelil and Ayachi, 1996; Lien et al., 1998; Shields et al., 2005; De Avila et al., 2008). Although the performance of birds grown on these materials can vary, other wood products such as bark chips, ground stumps, and hardwood pellets can also be utilized as poultry litter (Pearson et al., 2000). Different kinds of litter, including sawdust, rice husk, sugarcane bagasse, wheat straw, sand, and ash are used in Bangladesh as poultry bedding (Monira et al., 2003).

2.8 Wood as bedding material

A form of vegetal tissue called wood is made up of cellulose fibers that are encased in a lignin matrix. This abundant natural substance has well-known antibacterial qualities and considerable potential for absorption (Tsoumis, 1991). Additionally, wood includes resins, which are non-structural substances. These resins or extractives, which guard the wood against microbial decay, insect swarms, and animals, are primarily volatile organic compounds (VOCs) such as terpenoids, phenolics, alcohols, glycols, aldehydes, alkaloids, and esters. These VOCs release aromatic hydrocarbons, which are carcinogenic and cause cytotoxicity (Davey et al., 2003). The kind of tree has a significant impact on the amount and type of resin present in the wood. Terpenes and aldehydes, which are aromatic chemicals that give many plants, including pine, cedar, and citrus trees, their distinctive aromas, are mostly produced by softwoods (coniferous trees). Hardwoods (dicotyledon trees) including oak, maple, mahogany, and cherry are more abundant in alcohols and carbonyl compounds with strong antibacterial and insect-repellant qualities. Softwoods are frequently favored because it has been claimed that hardwoods are more cytotoxic and carcinogenic than those (Munir et al., 2019). Wood shavings, sawdusts as well as bark chips, ground

stumps, and hardwood pellets can be used as bedding materials. Chickens raised in wooden litter materials have been shown to have some significant consequences. The type of litter has an impact on the blood levels of alanine aminotransferase (ALT), hemoglobin content, white blood cell count, and mean corpuscular hemoglobin in broilers. In comparison to broilers raised on rice husks, broilers raised on wood shavings had significantly lower concentrations of red blood cells in their blood (Huang et al., 2009). Additionally, broilers raised on wood shavings had lower hemoglobin and red blood cell counts than broilers raised on various bedding materials (control, rice husk, groundnut hull) (James et al., 2019).

2.9 Wood dust

The surface area of the particle and the material's bulk density both affect the absorption capacity of wood. As a result, different wood presentations, including wood chips, wood shavings, and sawdust, have different capacities for absorbing moisture (Bilgili et al., 2009). Because of this, smaller particles have more surface area and absorption power. However, as particle size decreases, wood dust concentrations rise correspondingly, posing a risk to both human and animal health (Teixeira et al., 2015; Dorothy et al., 2018). A serious occupational hazard for nearly exclusively woodworking industry employees is wood dust. Cutting and processing timber materials primarily results in the production of wood dust. The largest risk of exposure to wood dust is among those working in sawmills, producing wood goods (floors, doors, and furniture), and carpentry (Whitehead, 1982; Li et al., 1990; Halpin et al., 1994; Mohan et al., 2013; Bislimovska et al., 2015). Furthermore, investigations on the particle size distribution and concentration of wood dust revealed that only 30% of the mass of dust is made up of particles in the $PM_{5.0}$ fraction. The numerical measurements, however, revealed that $PM_{5.0}$ particles are the most prevalent and the most hazardous because they can enter the lower, non-ciliated sections of the respiratory tract (Whitehead et al., 1981; Proto et al., 2010).

2.10 Wood dust's effects on poultry

The majorities of the inhalable and respirable airborne particles in poultry houses are organic in nature, with bedding, excrement, and feed making up the majority of these particles. Wood shavings and sawdust are commonly used in poultry houses. Compared to other bedding materials like wheat straw and rice husks, these materials

distribute more dust (Benabdeljelil and Ayachi, 1996). It is challenging to determine the precise impact of the wood dust fraction on the health of the birds because the amount of dust in the air of a poultry house depends on several factors, including relative humidity, moisture content of the litter, ventilation rate, and stocking density. A synergistic relationship between dust, ammonia, and other typical stressors including heat, relative humidity, and bacteria has been discussed by several writers. The combined effects of concurrent difficulties appear to be significantly more harmful than the effects of each stressor alone (Wolfe et al.,1968; Oyetunde et al., 1978; Willis et al., 1987; O'Connor et al., 1988; McFarlane et al., 1989; Hartung, 1993; Hamilton et al.,1996, 1999; Golbabaei and Islami, 2000; Donham et al., 2002; Amer et al., 2004; Wei et al., 2015).

Chapter 3: Materials and Methods

3.1 Study area and sample size

Samples were collected from the birds brought from different parts of Chattogram district to the department of Pathology and Parasitology, CVASU for post-mortem examination with the history of using dry sawdust as litter materials over the period of November 2021 to April 2022. After post-mortem examination, trachea, lung, and liver samples were collected and preserved for histopathological examination. A total of 168 samples (76 lungs, 73 tracheas, and 19 livers) were collected from 83 affected birds of 21 farms. Preserved tissues were processed considering standard procedure (Luna, 1968).

3.2 Sample collection and preservation

Grossly affected tissues were collected, identified, and preserved in Bouin's solution (10 Folds of the tissue size) in labeled plastic containers. The thickness of the tissue sample was 4-5 mm. Tissues were preserved for at least 7 days before processing.

3.3 Processing of tissue

Preserved tissues were processed following removal of fixative, dehydration, clearing, impregnation, and embedding.

Sample identification marks were made by a soft lead pencil and a garland (tissue string) of tissues was made considering the cut surface for sectioning. Then the tissue garlands were placed for an overnight wash in running tap water to remove the fixative. Dehydration was done by moving the tissues through ascending concentration of ethanol series (80% alcohol- two hours, 95% alcohol- two changes one hour each, 100% alcohol- three changes one hour each) for appropriate time to prevent shrinkage of cells. Clearing reagents should be miscible with the dehydrant and the paraffin. Xylene was used as a clearing reagent to replace alcohol (xylene- two changes one hour each, xylene- two hours). Impregnation of tissue by paraffin for complete removal of the clearing agent was done by three changes in paraffin bath (56-5^{8o}C), two hours each. The cooked tissues were kept overnight to rest. Embedding was done by placing the tissue in melted paraffin to make a block, which after

solidification provided a firm medium for keeping all parts of the tissue intact when sections were cut.

