

**PATHOLOGICAL INVESTIGATION OF AVIAN
ASPERGILLOSIS IN COMMERCIAL BROILER CHICKEN
AT CHITTAGONG DISTRICT**

**A THESIS
BY**

SHIRIN SULTANA

REGISTRATION NO. 1205098

SEMESTER: JULY-DECEMBER, 2013

SESSION: 2012-2013

**MASTER OF SCIENCE (MS)
IN
PATHOLOGY**



**DEPARTMENT OF PATHOLOGY AND PARASITOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY
UNIVERSITY, DINAJPUR-5200**

DECEMBER, 2013

745
28.1.15



**PATHOLOGICAL INVESTIGATION OF AVIAN
ASPERGILLOSIS IN COMMERCIAL BROILER CHICKEN
AT CHITTAGONG DISTRICT**

A THESIS

BY

SHIRIN SULTANA

REGISTRATION NO. 1205098

SEMESTER: JULY-DECEMBER, 2013

SESSION: 2012-2013

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

MASTER OF SCIENCE (MS)

IN

PATHOLOGY

**DEPARTMENT OF PATHOLOGY AND PARASITOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY
UNIVERSITY, DINAJPUR-5200**

DECEMBER, 2013

**PATHOLOGICAL INVESTIGATION OF AVIAN
ASPERGILLOSIS IN COMMERCIAL BROILER CHICKEN
AT CHITTAGONG DISTRICT**

**A THESIS
BY**

**SHIRIN SULTANA
REGISTRATION NO. 1205098
SEMESTER: JULY-DECEMBER, 2013
SESSION: 2012-2013**

Approved as to style and content by



Prof. Dr. S.M. Harun-ur-Rashid
Supervisor



Dr. Md. Nazrul Islam
Co-Supervisor



Dr. Md. Nazrul Islam
Chairman
Examination committee

**DEPARTMENT OF PATHOLOGY AND PARASITOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY
UNIVERSITY, DINAJPUR-5200**

DECEMBER, 2013



Dedicated
To My
Beloved Parents

ACKNOWLEDGEMENTS

The author is ever grateful and indebted to the Almighty Allah, without whose grace she would have ever been able to pursue her higher studies in this field of science and to complete her research work successfully for the degree of Master of Science (MS) in Pathology.

The author sincerely desires to express her heart felt respect, deepest sense of gratitude, sincere appreciation and profound indebtedness to her respected teacher and Research Supervisor, Prof. Dr. S. M. Harun-ur-Rashid, Department of Pathology and Parasitology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur, for his constant supervision, scholastic guidance, uncompromising principles, valuable advice, constant inspiration, affectionate feeling, radical investigation and constructive criticism in all phases of this study and preparing the manuscript.

The author finds it a great pleasure in expressing her heartfelt gratitude and immense indebtedness to her honorable and respected Research Co-Supervisor Dr. Md. Nazrul Islam, Associate Professor, Department of Pathology and Parasitology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur for his sympathetic supervision, kind cooperation, inspiration and valuable suggestion and valuable advice for the completion of the research work and preparation of the thesis.

The author wishes to express her cordial respect and profound gratitude to her teachers Dr. Md. Haydar Ali, Assistant Professor and Chairman, Dr. Md. Mominul Islam, Assistant Professor, and Dr. Md. Golam Azam, Lecturer, Department of Pathology and Parasitology, Faculty of Veterinary and Animal Science, HSTU, for their valuable advice and constant inspiration throughout the study.

The author deeply grateful to her friends, staffs of the department who have cooperated during the collection of her sample, taking autograph of her practical preparation and also provide some technical support along with her honorable Supervisor, Co-supervisor to complete her research work,

The Author

December, 2013

ABSTRACT

The present study was carried out to investigate the pathology of avian aspergillosis in commercial broiler chickens at Chittagong district. A total of 912 sick and dead chickens were collected from 20 commercial broiler farms and diagnosed for avian aspergillosis on the basis of clinical signs, symptoms and postmortem findings. The suspected birds were collected for necropsy examination and mycological culture. Gross lesions of multiple hard creamy to yellow colored, circumscribe plaques throughout the lungs surface and consolidated lung with necrotic areas were observed. Microscopically, the typical nodules consisted of caseous necrotic center were present. Identified the *Aspergillus spp* according to their color of colony growth on Potato Dextrose Agar media. The overall incidence of avian aspergillosis was found 6.14%. Among five upazilla, significantly ($p<0.007$) higher and lower incidence was found in Patenga and Sitakunda that were 9.25% and 3.43% respectively. It was observed that highest incidence (8.22%) in rainy and lowest (3.16%) in winter but moderate (5.16%) in summer season. The disease was significantly ($p<0.050$) higher (8.27%) in age between 6-10 days and lower (4.11%) in age between 0-5 days. It was also found that incidence of avian aspergillosis was significantly ($p<0.042$) higher in flocks reared on sawdust litter (7.69%) as compared to rice husk litter (3.46%).

CONTENTS

CHAPTER	SUBJECT	PAGE NO.
	ACKNOWLEDGEMENTS	iv
	ABSTRACT	v
	LIST OF CONTENTS	vi-viii
	LIST OF TABLES	ix
	LIST OF FIGURES	x
	LIST OF ABBREVIATIONS AND SYMBOLS	xi
CHAPTER 1	INTRODUCTION	1-2
CHAPTER 2	REVIEW OF LITERATURES	3-27
2.1	History	3
2.2	Epidemiology	4
2.2.1	Geographical distribution	4
2.2.2	Susceptible Host	4
2.2.3	Susceptible age	5
2.2.4	Seasonal occurrence	5
2.2.5	Incidence	6
2.2.6	Morbidity and mortality	6
2.2.7	Predisposing factors	7
2.3	Transmissions	8
2.3.1	Transmission from poultry house	8
2.3.2	Transmission through air	9
2.3.3	Transmission through poultry beddings	10
2.3.4	Transmission through poultry feedstuffs	10
2.3.5	Transmission through hatchery	11
2.4	Etiology	11
2.4.1	Taxonomy	12
2.4.2	Classification of <i>Aspergillus</i> species	12

CONTENTS (Contd.)

CHAPTER	SUBJECT	PAGE NO.
	2.4.3 Morphology of <i>Aspergillus</i> species	13
	2.4.4 Cultural properties	14
	2.4.5 Toxins of <i>Aspergillus</i>	15
	2.5 Clinical signs and lesions	16
	2.6 Pathogenesis	17
	2.7. Pathology	19
	2.7.1 Gross Lesions	19
	2.7.2 Organs affected	19
	2.7.3 Histopathological changes	21
	2.7.4 Generalized pathological changes	22
	2.8 Diagnosis	24
	2.8.1 Differential diagnosis	26
	2.9 Prevention	27
CHAPTER 3	MATERIALS AND METHODS	28-35
	3.1 Study area and study period	28
	3.2 Clinical examination of affected birds	28
	3.3 Collection of birds	28
	3.4 Necropsy examination of suspected birds	29
	3.5 Collection of samples	29
	3.6 Experimental layout	30
	3.7 Methods	31
	3.7.1 Histopathological study	31
	3.7.1.1 Materials required for histopathology	31
	3.7.1.2 Processing of tissues for histopathology	31
	3.7.1.3 Routine hematoxylin and eosin staining procedure	33

CONTENTS (Contd.)

CHAPTER	SUBJECT	PAGE NO.
	3.7.1.4 Preparation of Ehrlich's hematoxylin solution	33
	3.7.1.5 Preparation of eosin solution	33
	3.7.1.6 Staining protocol	34
	3.8 Mycological culture of samples	35
	3.8.1 Preparation of potato dextrose agar (PDA) media for cultivation of fungus	35
	3.9 Photography	35
	3.10 Statistical analysis	35
CHAPTER 4	RESULTS	36-45
	4.1 Incidence study	36
	4.1.1 Upazilla wise incidence study	36
	4.1.2 Season wise incidence	38
	4.1.3 Age wise incidence	38
	4.1.4 Litter wise incidence	39
	4.2 Clinical signs	41
	4.3 Pathological study of Avian Aspergillosis	41
	4.3.1 Gross pathology	41
	4.3.2 Histopathological examination	42
	4.4 Mycological examination	45
CHAPTER 5	DISCUSSION	46-48
CHAPTER 6	CONCLUSIONS AND RECOMMENDATIONS	49
	REFERENCES	50-71

LIST OF TABLES

TABLES	TITLE OF THE TABLES	PAGE NO.
Table 1	Upazilla wise Incidence of avian aspergillosis in commercial broiler chicken	37
Table 2	Seasonal incidence of avian aspergillosis in commercial broiler	38
Table 3	Incidence of avian aspergillosis in commercial broiler on the basis of age	39
Table 4	Incidence of avian aspergillosis in commercial broiler on the basis of litter used	40

LIST OF FIGURES

FIGURES	TITLE OF THE FIGURES	PAGE NO.
Figure 1	Schematic illustration of the experimental layout	30
Figure 2a	Rice husk litter status of experimental flock	40
Figure 2b	Saw dust litter status of experimental flock	40
Figure 3	a. Bird showing gasping and b. Birds showing depression	41
Figure 4	a. Lung shows the presence of creamy color nodules in plural surface, air sacs (arrow). b. Creamy to yellow color nodules shows throughout the lung (arrow).	42
Figure 5	Showing congestion of pulmonary, perialveolar blood vessel and diffuse edema of pulmonary tissues (arrow) in lung. H&E stain, 10×	43
Figure 6	Areas of caseous necrosis (black arrow) and cellular debris (red arrow) in lung. H&E stain, 10×	43
Figure 7	Aspergillosis showing granuloma formation with caseated center (arrow) in lung. H&E stain, 10×	44
Figure 8	Diffuse densification of the pleural parenchyma by congestion and an inflammatory cellular infiltration (arrows) in lung. H&E stain, 10×	44
Figure 9	a. PDA media shows whitish color colony of <i>A. fumigatus</i> (arrows). b. PDA media shows parrot green color colony of <i>A. flavus</i> (black color arrow) and dark black color colony of <i>A. niger</i> (red color arrow)	45