3.4 Preparation of sections

Tissue block embedded in paraffin was set in the rotary microtome machine and sections were cut at 3-5µm thickness until suitable ribbon was formed. The ribbon of tissue sections was placed in a warm water bath (55-58^{o}C) and allowed to spread. A small amount of gelatin was added to the water bath for better adhesion of the section to the slide. Sections were picked up on grease-free clear slides. Sections were the air-dried and placed on a rack.

3.5 Staining of tissue slides

A regressive staining procedure was followed to stain the tissue slides. In the regressive staining technique, the sections were first overstained with a relatively neutral solution of hematoxylin. Then the excess stain was removed by using an acid alcohol solution. After that sections were neutralized with an alkaline solution (weak ammonia water) for better differentiation. Then the sections were counterstained with eosin followed by the removal of excess eosin by alcohol.

After staining and mounting cover slip the slides were air dried and then examined under microscope.

Chapter 4: Results

4.1 Gross lesions

Ascites are found in 23 birds, where the abdomen is distended with straw color fluids (Figure 1a, 1b). In some cases, the fluid is clotted (Figure 1c, 1d). Tracheas are congested (Figure 2a, 2b) and mucus is present in the tracheal lumen of some birds (Figure 2c, 2d). Severe congestion is found in the lungs; the lungs are edematous and dark red in color (Figure 3). The liver is congested and enlarged (Figure 4), which become smaller than normal (Figure 1d) in the later stage of the disease condition.

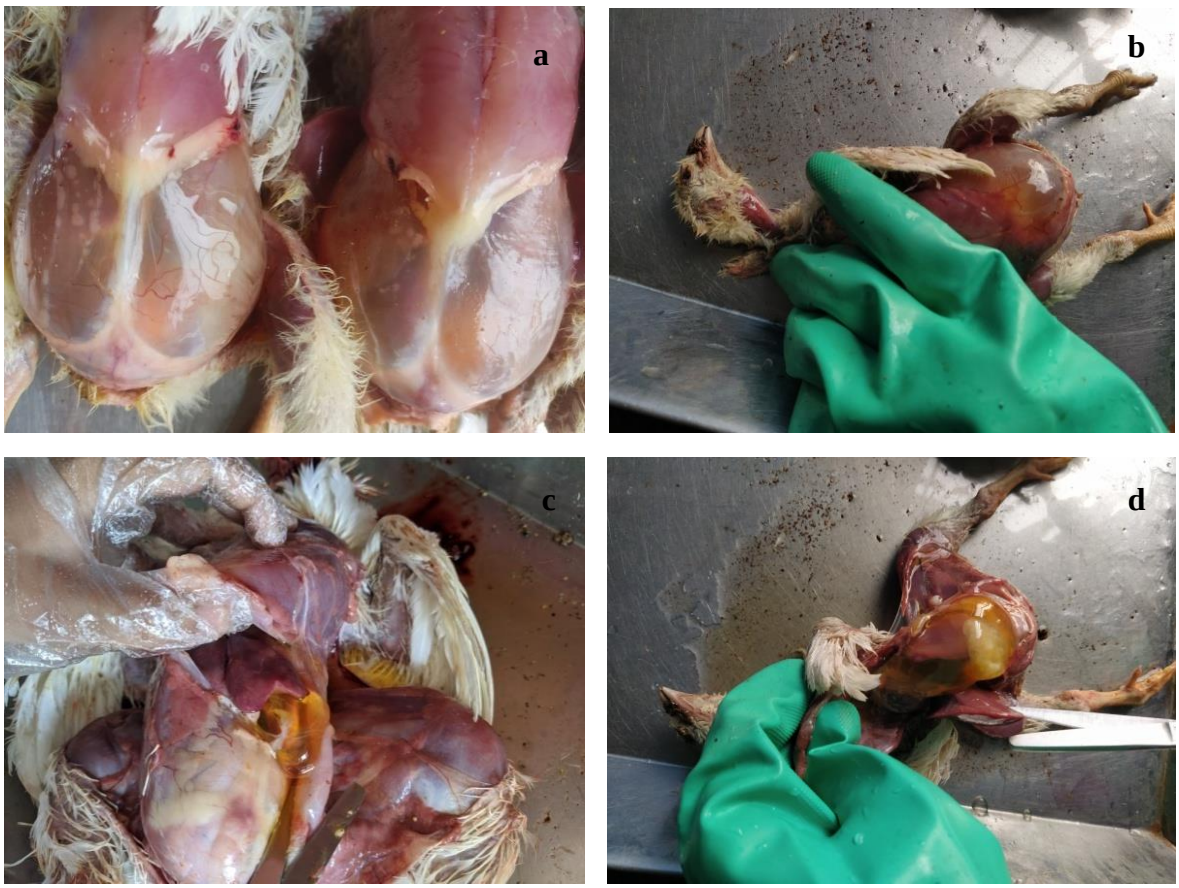

Figure 1: Gross pathological findings after post-mortem examinjations. Distended abdomen with fluid (a, b); straw-colored clotted fluids in the peritoneal cavity (c); liver become smaller in size and covered in straw-colored clotted fluids (d).

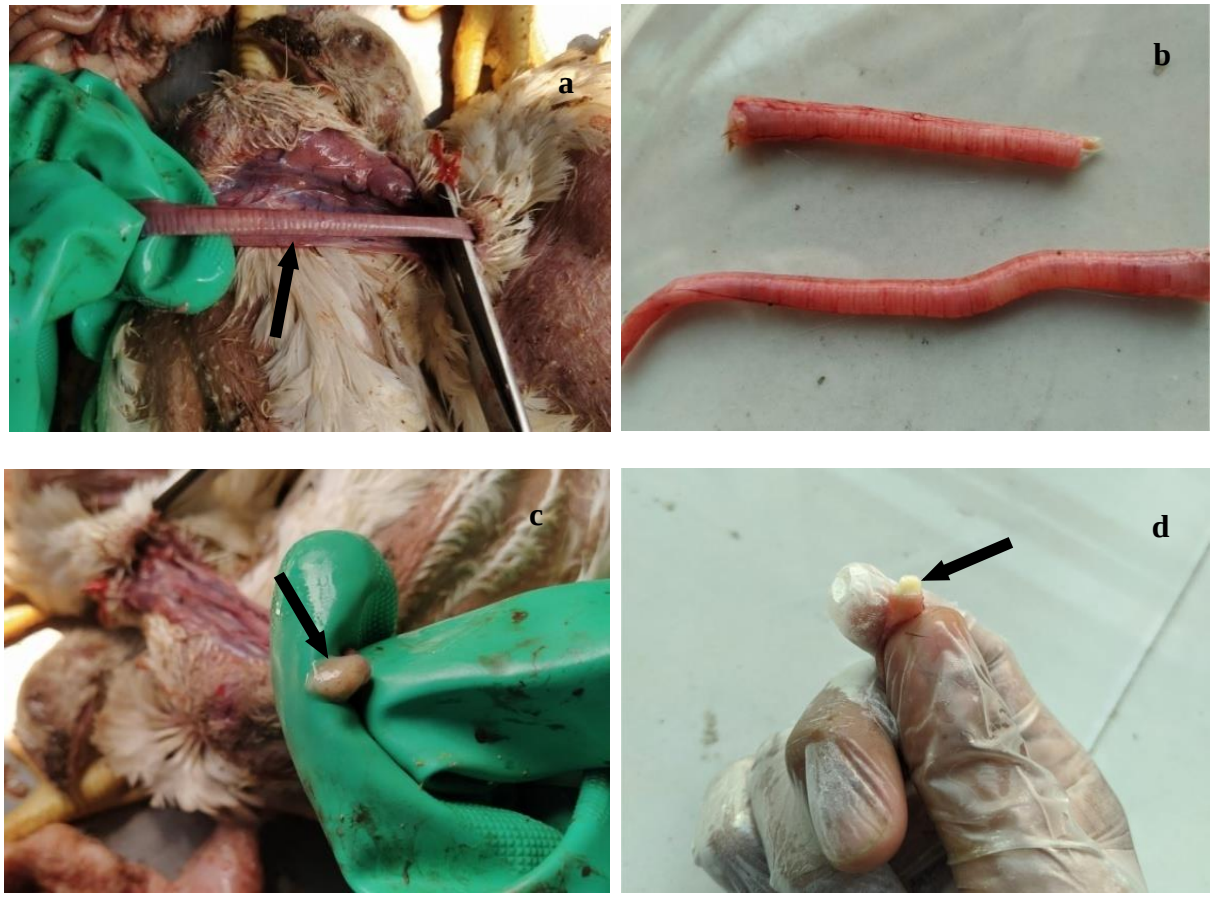

Figure 2: Congested trachea (a, b); Mucus in tracheal lumen (c, d).

Figure 3: Congested and edematous lungs.

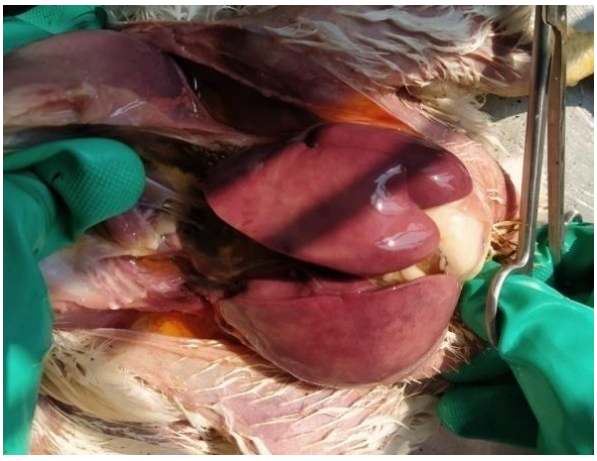

Figure 4: Congested and enlarged liver.

4.2 Microscopic lesions

Histopathological sections of the trachea show the presence of congestion, hemorrhage, and infiltration of inflammatory cells, desquamation of epithelial lining, loss of cilia (Figure 5a, 5b, 5c, 5d). Lung sections are severely congested (Figure 6a, 6b, 6d) and infiltrated with reactive cells (Figure 8). Pink colored edematous fluid is markedly present in the alveolar lumen (Figure 6a, 6c, 7a, 8a). Dark blackish dust particles are present in alveolar lining and in interstitial tissue (Figure 6c, 8a, 8b, 8c, 8d). Alveolar septa are thickened, presence of cuboidal cells in alveolar lining indicates metaplasia (Figure 7a, 7b). Emphysema is also found in lung sections (Figure 6a, 6b, 6d). Histopathological sections of liver show severe congestion in central vein, necrosis and loss of hepatocytes (Figure 9a, 9b, 9c, 9d).