LIST OF ABBREVIATION AND SYMBOLS

μ	= Micro
μl	= Micro liter
μm	= Micro meter
μg	= Micro gram
$^{\circ}\text{C}$	= Degree centigrade
%	= Percent
$\pm$	= Plus-minus
$\times$	= Multiplication
$<$	= Less than
$>$	= Greater than
@	= At the rate of
®	= Registered
AIDS	= Acquired immuno deficiency syndrome
cfu	= Colony forming unit
cm	= Centimeter
DPX	= Distrene, Plasticiser, Xylene
ELISA	= Enzyme Linked Immunosorbent Assay
Est.	= Establish
etc	= Etcetera
<i>et al.</i>	= and his associates
g	= gram
H & E	= Hematoxylin and Eosin stain
HSTU	= Hajee Mohammad Danesh Science and Technology University
ml	= Milliliter
mm	= Millimeter
nm	= Nanometer
PDA	= Potato dextrose agar
PCR	= Polymerase chain reaction
SDA	= Sabouraud dextrose agar
SE	= Standard error



CHAPTER 1

INTRODUCTION

CHAPTER 1

INTRODUCTION

Poultry farming is emerging as a strong agro-based industry from the backyard poultry rearing system to commercial intensive rearing systems during the last two decades in Bangladesh. This rapid growth of poultry industry to supplement their income with the fast development of poultry industry, the occurrence of diseases has increased many folds which remain the major problem affecting its economy as a results disease play a vital role to better understand the status and pattern of diseases (Islam *et al.*, 2003; Saleque *et al.*, 2003 and Uddin *et al.*, 2011).

In native type of poultry husbandry, the control of infectious diseases in poultry is difficult due to lack of proper biosecurity measures, intense farming and greater germ load in the farm premises. Among the infectious diseases fungal diseases have their own importance and seem to one of the great obstacle for the poultry farmers in the form of high incidence, morbidity, mortality and production losses.

Aspergillosis is the most common fungal disease of the avian respiratory systems, it is an infectious, non-contagious fungal disease caused by species in the ubiquitous opportunistic saprophytic genus *Aspergillus*, in particular *Aspergillus fumigatus* (Richard, 1991; Barnes and Denning, 1993). This mycosis was described many years ago, but continues to be a major cause of mortality in captive birds and, less frequently, in free-living birds. Although aspergillosis is predominantly a disease of the respiratory tract, all organs can be involved. It is believed that impaired immunity and the inhalation of a considerable amount of spores are important causative factors (Beernaert *et al.*, 2010).

The warm, humid environment of the farm sheds, feed stores, floor etc., favor its growth. It is a contaminant of virtually every environment because of its adaptability to growth substrates and the production of spores (conidia) that remain viable under extremely harsh conditions (Bardana, 1980). Inhalation of air

borne conidia is the principal mode of exposure (Richard and Thurston, 1983). The conidia are spheroidal and 2-3 μ m in diameter, once inhaled get deposited deep in the respiratory tract (Campbell, 1970). The constant exposure occasionally results in clinically apparent infection. Aspergillosis in young chicks and pullets is commonly associated with overwhelming exposure to large numbers of conidia from heavily contaminated feed, litter, or the hatchery environment (Dyar *et al.* 1984).

The disease can occur as an acute form with high mortality and morbidity especially in brooding age called brooder pneumonia (Badhy *et al.*, 2003), but also has the tendency of chronic form in older birds. Clinical signs such as dyspnea, gasping, cyanosis of un-feathered skin and hyperemia are usually associated with the disease. However, affected birds normally do not produce respiratory noise associated with other respiratory problems. Lesions in birds are commonly confined to lungs and air sacs, although oral mucosa, trachea, eyes may be affected. Typical lesions are fungal nodules or plaques within the lungs and on the air sacs. Occasionally, the syrinx may be also affected (Richard, 1991).

In recent past few years, aspergillosis has emerged as a significant poultry health concern for the poultry producers and humans health officials. The disease in human is common in immuno-compromised patients as a result of acquired immuno deficiency syndrome (AIDS), neoplasia and chemotherapy (Barnes and Denning, 1993). Therefore, the current study, was planned and executed to explore the incidence, gross pathology, histopathology and microbiological culture of avian aspergillosis in commercial broiler flocks in native type of poultry farming.

The present research was under taken with the following aims and objectives:

1. To investigate the incidence of avian aspergillosis in Chittagong district
2. To study the gross and histopathological changes in respiratory system caused by avian aspergillosis
3. Mycological culture for the determination of pathogens in affected birds



CHAPTER 2

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURES

2.1 History

Aspergillus has always played an important role in life and health of humans, mammals and mainly wild or domestic birds (Atkinson and Brojer, 1998; Friend and Franson, 1999; Forbes and Rapaces, 1999; Ramírez *et al.*, 2002). It was Micheli, who in 1729 for the first time made a microscopic study of these molds and was able to distinguish stalks (conidiophores) and spore heads. He observed that the spore chains or columns radiated from a central structure to suggest a pattern like that of *Aspergillum* (holy water sprinkler), with which he, as a priest, was familiar. Hence he gave the name *Aspergillus* to these molds. The generic description and some of the illustrations given by Micheli correctly represent the genus as has been long accepted. The first correct interpretation of structure of conidial head of *Aspergillus* appeared in the work of Corda (1997), who described and illustrated his studies from fresh materials. De Bary (1854), described and illustrated the ascigerous stage (cleistothecia), *Eurotium herbariorum* in *Aspergillus* (*A. glaucus*). In the last 273 years since the work of Micheli, considerable literature concerning *Aspergillus* has accumulated. The early literature was based on microscopic study of specimens collected from natural sources. After De Barry (1854) group began to investigate moulds in comparative culture in the early 1880's, the study of aspergilli received a great impetus. Eidam (1883) described the ascigerous stage of *A. nidulans*. Wehmer in Germany studied the biochemical aspects of aspergilli (Wehmer, 1991) and this culminated in the publication of a monograph on the genus *Aspergillus* in 1901 (Wehmer, 1992). Thom and Raper (1954) did an extensive re-examination of the genus *Aspergillus*, which led to the publication of a monograph *A Manual of Aspergilli* in 1945. Eighty species and ten varieties, assembled in 14 groups, were accepted as valid.

Aspergillus fumigatus is considered as a major respiratory pathogen in birds. This filamentous fungus was first found in the lungs of a bustard (*Otis tarda*) in 1863

by Fresenius (Richerd, 1997; Friend and Franson, 1999; Forbes *et al.*, 1999 and Schmidt, 2002).

2.2 Epidemiology

2.2.1 Geographical distribution

Aspergillus spp are found worldwide and widely distributed in the environment, having been reported in almost every farmed bird as well as in wild species (Kunkle, 2003; Ryan, 2004). *Aspergillus flavus* and *Aspergillus parasiticus* are frequently found in tropical and sub-tropical climates in Sub-Saharan Africa, Eastern Asia and parts of South America (Kew, 2013). In Brazil, some studies have focused on the diagnosis of aspergillosis in newly hatched birds (Tessari *et al.*, 2004; Spanamberg *et al.*, 2013).

2.2.2 Susceptible Host

Infection by *Aspergillus* sp. has been reported in almost all domesticated avian species and production types: broiler cockerels (Steinlage *et al.*, 2003), pullets in cages (Corkish, 1982), broiler breeders (Martin, 2007) and growers of chicken (Akan *et al.*, 2002 and Zafra *et al.*, 2008) or turkey poults (Cortes *et al.*, 2005; Singh *et al.*, 2009 and Olias *et al.*, 2010), common duck breeders (Planel *et al.*, 2001), goslings (Beytut *et al.*, 2004), great rheas (Copetti *et al.*, 2004), ostriches (Perelman and Kuttin, 1992), Japanese quails (Olson, 1969), or pigeons (Tokarzewski *et al.*, 2007). In spontaneous outbreaks, mortality ranged between 4.5% and 90%, whereas age of diseased birds varied from 3 days to 20 weeks (Islam *et al.*, 2003; Cortes *et al.*, 2005; Martin, 2007; Zafra *et al.*, 2008; Singh *et al.*, 2009 and Olias *et al.*, 2010). Beside direct losses related to mortality, feed conversion and growth rate in recovering birds remain poor. Indeed, air sacculitis is a major reason for carcass condemnation at slaughter inspection (Kunkle, 2003; Moretti *et al.*, 2010 and Lupo *et al.*, 2010). Economical significance of aspergillosis is most readily apparent in turkey production where disease occurs late in the growing cycle or primarily affects costly breeder toms (Kunkle, 2003).

Infection by *A. fumigatus* is also found in birds of prey, penguins, and parrots held in captivity (Redig, 2005 and Alvarez-Perez *et al.*, 2010). Incidence may be elevated in debilitated birds sheltered in wildlife centres and severely impair rehabilitation success (Balseiro *et al.*, 2005 and Xavier *et al.*, 2007).

2.2.3 Susceptible age

Acute aspergillosis typically occurs in very young birds and may be associated with considerable losses. About 10% of all deaths in chicks under 14 days old are caused by this disease (Watkins., 1940) 3 to 8 week-old ostriches (Perelman *et al.*, 1992), Mortality in chickens caused by aspergillosis was recorded highest in growing (>3 to 8 weeks old) chickens (0.98%) in comparison to other age groups of chickens in Bangladesh (Uddin *et al.*, 2011). Age of diseased birds varied from 3 days to 20 weeks (Akan *et al.*, 2002; Kunkle, 2003; Islam *et al.*, 2003; Martin, 2007; Zafra *et al.*, 2008; Singh *et al.*, 2009 and Olias *et al.*, 2010). Aspergillosis caused by *A. flavus* in 5 wk-old broiler breeder replacement pullets that were characterized by increased mortality, decreased growth rate, and absence of clinical respiratory signs (Martin *et al.*, 2007).