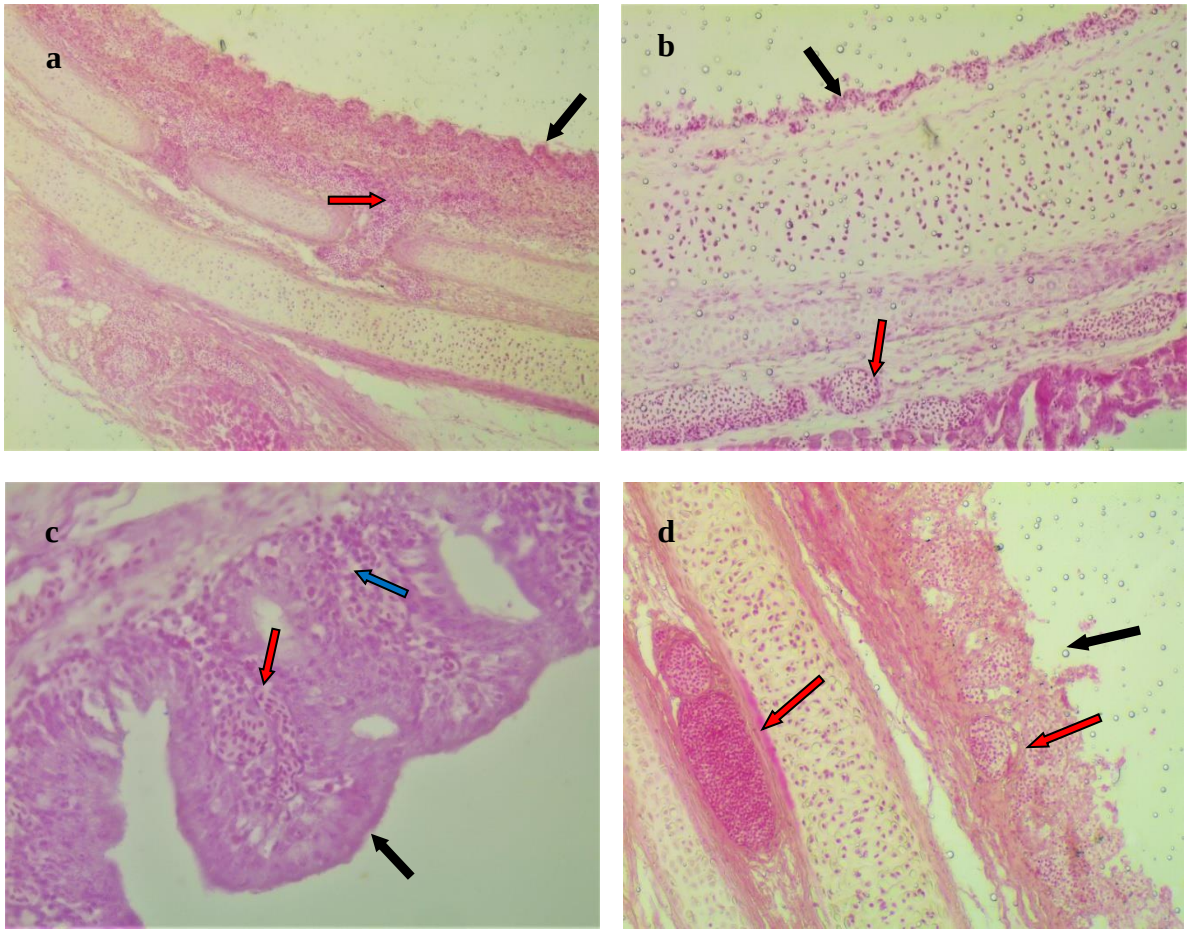

Figure 5: Congestion, hemorrhage and infiltration of inflammatory cells in tracheal mucosa (red arrow), desquamation of epithelial lining (black arrow) (a, b, d); loss of cilia in epithelial lining (black arrow), infiltration of inflammatory cells (blue arrow), congestion, hemorrhage (red arrow) (c).

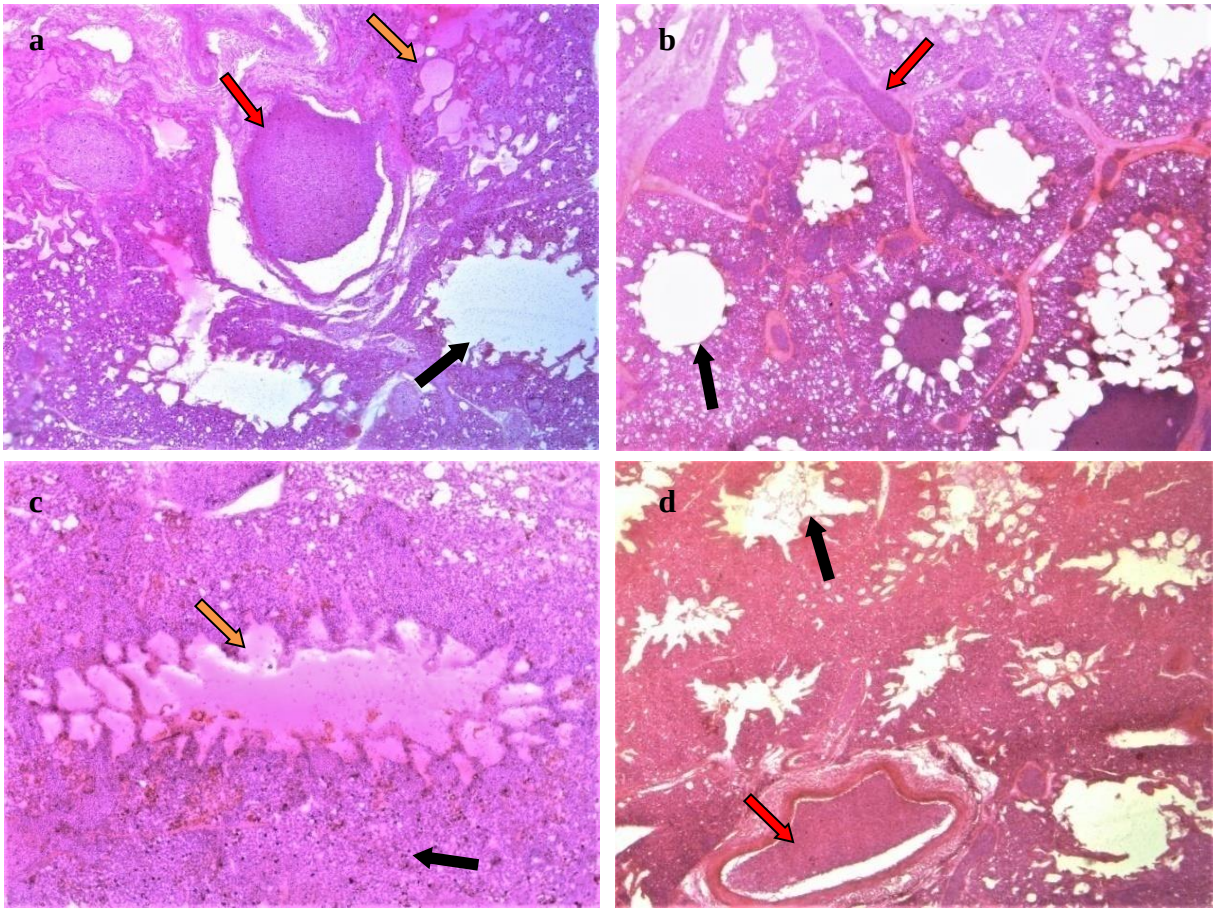

Figure 6: Congestion (red arrow), edema (orange arrow), emphysema (black arrow) in lung section (a); congestion (red arrow), emphysema (black arrow) (b, d); edema (orange arrow), dust particle in interstitial tissue (black arrow) (c).

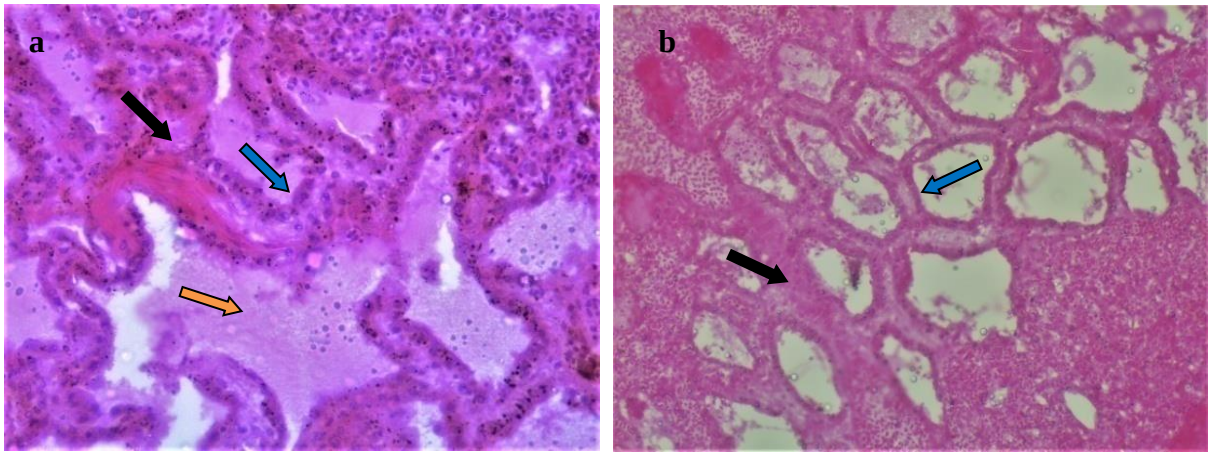

Figure 7: Alveolar lining completely made of cuboidal cells indicating metaplasia (blue arrow) (a, b); edematous fluid in alveolar lumen (orange arrow) (a); thickened alveolar septal wall (black arrow) (a, b).