2.2.4 Seasonal occurrence

Highest incidence of brooder pneumonia during hot and humid weather (Mahajan *et al.*, 1994 and Rahman and Samad, 2003). Seasonal mortality due to aspergillosis is highest during rainy (0.81%) in comparison to winter (0.49%) and summer (0.24%) seasons (Uddin *et al.*, 2011). Aspergillosis observed only in summer season and 1.6% birds are infected with this disease. In the summer season there is both strong sunlight and heavy rainfall causing an altering condition that provides an ideal situation for aspergillosis (Anonymous, 1997). Giasuddin *et al.*, (2002) and Talha *et al.*, (2001) who recorded highest episode was observed in summer (21.10%) followed by winter (18.30%) and rainy (10%) season.

2.2.5 Incidence

The regional incidence of avian aspergillosis have been described by Islam *et al.*, (2003) reported 17.53% in Sylhet, Uddin *et al.*, (2010) reported 7.98% in Narsingdi, Uddin *et al.*, (2011) reported 1.54%, Talha *et al.*, (2001) reported 4.20% in Mymensingh, Islam *et al.*, (1998) reported 4.03% in Mymensingh and Islam *et al.*, (2009) reported 1.60% in Gaibandha district of Bangladesh and Sajid *et al.*, (2006) reported 48.43% incidence in Pakistan. The seasonal incidence of avian aspergillosis have been described by Islam *et al.*, (2003) reported highest incidence of aspergillosis in rainy season (11.68%), than summer (5.33%) and winter (0.52%) season in Sylhet region of Bangladesh. The seasonal incidence also researched by Sajid *et al.*, (2006) reported highest incidence of avian aspergillosis in summer season (54.84%) than winter (25.80%), autumn (12.9%) and spring (6.45%) seasons in Pakistan. The incidence of avian aspergillosis according to age was analyzed by Islam *et al.*, (2003) and Uddin *et al.*, (2010) who reported highest incidence of aspergillosis in between 0-7 days of age in Sylhet and Narsingdi region of Bangladesh and in Pakistan, Bennet (1988) and Sajid *et al.*, (2006) who reported that increased susceptibility of young chickens to aspergillosis might be due to immaturity of phagocytes or to environmental factors.

2.2.6 Morbidity and mortality

Aspergillosis in poultry occurs as either severe outbreaks of high morbidity and mortality of young birds or sporadically in adults (Yokota *et al.*, 2004). Acute aspergillosis generally occurs in young birds resulting in high morbidity and mortality. The chronic form is sporadic. It causes lesser mortality and generally affects older birds, especially breeders in poultry, presenting a compromised immune system due to poor husbandry conditions (Kunkle, 2003 and Redig, 2005). Rahman and Samad (2003) was recorded aspergillosis was diagnosed in 59 chicks of 12 to 22 days of age and reported morbidity is 1.02-12.00%. The highest broiler proportionate mortality in Bangladesh is 77.97% due to aspergillosis was recorded by Rahman *et al.*, (2003). In spontaneous outbreaks, mortality ranged

between 4.5% and 90% (Islam *et al.*, 2003; Throne *et al.*, 2003; Zafra *et al.*, 2008 and Singh *et al.*, 2009). Mortality due to aspergillosis on various farms ranged from 1.54 to 4.20 in chickens (Talha *et al.*, 2001; Rahman and Samad, 2003; Uddin *et al.*, 2011 and Rashid *et al.*, 2013).

2.2.7 Predisposing factors

Aspergillosis is an opportunistic infection, causing the disease under immunocompromised situations of the host or when the bird is exposed to an overwhelming number of spores (Stone and Okoniewski, 2001; Jones *et al.*, 2000 and Forbes, 1991). Stress seems to be the major predisposing factor in the development of the disease and can be caused by shipping, heat, recently capture, or changes in management. Aspergillosis also occurs associated with prolonged disease or when immunosuppressive doses of corticosteroids are used (Atkinson and Brojer, 1998; Young *et al.*, 1998; Abrams *et al.*, 2001 and German *et al.*, 2002)

Factors impairing the bird's immunity can also predispose to mycosis. Examples of this include the administration of tetracyclines or long-term steroids (Verstappen and Dorrestein, 2005), vaccination (Barton *et al.*, 1992), metabolic bone disease (Vanderheyden, 1993), an inadequate diet (Bauck *et al.*, 1992 and Dorrestein, 1992) resulting in hypovitaminosis A (McMillan and Petrak, 1989 and De Herdt, 1996), overcrowding (McMillan and Petrak, 1989), shipping (Tsai *et al.*, 1992), quarantine or capture of wild birds (Abrams *et al.*, 2001), starvation, thermal discomfort, migration (Young *et al.*, 1998), inbreeding (Low *et al.*, 2005), circovirus infection and lymphoproliferative disorders (Kelly *et al.*, 2004), toxicosis (Young *et al.*, 1998; Carrasco *et al.*, 2001), traumatic injuries (Xavier *et al.*, 2007) and reproductive activity (Jones and Orosz, 2000).

Environmental factors play an important role in the development of the disease, increasing the number of spores to which the birds are exposed. Poor sanitation in nests as well as food or fecal accumulation promotes fungal growth. Poor ventilation, in conjunction with these factors, increases the possibility that

airborne spores may invade and infect a bird's respiratory system. Wood shavings used as bedding material have been frequently implicated in outbreaks of the disease (Roskopf and Woerpel, 1997).

2.3 Transmission

Aspergillosis is primarily an infection of the respiratory tract (Kunkle, 2003; Fedde, 1998). Birds placed in environments contaminated with aerosolized conidia may show significant pathology after only a short duration of exposure (Fedde, 1998 and Reese *et al.*, 2006). Inhalation of airborne conidia, through contaminated water is a significant transmission factors (Verweij *et al.*, 2007).

2.3.1 Transmission from poultry house

Initial contamination of poultry farms may occur through use of a moldy litter or introduction of one-day-old birds that's down has retained conidia in hatchery facilities. Further contamination may involve inappropriate bedding management (Perelman and Kuttin, 1992 and Zafra *et al.*, 2008), poor quality feedstuffs, or admission of outside air loaded in conidia (Martin *et al.*, 2007). Organic substrates like litter, feed, and even feathers (Santos *et al.*, 1996) can easily fulfil nutrient requirements of *A. fumigatus* (Kunkle, 2003). Humidity and temperature conditions encountered in poultry farms promote the rapid growth of hyphae and efficient asexual multiplication resulting in a copious production of easily airborne hydrophobic conidia, which are subsequently dispersed and inhaled by the birds (Pinello *et al.*, 1977 and Kunkle, 2003). Transfers of conidia between the putative bedding reservoir (Pinello *et al.*, 1977 and Dyar *et al.*, 1984) and indoor atmosphere are still poorly understood (Debey *et al.*, 1995). Constant animal movements under high stocking densities, litter refreshing (Bacon and Burdick, 1977 and Dyar *et al.*, 1984), or deficient ventilation (Debey *et al.*, 1995 and Pinello *et al.*, 1977) may contribute to generate a conidial aerosol. A short-time exposure to heavily contaminated wood shavings induced an experimental pulmonary aspergillosis in chickens (Julian and Goryo, 1990) and turkeys (Fate *et al.*, 1987). Birds inhale the air and contact litter with continual exposure to the

conidia. Numerous longitudinal surveys have been conducted in different production systems including broilers (Lugauskas *et al.*, 2004) and broilers in order to characterize the mycoflora of litter, feedstuffs, and air inside the buildings (Pinello *et al.*, 1977; Bacon and Burdick, 1977).

2.3.2 Transmission through air

Incidence and relative importance of *Aspergillus* species (*A. fumigatus*, *A. flavus*, *A. nidulans*, or *A. amstelodami*) can vary significantly (Debey *et al.*, 1995; Nichita *et al.*, 2010 and Lugauskas *et al.*, 2004). In fact, air contamination is characterized by cyclic variations as evidenced by weekly sampling (Sauter *et al.*, 1981 and Lair-Fullerenger *et al.*, 2006). These fluctuations may be related to season or husbandry management (Debey *et al.*, 1995 and Lair-Fullerenger *et al.*, 2006). In several healthy poultry flocks, air concentration of *Aspergillus* spp. measured in the winter was fifteen times higher than in summer (Debey *et al.*, 1995). Concentration of *Aspergillus* spp. which predominated in air and litter in a turkey farm decreased drastically when the windows were opened (Pinello *et al.*, 1977), whereas no significant quantitative differences were attributable to house ventilation design in other surveys (Debey *et al.*, 1995 and Gigli *et al.*, 2005). The negative correlation between relative humidity and the number of *Aspergillus* conidia in air may indicate that xerophilic *Aspergillus* conidia more readily discharge in dry conditions than in humid atmosphere. Interestingly, high counts of *A. fumigatus* conidia in air coincided with high levels of respirable dust particles suggesting a possible physical association or a similar response to indoor conditions (Debey *et al.*, 1995). Sawdust generated by both litter and feed, harboured numerous fungi as dormant propagules due to low moisture that could serve as an inoculum for fresh litter (Dennis and Gee, 1973; Hamet, 1990; Bacon and Burdick, 1977).

2.3.3 Transmission through poultry beddings

The biodiversity of litter mycoflora depends on material choice, litter aging, and handling techniques (Dennis and Gee, 1973). More than thirty different taxa were identified in wood chips of turkey facilities (Pinello *et al.*, 1977) and wood shavings in broiler houses with a predominance of *Aspergillus*, *Scopulariopsis*, and *Penicillium* (Pinello *et al.*, 1977; Bacon and Burdick, 1977; Thiso *et al.*, 1978). Using an immersion technique with strip baits, Bacon and Burdick (Bacon and Burdick, 1977) isolated 18 fungal species from poultry litter. The same fungal species were isolated from both litter and air (Pinello *et al.*, 1977). The mean total fungal counts in wood shavings or wood chips from broiler and broiler houses ranged between 102 and 108/g with many samples that exhibited variable counts for most of the species defining contrasted growth patterns (Dennis and Gee, 1973; Bacon and Burdick, 1977). Conidial populations in five broiler litters showed significant differences varying from 1.4×10^5 to 7.8×10^5 /g (Thiso *et al.*, 1978). The role of variations in pH and moisture content on fungal population densities remained controversial although localized damp and soiled areas under troughs or feeders might promote fungal development (Bacon and Burdick, 1977 and Thiso *et al.*, 1978). In two broiler houses, the replacement of rice-hull beddings by *A. fumigatus* contaminated sunflowers shell was associated with a severe aspergillosis. Removal of mouldy litter resulted in health improvement. The very high moisture of the fresh material seemed to be highly favourable for *A. fumigatus* growth and sporulation (Zafra *et al.*, 2008).