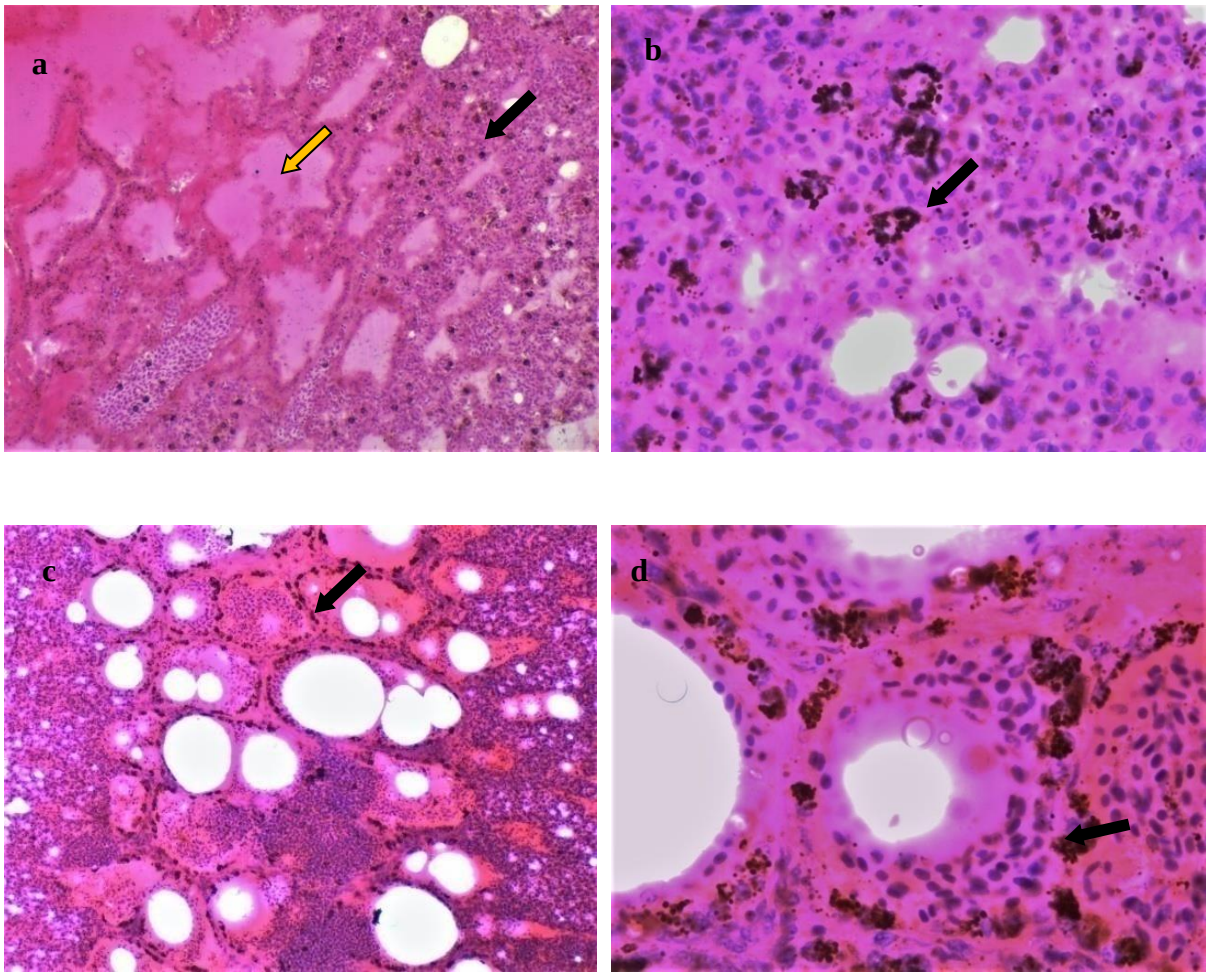

Figure 8: Lung tissue containing dust particles as well as inflammatory cellular infiltration, dust particle in interstitial tissue (black arrow) (a, b); edema (orange arrow) (a); dust particles surrounding the alveolar lumen (black arrow) (c, d).

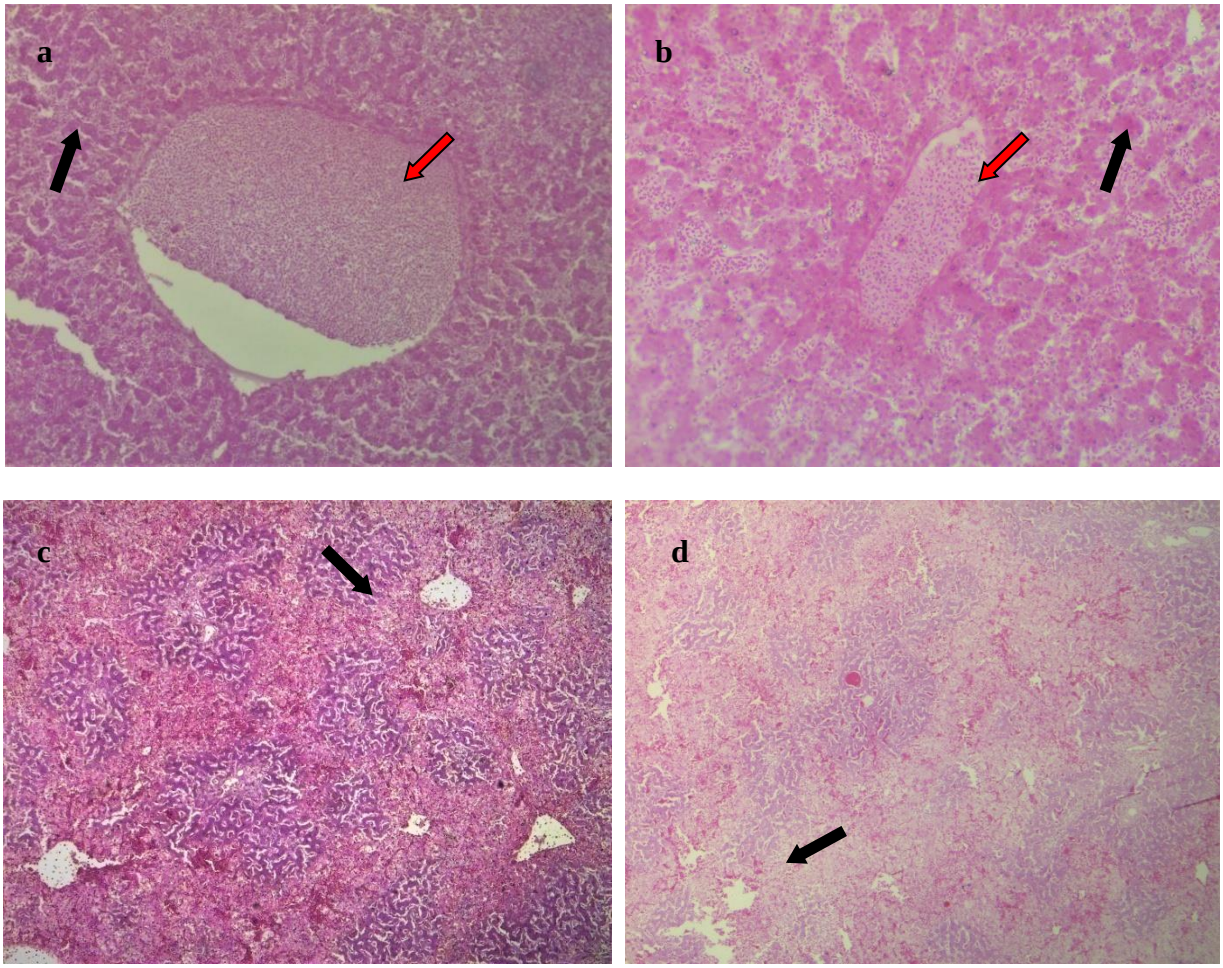

Figure 9: Congested central vein in liver section (red arrow), coagulation necrosis of hepatocyte (black arrow) (a, b); loss of hepatocytes near central veins (black arrow) (c, d).

Table 4.1- Microscopic lesions among samples

Tissue sample (No. of samples)	Lesions	Number of observations
Trachea (73)	Congestion	73 (100%)
	Hemorrhage	43 (58.9%)
	Inflammatory cellular infiltration	20 (27.4%)
	Desquamation of lining epithelia	40 (54.8%)
	Loss of cilia in epithelial lining	23 (31.5%)
Lung (76)	Congestion	76 (100%)
	Inflammatory cellular infiltration	71 (93.4%)
	Edema	67 (88.2%)
	Dust accumulation	34 (44.7%)
	Metaplasia	31 (40.8%)
	Emphysema	22 (28.9%)
Liver (19)	Congestion	14 (73.7%)
	Hepatic necrosis	10 (52.6%)
	Loss of hepatocytes	3 (15.8%)

Table 4.2- Development of ascites in broiler chickens

Number of farms sampled	Number of farms with ascitic birds	Number of birds sampled	Number of birds with ascites
21	8 (38%)	83	23 (27.7%)

Chapter 5: Discussion

The focus of the study was to determine what kind of pathological alterations take place in birds exposed to dust during the dry season when environments are dustier. Following investigation, this study identified several gross and microscopic lesions that would help in diagnosing disease conditions caused by dust exposure and would play an important role in managing those condition.