2.3.4 Transmission through poultry feedstuffs

A wider variety of fungal genera were isolated from litter than from feed. Up to twelve fungal genera, with dominant *Aspergillus*, *Fusarium*, *Mucor* and *Penicillium* were isolated in feed with total densities varying from 7×10^2 to 3.2×10^5 cfu/g. The species *A. fumigatus* yielded at a maximum of 2.3 cfu/g in turkey commercial feed (Lair-Fullerenger *et al.*, 2006).

2.3.5 Transmission through hatchery

A case of egg-borne aspergillosis was reported by Eggert and Barnhart (1953). They suggested that the fungus had penetrated through the eggshell during incubation and the recently hatched chicks were infected (Williams and Brake, 2000). Another case which was shown to be hatchery-borne was reported by Clark *et al.* (1954). From 21 ranches where 210,000 chicks were involved, there was mortality of 1-10%. The infection could not be traced to the hatching eggs but was readily found in the incubators, hatchers, incubator rooms, and intake ducts. Symptoms and lesions were noted in some day-old chicks, but generally classical lesions were observed in chicks 5 days of age.

O'Meara and Chute (1959) produced aspergillosis experimentally in hatching chicks. Chicks in the process of hatching and up to 2 days of age were easily infected with *A. fumigatus* spores by contaminating the forced draft incubator with wheat which had been seeded with *A. fumigatus*. Chicks older than 3 days of age were resistant to infection. Chute and O'Meara (1962) reported some unusual cases of avian mycosis. Two cases of fungous tracheitis were observed, and in each case the mycelial growth extended through the cartilage

2.4 Etiology

Aspergillosis is mostly caused by *Aspergillus fumigatus* but *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus glaucus*, *Aspergillus nidulans*, and other *Aspergillus* species or mixed infections can play a role in the disease (Barton *et al.*, 1992; Perelman and Kuttin, 1992 and Joseph, 2000). The reason why *A. fumigatus* is the predominant species of airborne fungal infections might be that the spores are much smaller than the spores of other *Aspergillus* species (Richard and Thurston, 1983).

2.4.1 Taxonomy

Kingdom: Fungi

Phylum: Ascomycota

Order: Eurotiales

Family: Trichocomaceae

Genus: *Aspergillus*

2.4.2 Classification of *Aspergillus* species

Currently species in the genus *Aspergillus* are taxonomically placed in well-characterized sub genera, viz. *Aspergillus*, *Fumigatus*, *Ornati*, *Clavati*, *Nidulantes* and *Circumdati*. Each of these sub genera is divided into several sections containing one or more species (Powell *et al.*, 1994). For instance, *A. fumigatus* belongs to the section *Fumigati* of sub genus *Fumigati*, while *A. clavatus*, *A. nidulans*, *A. versicolor* and *A. terreus* are placed in the sections *Clavati*, *Nidulantes*, *Versicolores*, *Usti* and *Terrei* respectively in the sub genus *Nidulantes*. *Aspergillus glaucus* and *A. restrictus* belong to the sections *Aspergillus* and *Restrictus* respectively, in the subgenus *Aspergillus*. Likewise, *A. flavus*, *A. niger*, *A. ochraceus*, and *A. candidus* belong to the respective sections *Flavi*, *Nigri*, *Circumdati* and *Candidi* in the sub genus *Circumdati*. A very large number of species in the genus and variations in the morphological characteristics of individual species have created the need for alternative approaches. Determination of isoenzyme patterns has facilitated classification of some species, which are difficult to identify by morphological criteria (Cruickshank and Pitt, 1990). Other approaches used to delineate species include the presence of extracellular polysachharides (Debeaupuis *et al.*, 1990), biotyping by the yeast killer system (Polonelli *et al.*, 1990), the ubiquinone system, especially Q-9 and Q-10 (Kuriahsi *et al.*, 1990), serological typing (Fuhrmann *et al.*, 1990). Application of combination of different types of biochemical, physiological and morphological characteristics and molecular approaches to taxonomy of *Aspergillus* has been discussed by several workers (Samson and Pitt, 2000). This approach has not yet found application for routine species identification of

Aspergillus. Practical identification is still essentially based on a detailed study of morphological and cultural characteristics of the isolates (Powell *et al.*, 1994 and Hoog *et al.*, 2000).

2.4.3 Morphology of *Aspergillus* species

Colony characters studied include pigmentation of the aerial mycelium or the underlying medium, rate of growth, appearance of the colony margin, whether heavy or sharply limited or thin and diffuse, smooth and entire or irregularly lobed or submerged and the texture of surface growth, whether velvety (consisting of submerged mycelium, from which only fruiting structures arise above the surface) or floccose (producing a conspicuous aerial felt of branching and interlacing hyphae and conidiophores) (Richardson, 1998; Gugnani, 2003). The characters of the conidial head include its colour and shape i.e. conidia produced in dry chains forming columns (columnar), or diverging (radiate) observed in a mature sporing culture, colour, size, shape (globes, sub-globes or oval) and wall texture of the conidia i.e. smooth-walled or ornamented, shape of the vesicle (sub-spherical, hemispherical, clavate, or flask-shaped), roughness or smoothness of the conidiophore wall, phialides being in single row (uni-seriate) or two rows (bi-seriate) (Richardson, 1998; Gugnani, 2003; Tortora *et al.*, 2010;). These morphological features of conidial heads are characteristics of the species and are better studied in slide culture. Examination of several heads in colonies (often on several media) is essential for complete description and interpretation of their size and shape (Jordan and Pattison, 1997). Cells with extremely thick walls (Hülle cells) of unknown function may be present in certain species of *Aspergillus*. The close and consistent association of globose Hülle cells with cleistothecia of *A. nidulans* suggests that presence of such structures in other species may indicate a potential for cleistothecia formation. True sclerotia consisting of thick-walled, parenchyma-like cells occur regularly in some isolates of *A. candidus*, *A. niger*, *A. flavus* and *A. ochraceus* (Richardson, 1998).

2.4.4 Cultural properties

For mycotic examination, samples from air sacs, lung and liver were cultured aerobically on Sabouraud dextrose agar (SDA) or Potato dextrose agar (PDA) at 25°C of 6-7 days in dark place. The isolated fungus were suspended in lactophenol and examined microscopically by slide culture (Yokota *et al.*, 2004).

Trachea culture is done using a sterile nasopharyngeal swab, introducing it deep into the trachea during the inspiratory process, when glottis is open and trying to not make contact with oral cavity to avoid sample contamination (Stone and Okoniewski, 2001).

The recovered material in the swab is immediately transferred to an appropriate culture media and incubated at 37°C. Also, a tracheal wash can be performed, using a syringe, a feline urinary catheter and 3ml/kg of saline. The catheter is introduced into the trachea, the liquid is injected and recovered immediately for culture and cytological study (Stone and Okoniewski, 2001; Akan *et al.*, 2002 and Yokota *et al.*, 2004).

Aspergillus species grow at temperatures from 25 to 37°C, some species can grow at 45°C in Sabouraud dextrose agar or blood agar and antibiotics like chloramphenicol must be used to avoid bacterial contamination, however, *Aspergillus* species are sensitive to cycloheximides (Richard, 1997 and Akan *et al.*, 2002).

Aspergillus fumigatus colonies have a diameter of approximately 3 to 4cm in 7 days. The flat colonies are white at first, then bluish green as conidia begin to mature, especially near to the center of the colony. As the colony matures, conidial masses become gray-green, while the colony edge remains white. A distinctive feature of *A. fumigatus* is the development of columnar masses of chains of conidia arising from the vesicle. These conidial chains can reach a length of up to 400µ (Yokota *et al.*, 2004). This organism is resistant to high temperatures and can grow well at 45°C. These are the most typical

characteristics, but variation occurs in colony color, both surface and reverse and colony morphology (Oglesbee, 1997; Richard, 1997 and López *et al.*, 1995).

A. flavus grows very rapidly, obtaining a colony diameter of 6-7cm in 10 days at $25\pm 2^{\circ}\text{C}$. The colony begins with a white color, turning yellowish to yellow-green with a white edge as conidia develop. Mature colonies may become somewhat olive-green (López *et al.*, 1995 and Reddy *et al.*, 2010).

A. niger begins with a white color, but rapidly develops a black color as the conidia mature. The colony reverse is yellowish (Yokota *et al.*, 2004 and Reddy *et al.*, 2010).

2.4.5 Toxins of *Aspergillus*

Aflatoxins, metabolites of the fungi *Aspergillus flavus* and *Aspergillus parasiticus* are frequent contaminants of a number of staple foods, particularly maize and ground nuts, in subsistence farming communities in tropical and sub-tropical climates in Sub-Saharan Africa, Eastern Asia and parts of South America (Kew, 2013). It's a natural contaminant of feeds, toxic metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus* (Giambrone *et al.*, 1985; Balachandran and Ramakrishnan, 1987; Demet *et al.*, 1995).

A. fumigatus produces several toxic secondary metabolites (Cui *et al.*, 1997; Frisvad and Samson, 1990 and Turner and Aldridge, 1983). The most intensively studied is gliotoxin, a metabolite of the epipolythiodioxopiperazine family (Mu"llbacher and Eichner, 1984 AND Mu"llbacher *et al.*, 1985). This compound, which is acutely toxic (Frame and Carlton, 1988), also has a broad range of immunosuppressive properties.