Postmortem examinations of dead birds with the history of dust exposure from dry litter materials have shown some pathological changes in broiler chickens. Gross pathological changes include congested trachea with or without mucus in the tracheal lumen due to irritation from dust particles. Lungs were found severely congested and edematous. Neumann et al. (1975), Huchzermeyer (1984), Lopez-Coello et al. (1985), Julian (1993), and Gupta (2011) also mentioned in their studies about extremely congested and edematous lungs in birds with ascites problems. Their findings resemble the findings of this study.

The liver was found dark, congested, enlarged, and in some cases smaller than normal size. Julian (1993), and Gupta (2011) also mentioned similar changes which are swollen and congested liver that sometimes had a shrunken appearance, in birds with ascites; which are supportive of the findings of this study.

Development of ascites was also found, where abdominal cavities were filled by clear, yellow-colored fluid sometimes which was clotted. This finding matches with the findings mentioned by Julian (1993) and Gupta (2011) in ascitic birds. Pulmonary congestion along with reduced oxygen intake leads to the development of ascites. Similar findings were found in other studies (Broom, 1988; Jensen et al., 2000), according to which ascites develops as pulmonary pressure rises, leading birds to feel severe pain and discomfort. Julian (1993) described that the accumulation of fluids in the peritoneal cavities happens due to increased intravascular pressure in the hepatic portal system which arises from right ventricular valvular insufficiency caused by right ventricular hypertrophy in response to pulmonary hypertension. A similar process was also found in this study where pulmonary hypertension eventually caused portal hypertension in liver which in turn led to the development of ascites.

Although the gross pathological changes match with these studies (Neumann et al., 1975; Huchzermeyer, 1984; Lopez-Coello et al., 1985; Julian, 1993; Gupta, 2011), they focused mainly on high altitude, higher feed conversion ratio, high energy contents of poultry diet, fast growth rate and higher metabolic rate of broiler chicken to cause a hypoxic condition which in turns leads to ascites, whereas the ascitic condition found in this study developed due to severe pulmonary congestion caused by the bird's exposure to dust in poultry houses that used dry sawdust as litter materials.

Histopathological changes observed under the microscope revealed lesions in trachea, lung and liver tissues. Histopathological sections of trachea showed hemorrhage, congestion, infiltration of inflammatory cells, loss of cilia in lining epithelial cells, and desquamation of lining epithelia; all of these changes are indicative of tracheitis. These findings are consistent with the findings of some studies (Anderson et al., 1964, 1966, 1968; Oyetunde et al., 1978; Al-Mashhadani and Beck, 1985), where it was found that birds exposed to ammonia and poultry dust regularly experienced respiratory mucosa inflammation, deciliation, and loss of structure.

There is marked congestion, infiltration of inflammatory cells, diffuse edema, and thickening of the alveolar septal wall in lung tissue. Julian (1993) and Gupta (2011) mentioned in their studies about congestion, edema, thickening of the alveolar septal wall in lung tissue which match the findings of this study.

Dust particles were found surrounding the alveolar lining and also in interstitial tissue of the lungs, most of which were within macrophages, as well as discrete tiny dust particle. History of dusty house environment with dry sawdust litter along with microscopic observation of tiny granular texture and dark blackish color were considered during identifying these granules as dust particles. Smith et al. (1973), Brambilla et al. (1979) and Roperto et al. (2000) reported the presence of dust within macrophages in the lungs of different avian species exposed to various types of dust which are similar to the findings observed in this study.

In some cases, alveolar lining epithelia had fully transformed from squamous cells (Type 1 pneumocytes) to cuboidal cells (Type 2 pneumocytes), which indicate metaplasia. Emphysema was also observed in some congested lung tissues. These changes (metaplasia and emphysema) were not mentioned that much in other studies

(Anderson et al., 1964, 1966, 1968; Smith et al., 1973; Oyetunde et al., 1978; Brambilla et al., 1979; Al-Mashhadani and Beck, 1985; Julian, 1993; Roperto et al., 2000; Gupta, 2011).

Dust in alveolar lining epithelia makes it difficult to exchange oxygen between alveolar air and capillary blood. Therefore to meet oxygen demand more blood flows through pulmonary capillaries leading to the development of pulmonary congestion. Pulmonary congestion is responsible for secondary congestion in other organs, especially in the liver. In liver sections congestion was prominent. Liver lesions included blood stasis and dilatation of central veins and near the hepatic sinusoids. Hepatocytes around the central vein had undergone coagulation necrosis, followed by loss of hepatocytes which might lead to fibrous atrophy of the liver making the liver smaller than normal. Julian (1993) and Gupta (2011) also mentioned in their studies about congestion in central veins and hepatic sinusoids along with necrosis of hepatocytes in liver tissue of broilers with ascites which support the findings of this study. These histopathological findings correlate with gross pathological findings where congested, enlarged liver was observed in birds with congested lungs.

Although Anderson et al. (1966, 1968) found little histological changes in their test birds while conducting an experimental study on the effects of poultry house dust on the respiratory tracts of chicken and turkey, the histological changes found in the respiratory system of birds predisposed to dust were much prominent in this study. This difference may be due to the use of softwood shavings as bedding materials in their studies (Anderson et al., 1966, 1968), whereas in this study tissue samples were collected from birds reared on dry sawdust beddings. Therefore, this could be taken into consideration that compared to wood-shaving litter materials, sawdust litter materials have a greater impact on the respiratory system of chickens.