Aspergillus sp. also produce Ochratoxin by *Aspergillus ochraceus*, *A. westerdijikiae*, *A. steynii* (Martins *et al.*, 2012; Ostry *et al.*, 2013 and Malir *et al.*, 2013), Verruculogen by *Aspergillus fumigatus* (Frisvad *et al.*, 2009), Viriditoxin by *Aspergillus fumigatus* (Lillehoj and Milburn, 1973), Fumagilin by *Aspergillus*

fumigatus (Rementeria *et al.*, 2005), Sterigmatocystin *Aspergillus flavus*, *A. nidulans*, *A. versicolor* (Cary *et al.*, 2005).

2.5 Clinical signs and lesions

Clinical manifestations depend on the infective dose, the spore distribution, pre-existing disease, and the immune response of the host (Dahlhausen *et al.*, 2004). Avian aspergillosis is often classified as acute or chronic. Acute aspergillosis is thought to be the result of inhaling an overwhelming number of spores, while chronic Aspergillosis is generally associated with immune suppression (Vanderheyden, 1993). Although aspergillosis is predominantly a disease of the respiratory tract, any organ can be infected. Nasal aspergillosis causes exudative rhinitis (Tsai *et al.*, 1992), possibly accompanied by malformation of the nostrils and beak (Bauck *et al.*, 1992). Mycotic keratitis can cause blepharospasm, photophobia, periorbital swelling, turbid discharge, swollen and adhered eyelids, cloudy cornea and cheesy yellow exudates within the conjunctival sac (Beckman *et al.*, 1994 and Hoppes *et al.*, 2000). Neurological signs can be caused by *Aspergillus spp* (Jensen *et al.*, 1997). Epidermal cysts associated with *Aspergillus fumigatus* have been described in the comb of a silky bantam chicken (Suedmeyer *et al.*, 2002). Blepharitis and dermatitis involving the eyelids and the head have been described in a peregrine falcon gyrfalcon hybrid (*Falco peregrines* x *Falco rusticolus*) (Abrams *et al.*, 2001). Right ventricular dilatation (corpulmonale) due to pulmonary hypertension, with or without ascites and congestion of the lungs caused by ventricular failure occasionally occurs (Julian and Goryo, 1990). Birds suffering from aspergillosis do not always show respiratory problems. The owner of a trained raptor, for example may rather observe that the bird's reactions are impaired. Unilateral drooping of the wing (due to infection of the thoracic air sac, the clavicular air sac or the proximal humerus) or repeated vomiting (due to lesions in the anterior air sacs) is also seen (Forbes, 1991, 1992).

2.6 Pathogenesis

Inhalation is considered the main infection route for *A. fumigatus* in birds (Oglesbee, 1997), and because *A. fumigatus* spores are too small to be trapped completely in the nasal cavity or trachea, some are able to reach the lungs and air sacs (Fedde, 1998). The air sacs are usually the primary infection sites, since inhaled air reaches the posterior thoracic and abdominal air sacs prior to contacting epithelial surfaces in the lungs (Nardoni *et al.*, 2006). In the lung parenchyma, spores get embedded in the atria and parts of the infundibula in the parabronchus and are engulfed by (surface) phagocytic epithelial cells (Maina, 2002). When there are too many spores or the bird has an impaired immune response, the innate defense mechanisms do not succeed in eliminating infection at the site of the air capillaries. This may lead to the development of loosely attached plaques, which may or may not become overgrown by connective tissue of the host. These plaques or necrotic debris in the respiratory tract can obstruct the trachea or bronchi and/or fill up the air sacs (Oglesbee, 1997).

Occasionally, sporulation occurs in the lungs and air sacs (Nardoni *et al.*, 2006; Cacciuttolo *et al.*, 2009). Hyphae containing fruiting bodies can fill the lumen and may penetrate the air sacs, causing serositis and superficial necrosis in the adjacent organs (Tsai *et al.*, 1992). Besides direct extension of the infection through the air sac wall, disseminated mycosis also occurs by haematogenous spread. Hyphae are known to be tissue and angio-invasive (Dahlhausen *et al.*, 2004), as well as host cells play a role in this spreading mechanism. Macrophages in the respiratory tract ingest spores and find their way through the interstitium into the blood and lymphatic stream and thus to other organs (Richard and Thurston, 1983). The relevance of virulence factors in avian Aspergillosis is not well known, as research on this is minimal (Peden and Rhoades, 1992; Richard *et al.*, 1994, 1996). Ongoing studies suggest that *A. fumigatus* conidia may be able to resist killing by the avian respiratory macrophage. Two types of tissue reaction are recognized: the granulomatous or deep nodular form, and the infiltrative or superficial diffuse form. In the first type, neither exudative inflammation nor

vascular lesions in the neighboring tissues are seen. This type of encapsulated reaction develops both in non-aerated and aerated organs (Nardoni *et al.*, 2006; Femenia *et al.*, 2007 and Cacciuttolo *et al.*, 2009). In the non-ridged, non-encapsulated infiltrative type, the fungus frequently invades blood vessels. In aerated organs such as the lungs and the air sacs, the fungus may form aggregates of radiating hyphae containing large numbers of conidiophores and conidia in the absence of a structured granuloma formation (Nardoni *et al.*, 2006; Cacciuttolo *et al.*, 2009). A mixed type composed of both tissue reactions in the same tissue section is also possible (Tsai *et al.*, 1992; Atasever and Gumussoy, 2004). The popular assumption that the paucity of free avian respiratory macrophages results in an inadequate or incompetent avian respiratory immune system and is therefore responsible for the high susceptibility of birds to respiratory pathogens (Toth and Siegel, 1986) lacks scientific foundation. Instead, the susceptibility of birds to *Aspergillus* spp. may be attributed to differences in anatomical, physiological and respiratory immune system characteristics compared with mammals (Toth, 2000; Maina, 2002 and Tell, 2005).

2.7 Pathology

2.7.1 Gross Lesions

The severity and the degree of development of the disease determine both morphology and extension of macroscopic lesions. Extensive involvement of the respiratory tract can occur before clinical signs are apparent. Typically, lesions consist of white to yellowish granulomas ranging from miliary (<1mm in diameter) to large roughly spherical granulomatous nodules (>2 cm) involving serosae and parenchyma of one (Corkish, 1982) or multiple organs. Single or multiple necrotic areas are visible on cut surfaces. The primary location of lesions is the air sacs and lungs although oesophagus, proventriculus, gizzard, small intestine, liver, kidney, spleen, skin, trachea, peritoneum, brain, eye, muscle, or heart may be involved (Akan *et al.*, 2002; Steinlage *et al.*, 2003; Martin *et al.*, 2007 and Singh *et al.*, 2009). Lung parenchyma is either consolidated or has focal granulomas of varied size (Perelman and Kuttin, 1992; Copetti *et al.*, 2004 and Beytut, 2007). When coalescing in air sacs, these masses form cheesy caseous plaques covering the thickened membranes and even obstructing the entire lumina where fungal sporulation may occur as evidenced by grey-greenish velvet (Akan *et al.*, 2002; Steinlage *et al.*, 2003; Beytut *et al.*, 2004; Singh *et al.*, 2009; Cacciuttolo *et al.*, 2009). Nonulcerative or mildly ulcerative keratitis was reported in a turkey flock (Dyar *et al.*, 1984). Broiler pullets presented periorbital and eyelid swelling with cheesy yellow exudates in the conjunctival sac (Akan *et al.*, 2002). Circumscribed white to grayish areas were observed in the cerebellum of broiler breeders (Akan *et al.*, 2002) and turkeys (Jensen *et al.*, 1997). Ribs of ostriches (Perelman and Kuttin, 1992), sternum of broiler breeders (Martin *et al.*, 2007), yolk sacs of poults (Cortes *et al.*, 2005), and hip joints of turkeys (Olias *et al.*, 2010) constituted unusual locations of aspergillosis lesions.

2.7.2 Organs affected

Although aspergillosis is predominantly a disease of the respiratory tract, all organs can be involved, leading to a variety of manifestations ranging from acute

to chronic infections (Beernaert *et al.*, 2010). The respiratory tract is the primary site of *A. fumigates* development leading to severe respiratory distress and associated granulomatous airsacculitis and pneumonia (Pascal *et al.*, 2011). *Aspergillus* spp. has an affinity for the respiratory region of the nose, inducing an exudative rhinitis (Tsai *et al.*, 1992).

In lungs mainly found grossly white to yellow caseous granulomatous nodules (Akan *et al.*, 2002; Tsai *et al.*, 1992; Martin *et al.*, 2007). Small white nodular lesions 1 millimeter diameter is occur in the congested lungs and thoracic air sacs (Sajid *et al.*, 2006). On necropsy large, multifocal, firm, tan, nodular lesions occur in the lungs air sacs, peritoneum, livers, spleens and kidneys. On histopathology, mixed populations of zygomycetes and, and zygomycete hyphae were observed in the granulomas in the air sacs, peritoneum, livers, spleens and kidneys (Mukaratirwa, 2006).

The serous membrane of the air-sacs becomes thickened in places by the development of small yellowish-white plaque-like lesions which enlarge rapidly, become raised in the centre, and then coalesce to form a complete membranous or caseous lining to the serosa up to 5 mm. thick (Spanamberg *et al.*, 2013). Two types of tissue reaction, invasive and granulomatous, are found in the air sac (Tsai *et al.*, 1992). Small white nodular lesions 1 millimeter diameter is occur in the thoracic air sacs (Sajid *et al.*, 2006). Large, multifocal, firm, tan, white to yellow caseous nodules occur on thoracic air sac (Akan *et al.*, 2002 and Mukaratirwa, 2006).

In liver, spleen, peritoneum and kidney, occurs a large, multifocal, firm, tan, nodular lesion (Mukaratirwa, 2006).

In alimentary canal, plaque-like lesions may occur in the mouth, the gizzard and the intestines especially in turkeys (Charlton *et al.*, 2008 and Ceolin *et al.*, 2012).

In nose, hyperkeratotic change is found in the nasal cavity (Tsai *et al.*, 1992).