The season is another factor to take into account in this investigation. This study was done in the dry season when the environment temperature and humidity are lower, which makes litter materials become dry more frequently leading to an increase in the dust level of the poultry house. Takai et al. (1998) also found that dust emission rates in poultry houses were higher in the winter season. Dust makes ascites more likely to happen. As dust level increases in poultry houses during the dry season, these dust particles get inhaled by the birds and irritate bird’s respiratory mucosa. Dust then

further causes pulmonary hypertension that eventually leads to the development of ascites as a result of hepatic portal hypertension, which have been found in this study.

Issac et al. (2010) also supported that dusty environments can contribute to the development of ascites in poultry by reducing the transfer of oxygen between the bird and the environment. According to McGovern et al. (1998), dust particles can spread pathogenic bacteria, irritate or infect the lungs, and decrease oxygen transfer between the birds and their environment, which is also relatable to the role of dust in causing ascites in birds. Islam et al. (2009, 2012) reported that among non infectious diseases of broiler chicken, the occurrence of ascites is higher during the winter season in Bangladesh. The current study found that exposure to poultry house dust in the dry season has a great impact on poultry respiratory system that may lead to the development of ascites in broiler chickens which are consistent with the findings of above mentioned studies.

The findings of this study will play an important role in diagnosing disease conditions caused by dust exposure and will invoke the urgency to maintain proper litter management to reduce dusty litter and treat the affected birds accordingly.

Chapter 6: Conclusion

The current study investigated the pathological changes developed in broiler chickens predisposed to dust during dry periods. This study suggests that pathological changes do happen when birds are exposed to dust. Litter materials in poultry housing become dry more frequently in the dry season resulting in the formation of dust particles that present in the air because of frequent movement of litter. Specks of dust inhaled by bird cause respiratory tissue alterations because of the presence of dust in their respiratory system, the liver also become affected. Ascites may develop along with these changes. These conditions put the bird in distress and may cause mortality, which may put the poultry farmers under great challenge.

Chapter 7: Recommendations and Future Perspectives

This study found that using dry sawdust litter during the dry season may lead to pathological changes in the respiratory system and liver as well as the development of ascites in broiler chicken. To minimize this problem poultry house management should be proper to reduce dusty environment. There are some limitations in this study. Samples were taken only from dead birds brought to the department of Pathology and Parasitology for postmortem examination. Visiting those farms could give more information about housing condition, as well as taking blood samples for hematological tests could give information about blood profiles in birds with ascites. For future study, these options could be taken under consideration. Furthermore, pathological changes could also be compared among broiler chickens exposed to different types of litter dust.

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Appendix

Questionnaire for rearing system of chickens

1. Serial no. : ……... Date: ……..
2. Name of the Owner/ Farm …….……..
3. Location of the farm ……...…….
4. Mobile No ……
5. Species ……..… Age ……..
6. Flock size …….
7. Feeding system …… Watering system.....
8. Litter materials……... Litter condition……
9. Onset of clinical signs (Date)…….. Symptom ……...
10. No. affected …… No. of death ……Time of Death ……..
11. Vaccination ……Treatment (given any) …….……
12. Necropsy findings …….....
13. Tentative diagnosis …….....
14. Collected samples (organs) …….
15. Tag No …….

Compositions of fixative used to preserve tissue sample

Bouin's solution (fixative)	
Composition	**Amount**
1. Picric acid, saturated aqueous solution	750 ml.
2. 37-40% formalin	250ml.
3. Glacial acetic acid	50ml.

Reagents and solutions used in staining of tissue sections

Harris Hematoxylin	**1% stock alcoholic eosin**
Hematoxylin crystals……... 5 gm. Alcohol 100% .....,..... 50 ml. Ammonium or potassium alum….. 100 gm. Distilled water……...……. 1000 ml. Mercuric oxide……..... 2.5 gm. After preparation of the stain 2-4 ml glacial acetic acid per 100 ml of solution was added. Stain was filtered before use.	Eosin Y ……...…. 1 gm. Distilled water ……...… 20 ml. Dissolve and add; Alcohol 95% ……..…. 80 ml. For working solution 1 part of Eosin stock solution was mixed with 3 parts of 80% alcohol. Just before use 0.5 ml of glacial acetic acid per 100ml of stain solution was added.
Acid alcohol	**Ammonia water**
Alcohol 70%……..1000 ml. Hydrochloric acid, concentrated ……10 ml.	Distilled water…….1000 ml. Ammonium hydroxide, 28%.....2-3 ml.

Hematoxylin and Eosin staining procedure

i. Deparaffinization:
    a. Xylene ……...2 changes, 5-10 minutes each.
ii. Rehydration through graded alcohol:
    a. Alcohol 100%.....2 changes, 5 minutes each.
    b. Alcohol 95%.....2 minutes.
    c. Tap water ……..……5 minutes.
iii. Harris hematoxylin……..…..10-15 minutes.
iv. Rinse in tap water…….…….10 minutes.
v. Differentiate in acid alcohol……..…3-10 quick dips.
vi. Wash in tap water…….….5 minutes.
vii. Ammonia water (for bluing)…….3-5 dips.
viii. Wash in tap water…….10 minutes.
ix. Eosin…….15 seconds to 2 minutes.
x. Alcohol 95% ……2 changes, 2 minutes each.
xi. Alcohol 100% ……..2 changes, 3 minutes each.
xii. Xylene ……..2 changes, 2 minutes each.
xiii. Cover slip was placed on stained tissue after putting DPX.

The slides were then dried at room temperature and examined under microscope.

Biography

Subrata Paul, son of Pradip Kumar Paul and Shilpi Rani Paul was born in Feni, Bangladesh. He completed his Secondary School Certificate (SSC) examination from Victory Adarsha High School in 2012 and Higher Secondary Certificate (HSC) examination from Govt. Hazi Muhammad Mohsin College in 2014. He obtained his Doctor of Veterinary Medicine Degree in 2020 from Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. Currently, he is a candidate for the degree of Master of Science in Veterinary Pathology, under the Department of Pathology and Parasitology, Faculty of Veterinary Medicine, CVASU.