In eye, white to yellow caseous nodules are observed on eyes (Akan *et al.*, 2002). Lesions have been noted in the eyes of baby chicks by Hudson (1947) and in turkeys by Moore (1953), the latter author finding the condition significantly more common in males than in females.

In bones and joints, osteo-arthritis may be set up in the joints with subsequent lameness (Bizard and Pommay, 1887) and lesions in the long bones were observed very early by Dsa *et al.*, 2013.

In brain, white to yellow caseous nodules are observed on cerebellum (Akan *et al.*, 2002). There are several reports of abscesses in the cerebrum and cerebellum containing the hyphae of *A. fumigatus* both in the presence and absence of pulmonary and other lesions (Jungherr and Gifford., 1944 and Raines *et al.*, 1956).

In crop, gizzard and proventriculus, hyperkeratotic change in the mucous membrane is found (Tsai *et al.*, 1992).

2.7.3 Histopathological changes

Lungs: Caseous necrotic mass surrounded by fewer inflammatory cells in nodular lesions are present (Badhy *et al.*, 2003; Akkoc *et al.*, 2009). Presence of radiating eosinophilic hyphae in coagulative necrotic center (Medani *et al.*, 2004). Inflammatory lesions were focally present on the pleura and the underlying pulmonary lobules lungs (Femenia *et al.*, 2007).

Nasal: Nasal is presented as a solid mycotic plug or aspergilloma localized in nares or choana. Histopathological examination of granulomas generally shows a necrotic foci surrounded by macrophages, heterophils and giant cells, sometimes within a connective tissue capsule (Mycoses, 1997).

Tracheal: Trachea is characterized by the presence of mycotic colonies above the syrinx or at the bifurcation due to air turbulence which deposits spores in this section of the air ways; in addition, the narrowing in the respiratory tract at this point may predispose it to blockage with necrotic debris and caseous exudates.

The lesions reduce air movement through the lower airway, so inspiratory dyspnea exists. Mycelia penetrate walls and in combination with inflammatory cells and connective tissue, granulomatous nodules are formed (Roskopf and Woerpel, 1997; Forbes and Rapaces, 1999).

Cutaneous: Necrotic granulomatous dermatitis has been described and *A. fumigates* was isolated from infected tissue. Skin lesions have been encountered under moisture retaining bandages. Cutaneous lesions and their manifestations are rare in birds. Atkinson (1998) reports one case in a wing of a Great horn owl (*Bubo virginianus*) on the head of a hybrid peregrine-gyr Falcon (*Falco peregrinus-Falco rusticolus*) (Atkinson and Brojer, 1998; Friend and Franson, 1999; Abrams *et al.*, 2001).

Ophthalmitis: Superficial and the affected tissues are conjunctiva and external surfaces of the eye with the development of a cheesy exudates or plaque forming beneath the nictitating membrane (Richard, 1997; Abrams *et al.*, 2001; Akan *et al.*, 2002). Pathological changes can occur in the vitreous humor and extend into adjacent tissue. Pectin edema can be present with heterophil infiltration and mononuclear cells. Also it is possible to observe fungal hyphae, heterophils, macrophages and cell debris in the retina. In rare cases, well-encapsulated *Aspergillus* granulomas can be found in the trachea, esophagus or under the skin. These probably represent an effective walling-off of early fungal infection on the part of the bird (Heidenreich, 1997).

2.7.4 Generalized pathological changes

Pulmonary: Yellowish granulomas are seen disseminated throughout the pulmonary tissue. Lesions may also be seen in air sacs and their extensions. Characteristic lesions are granulomas that are larger and more diffuse than other presentations possibly indicating the primary site of infection. Granulomatous lesions become caseated as the disease progresses, and it is possible to find suppurative exudate accumulated in the bronchi (Young *et al.*, 1998 and Friend and Franson, 1999;). In chronic cases, there is pulmonary hypertension caused by

granulomas and necrosis can occur in the tissue leading to right ventricular dilatation or ascites. In well oxygenated sections of tissue like bronchi the organism can sporulate (Pérez and Carrasco, 2000; Richard, 1997). After inhalation, spores can disseminate through the blood producing lesions in brain, bones, pericardium, pectin and other tissues (Richard, 1997; Roskopf and Woerpel, 1999; Akan *et al.*, 2002). Cerebral presentation is described by encephalitis or meningoencephalitis in different bird species. Necrotic caseous lesions surrounded by giant cells in the brain or cerebellum may be observed causing granulomatous encephalitis with hyphae can be seen in the central area of some lesions. There are reports in the literature that birds presenting with the infection in their lungs and air sacs in many cases have kidneys and liver involvement. In bones, the infection can cause vertebral deformities resulting in paralysis. The extension of the fungal colonization dependent upon host immune system integrity. Clinical signs are often not apparent until fungal colonization is largely extended (Richard, 1997 and Akan *et al.*, 2002).

In air sacs: Initial lesions are found in zones with high oxygen tension and poor blood circulation, like air sacs. Specifically the caudal thoracic, and the abdominal air sacs where abundant caseous and necrotic debris may be found (Richard, 1997; Roskopf and Woerpel, 1999 and Young *et al.*, 1998). Hyphae can come together and create plaques over the connective tissue and can completely cover air sacs, in the case of *A. fumigatus* these plaques are grayish-green in color. In some cases, sporulation can occur in air sacs after a growing period and when oxygen conditions are optimal (Richard, 1997; Dykstra *et al.*, 1997 and Young *et al.*, 1998; Friend and Franson, 1999). Fungus dissemination can be the result of direct contact with pneumatic bones, because there are diverticula of various air sacs that connect to lungs, like humerus, coracoids and femur (Atkinson and Brojer, 1998) also, lungs, liver and adjacent structures can contain yellowish-white granulomas (Richard, 1997). This is the most chronic and debilitating form of the disease and is the least responsive to treatment (Atkinson and Brojer, 1998).

2.8 Diagnosis

The signs of aspergillosis are non-specific, making diagnosis difficult (Dahlhausen *et al.*, 2004). Moreover, no single test provides certainty. Diagnosis usually relies upon an accumulation of evidence from the history, clinical presentation, haematology and biochemistry, serology, radiographic changes, endoscopy and culture of the fungus (Jones and Orosz, 2000).

The history of the bird can reveal a stressful event and/or some underlying environmental factors and/or an immunosuppressive condition or treatment. It may also reveal chronic debilitation, voice change or exercise intolerance (Jenkins, 1991). The clinical signs depend on which form of Aspergillosis the bird develops and which organs are involved (Jones and Orosz, 2000). Hence, aspergillosis should be included in the differential diagnosis of respiratory tract and systemic diseases (Jenkins, 1991; Jones and Orosz, 2000).

Results of haematology and plasma biochemistry can be considered indicative rather than diagnostic (Jones and Orosz, 2000). Leukocytosis of 20,000 to more than 100,000 white blood cells per microlitre (Jenkins, 1991), heterophilia with a left shift (degenerative shift), monocytosis and lymphopenia have been described in aspergillosis cases (Forbes, 1992). In addition, non-regenerative anaemia, increased total protein and globulin fraction can be observed (Vanderheyden, 1993; Reidarson and McBain, 1995 and Jones and Orosz, 2000). Acute infections often present an increase in b-globulins, while chronic infections show an increase in b-globulin and/or g-globulin fractions. However, immunosuppressed birds may have hypoproteinaemia (Ivey, 2000) and white blood cells may be in the normal range (Flammer and Orosz, 2008). Overall, changes in protein electrophoresis are non-specific, but can be useful to estimate disease progression and the response to therapy (Ivey, 2000).

Serological tests have been developed to confirm an early and more definite diagnosis of aspergillosis (Peden and Rhoades, 1992). However, in the acute stage, antibody production trails behind antigen exposure by 10 to 14 days

(Brown and Redig, 1994); and if the bird is immunosuppressed, the low antibody production results in false negative results (Redig, 1994). In these cases, detection of circulating *Aspergillus* antigen in the serum may be more helpful. In chronic cases in which antigen levels may be low, detection of antibodies may be useful (Jones and Orosz, 2000). A number of serological test methods have been applied in birds, including counter-immunoelectrophoresis, agar gel immunodiffusion and enzyme-linked immunosorbent assays but, in general, negative serological tests do not rule out aspergillosis, and positive tests are only considered diagnostic when backed up by other evidence (Peden and Rhoades, 1992; Brown and Redig, 1994 and Arca- Ruibal *et al.*, 2006).

Although radiographs may not be helpful, lateral and ventrodorsal views can be taken in a bird suspected of having aspergillosis. For those that are unlikely to survive anesthesia, standing or perching lateral views as well as dorsoventral views are helpful (Jones and Orosz, 2000). The radiographic changes of pneumonia and consolidating airsacculitis are non-specific (McMillan and Petrak, 1989). Consistent with radiography, computed tomography scans reveal the extent of lesions but the diagnosis of aspergillosis still requires identification by biopsy, smear or culture (Phalen, 2000).

Endoscopy is invasive and requires anesthesia, but it allows the extent of the lesions to be seen as well as the progress of infection during treatment (Jones and Orosz, 2000). This technique enables evaluation of the entire respiratory tract. Tracheal endoscopy is useful for showing a single lesion, such as a thick white discharge or plaque occluding the trachea or syrinx (Marks *et al.*, 1994). The lower respiratory tract is best evaluated by laparoscopy (Jones and Orosz, 2000). Endoscopy of the abdominal air sac can show a diffuse cloudiness or white or yellow plaques. In the event of sporulation, plaques are covered with green gray pigmented mould. Samples for culture and cytology should be taken directly with biopsy forceps or via air sac lavage (Taylor, 1993). On necropsy, the yellow, green or white granulomatous foci can be noted in chronic aspergillosis patients

(Vanderheyden, 1993). Acute Aspergillosis causes numerous miliary granulomatous foci (McMillan and Petrak, 1989).

Definitive diagnosis requires demonstration of the presence of the organism by cytology or histopathology and its identification by culture (Dahlhausen *et al.*, 2004). It is important to mention that isolation of the fungus alone does not confirm the infection because *Aspergillus* organisms are ubiquitous and can be contaminants (Jensen *et al.*, 1997 and Flammer and Orosz, 2008). However, an abundant culture from any organ should be regarded as diagnostic. On the contrary, a negative culture does not rule out aspergillosis (Jensen *et al.*, 1997). Histopathological lesions can be suggestive, but because in vivo hyphae of hyaline filamentous fungi are very similar and there in situ manifestations are not pathognomonic, this technique does not allow fungal species identification (Kaufman *et al.*, 1997).

Thus the etiological diagnosis should ideally be confirmed by immunohistochemistry, although few reports on immunohistochemical techniques using monoclonal or polyclonal antibodies in birds with Aspergillosis are available (Jensen *et al.*, 1997; Beytut *et al.*, 2004 and Beytut, 2007).

A few reports of different polymerase chain reaction assays (including real-time polymerase chain reactions) tested on heparinized whole blood, tracheal washings, air sac fluids, respiratory tract granulomas or (biopsy) tissue samples from birds support the value of this assay in diagnosing avian aspergillosis (Dahlhausen *et al.*, 2004).

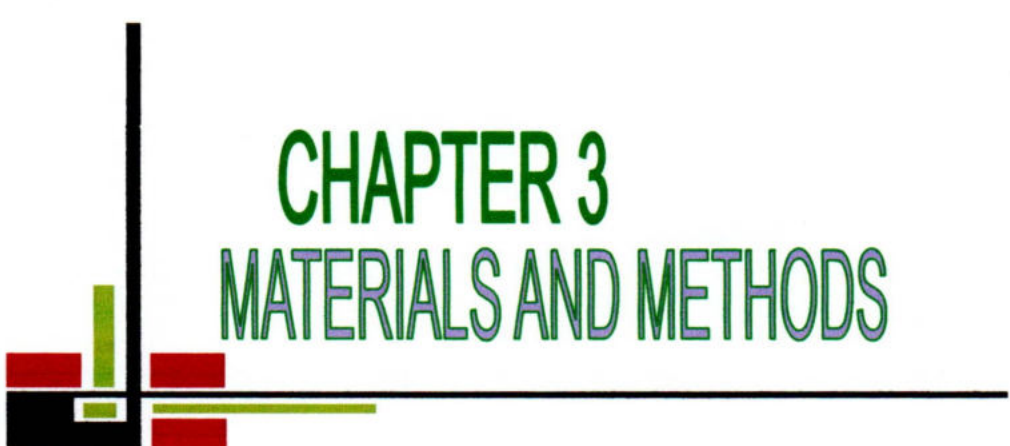
2.8.1 Differential diagnosis

Differential diagnosis in a mature bird showing weight loss, and severe heterophilia can include chlamydiophylosis and tuberculosis (Roskopf and Woerpel, 1997; Ramírez *et al.*, 2002). Neoplasias can sometimes cause weight loss and heterophilia. Differential for dyspnea include increase in abdominal pressure from a mass, ascites, hepatomegaly, pneumonia and inhalation of foreign bodies.11 Vitamin A deficiency can cause ocular lesions. Some granulomas can

be produced by bacteria. Other mycotic infections must be suspected like candidiasis (Roskopf and Woerpel, 1997; Atkinson and Brojer, 1998).

2.9 Prevention

Protective immunity following vaccination is thought to be useful in treating and preventing avian aspergillosis. However, attempts to devise vaccination strategies appear questionable in immunosuppressed animals that would need passive immunization with immunoglobulin's (Schmidt, 2002). Nevertheless, a number of different vaccination strategies have been attempted in birds using different vaccine preparations, but with inconsistent results (Richard *et al.*, 1991; Bauck *et al.*, 1992 and Meredith, 1997). Future knowledge regarding virulence factors and the role of cellular immunity in avian aspergillosis may contribute to newer and more effective vaccination strategies. The use of immune stimulants has been suggested (Jenkins, 1991). Levamisole, a derivative of imidazothiazole, has been found to possess an immunostimulant effect as an adjunct in the treatment of chronic infections. However, levamisole did not decrease Aspergillosis associated lesions in turkeys (Perelman, 1993). In humans, clinical improvement of aspergillosis has been documented after the addition of interferon- γ and granulocyte macrophage colony-stimulating factor to the antifungal treatment (Bandera *et al.*, 2008). Whether the favorable effect of interferon- γ and granulocyte macrophage colony-stimulating factor could also play a role in future treatment protocols for avian Aspergillosis needs to be investigated.



CHAPTER 3

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

The present study was carried out to ascertain the pathological investigation of avian aspergillosis in commercial broiler at Chittagong district of Bangladesh. The materials and methods which were used to perform this research work are described below:

3.1 Study area and study period

The study was conducted on 20 randomly selected commercial broiler farms from five Thana (Sitakunda, Patenga, Anwara, Boaklhali and Pahartali) of Chittagong district during the period from January to December, 2013. Samples were collected from the selected broiler farm and brought to the department of Microbiology and Hygiene, Chittagong Veterinary and Animal Science University, Chittagong. Postmortem examination and microbiological work was done in the laboratory of Department of Microbiology and Hygiene, Chittagong Veterinary and Animal Science University. Histopathological examination was done in the laboratory of Department of Pathology and Parasitology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur.

3.2 Clinical examination of affected birds

The general health condition and age of the chicken were recorded. The clinical signs were observed from the visual examination in case of diseased broiler birds. The clinical signs were recorded during the physical visit to the affected flocks. Farmer's complaints about the affected birds were considered in some cases

3.3 Collection of birds

A total of 912 sick and dead chickens were collected from 20 commercial broiler farms in 5 Upazilla of Chittagong district and diagnosed for avian aspergillosis on the basis of clinical signs, symptoms and postmortem findings.

3.4 Necropsy examination of suspected birds

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

- At first the bird was laid on its back and each leg, in turn drawn outward away from the body while the skin was incised between the leg and abdomen on each side.
- Then the both legs were then grasped firmly in the area of the femur and bent forward, downward and outward, until the heads of both femurs were broken free of the acetabular attachment so that both legs lied flat on the table.
- The skin was cut between the two previous incisions at a point midway between keel and vent.
- The cut edge was then forcibly reflected forward, cutting was necessary until the entire ventral aspect of the body including the neck was exposed.
- For exposing of the viscera, knife was used to cut through the abdominal wall transversely midway between the keel and vent, then through the breast muscle on each side.
- Positioning shears were used to cut the rib cage, the coracoid and clavicle on both sides.
- This was done carefully without severing the large blood vessels and thorough examination of the organs was done.
- The liver and intestine were found just by opening the chest of carcass the lung attached with ribs distal position was examined.

3.5 Collection of samples

Collection of lung for histopathological examination and tracheal swab for mycological culture from suspected chickens was done. The collected lung was examined for gross abnormalities of avian aspergillosis and lesion containing lungs were kept in plastic jar containing 10% formalin solution.

3.6 Experimental layout

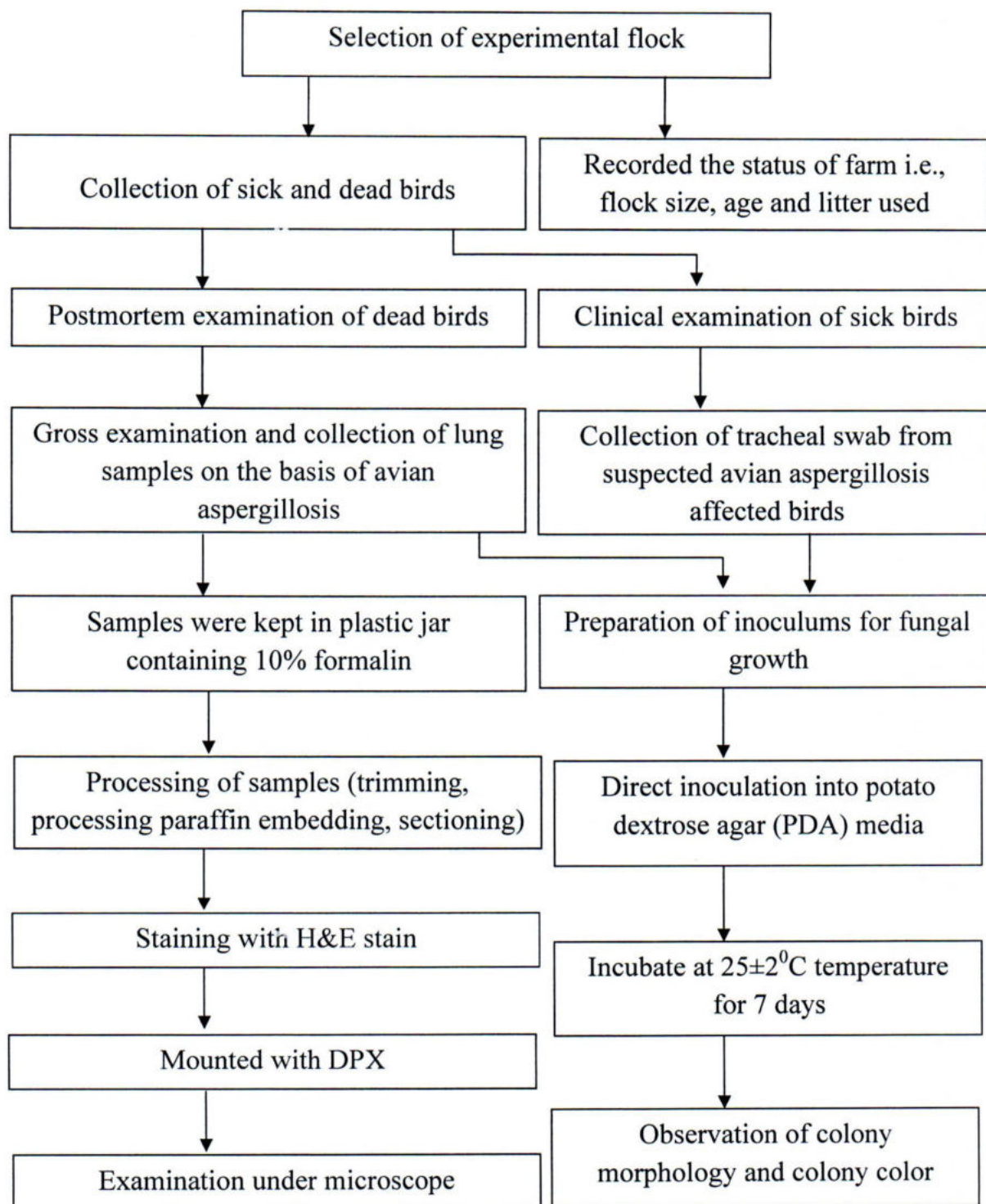


Figure 1. Schematic illustration of the experimental layout

3.7 Methods

3.7.1 Histopathological study

Lungs having gross lesions were collected from selected farm then preserved at 10% formalin, after that processed, sectioned and stained for histopathological studies following a standard procedure (Luna, 1968).

3.7.1.1 Materials required for histopathology

- Samples (Lungs)
- 10% formalin
- Chloroform
- Paraffin
- Alcohol
- Tape water
- Microtome
- Xylene
- Hematoxylin and Eosin stain
- Distilled water
- Clean slides
- Cover slips
- Mounting media (DPX).
- Microscope

3.7.1.2 Processing of tissues for histopathology

1. Collection of tissues and processing

During tissue collection the following point were taken into consideration-

The tissues were collected in conditions as fresh as possible. Normal and diseased tissues were collected side by side. The thickness of the tissues were as less as possible (5mm approximately).

The tissues (lungs) were collected from the chicken in the Laboratory of Department of Pathology and Parasitology, Hajee Mohammad Danesh Science and Technology University, Dinajpur.

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

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

4. Dehydration: The tissues were dehydrated by ascending ethanol series to prevent shrinkage of cells as per following schedule.

- i. 50% alcohol – one hour
- ii. 70% alcohol – one hour
- iii. 80% alcohol – one hour
- iv. 95% alcohol – one hour
- v. Absolute alcohol – three changes (one hour for each change)

5. Cleaning: The tissues were cleaned in chloroform for 3 hours to remove ethanol (1 and half hr in each, two changes).

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

7. Embedding: Paraffin blocks containing tissue pieces were made using templates and molten paraffin

8. Sectioning: Then the tissues were sectioned with a microtome at 5-6µm thickness. The sections were allowed to spread on lukewarm water bath (40-45°C) and taken on a glass slide. A small amount of gelatin was added to the water bath for better adhesion of the section to the slide. The slides containing section were air dried and stored in cool place until staining.

3.7.1.3 Routine hematoxylin and eosin staining procedure

3.7.1.4 Preparation of Ehrlich's hematoxylin solution

Reagents	Quantity
Hematoxylin crystals	4.0 gm
Alcohol, 95%	200.0 ml
Ammonium or potassium alum	6.0 gm
Distilled water	200.0 ml
Glycerin	200.0 ml
Glacial acetic acid	20.0 ml

Hematoxylin was dissolved in the alcohol and the alum was dissolve in distilled water and mixed thoroughly. After these were in complete solution the glycerin and acetic acid were added.

3.7.1.5 Preparation of eosin solution

1% stock alcoholic eosin

Reagents	Quantity
Eosin Y, water soluble	1 gm
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

Reagents	Quantity
Eosin stock solution	1 part
Alcohol (80%)	0 parts

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

3.7.1.6 Staining protocol

The sectioned tissues were stained as described below-

- i. The sectioned tissues were deparafinized in three changes of xylene (three minutes in each)
- ii. Then the sectioned tissues were rehydrated through descending grades of alcohol as per following schedule.
 - Absolute alcohol - three changes (three minutes for each)
 - 95% alcohol - two minutes
 - 80% alcohol - two minutes
 - 70% alcohol - two minutes
 - Dipping with distilled water for 10 minutes.
- iii. The tissues were stained with Harris hematoxylin for 2-10 minutes.
- iv. Washed in running tap water for 10-15 minutes.
- v. Then the tissues were dipped in ammonia water (few dips).
- vi. Stained with eosin for one minute.
- vii. Differentiated and dehydrated in ascending grade of alcohol.
 - 95% alcohol – three changes (2-4 dips for each.)
 - Absolute alcohol – three changes (2-3 minutes for each)
- viii. Cleaned in xylene: three changes (five minutes each).
- ix. Tissues were mounted with cover slip by using DPX
- x. The slides were dried at room temperature and examined under a low (10×) and high (40×, 100×) power objectives.

3.8 Mycological culture of samples

3.8.1 Preparation of potato dextrose agar (PDA) media for cultivation of fungus

Commercial potato dextrose agar media were purchased from local agent HIMEDIA[®] (manufactured by HiMedia Laboratories Pvt. Ltd., 23, Vadhani Ind. Est., LBS Marg, Mumbai-400086, India). A total of 39.0 gm of media were properly mixed with 1000 ml distilled water and boiled to dissolve the media completely. It was sterilized using autoclaving at 121⁰C temperature for 15 minutes, 15lbs/ cm² and dispense in petridishes aseptically for solidification.

An inoculum was prepared from lung and swab samples and directly streaked on PDA media. Then the petridishes were incubated at 25±2⁰C temperature for 7 days. After 7 days, observed the colony morphology and colony color to identify the *Aspergillus* sp.

3.9 Photography

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

3.10 Statistical analysis

All the data obtained during the study were analyzed statistically by using SPSS (Statistical Package for Social Science) software to find out the P-value. The data in relation to age wise incidence and litter wise incidence of avian aspergillosis were calculated by t-test and overall incidence and seasonal incidence calculated by F-test and Chi-square test, respectively.



CHAPTER 4 RESULTS

CHAPTER 4

RESULTS

4.1 Incidence study

4.1.1 Upazilla wise incidence study

In this study a total of 912 sick and dead commercial broiler chickens from 20 broiler farms under five Upazilla of Chittagong district of Bangladesh were examined grossly for visible lesions of avian aspergillosis under day old to 20 days of age. The overall incidence of avian aspergillosis was found 6.14%. Among five Upazilla of selected area highest and lowest incidence was found in Patenga and Sitakunda that were 9.25% and 3.43% respectively and another three Upazilla was 6.96%, 6.07% and 4.99% of Boalkhali, Anwara and Pahartali, respectively. The F-test value indicates a significant ($p < 0.01$) association of avian aspergillosis in Upazilla wise incidence. The details of the results are shown in Table 1.

Table 1. Incidence of avian aspergillosis in commercial broiler chicken

Name of the Upazilla	Farm	No. of birds observed	No. of infected birds %	Incidence (%) ±SE	
				In Upazilla	Overall
Sitakunda	1	1200	22 (1.83)	3.43 ^c ±0.69	6.14±0.58
	2	550	28 (5.09)		
	3	650	25 (3.85)		
	4	1050	31 (2.95)		
Patenga	1	550	38 (6.90)	9.25 ^a ±0.88	
	2	650	72 (11.07)		
	3	690	68 (9.86)		
	4	525	48 (9.14)		
Anwara	1	550	27 (4.90)	6.07 ^{bc} ±0.87	
	2	450	38 (8.44)		
	3	650	41 (6.30)		
	4	950	44 (4.63)		
Boalkhali	1	850	33 (3.88)	6.96 ^{ab} ±1.29	
	2	700	69 (9.85)		
	3	680	55 (8.08)		
	4	1100	66 (6.00)		
Pahartali	1	1350	41 (3.07)	4.99 ^{bc} ±0.91	
	2	1000	69 (6.9)		
	3	750	46 (6.13)		
	4	1300	51 (3.92)		
Total=	20	16,195	912 (6.14)		
F value				5.27	
P value				0.007	
Level of significant				**	

** Significant (p<0.01)

4.1.2 Season wise incidence

Seasonal incidence of avian aspergillosis was also analyzed. It was observed that the highest incidence (8.22%) was observed in rainy and lowest (3.16%) in winter but moderate (5.16%) in summer (Table 2). The Chi-square value indicates a significant ($p < 0.05$) association of avian aspergillosis in season wise incidence.

Table 2. Seasonal incidence of avian aspergillosis in commercial broiler

Season	Total No. of farm observed	Total No. of birds observed	No. of infected birds	Incidence (%)
Rainy	9	5995	493	8.22
Summer	6	4800	248	5.16
Winter	5	5400	171	3.16
Chi-square value		6.00		
P value		0.019		
Level of significant		*		

* significant ($p < 0.05$)

4.1.3 Age wise incidence

Incidence of avian aspergillosis was analyzed on the basis of age of broiler chicken. The incidence was higher (8.27%) in 6-10 days of age than 11-15 days (6.85%) and 16-20 days (5.33%) of age and the incidence rate was lower (4.11%) in 0-5 days of age range. The t-test value indicates a significant ($p < 0.05$) association of avian aspergillosis in age wise incidence.

Table 3. Incidence of avian aspergillosis in commercial broiler on the basis of age

Age (Day)	Total No. of farm observed	Total No. of birds observed	No. of infected birds	Incidence (%) $\pm$ SE
0-5	5	4900	178	4.11 ^b $\pm$ 0.86
6-10	5	3350	276	8.27 ^a $\pm$ 1.06
11-15	5	3420	235	6.85 ^{ab} $\pm$ 1.01
16-20	5	4525	223	5.33 ^{ab} $\pm$ 1.07
P value				0.050
Level of significant				*

* Significant ($p < 0.05$)

4.1.4 Litter wise incidence

In the present study the incidence of avian aspergillosis was considerably higher in farms with saw dust as litter. The result revealed that 7.69% incidence of avian aspergillosis in farms were being reared on saw dust as litter and 3.46% incidence were found in which rice husk was used as litter (Table 4). The t-test value indicates a significant ($p < 0.05$) association of avian aspergillosis in litter wise incidence. It is speculated that the saw dust is more favorable for fungal growth as compared to rice husk because of its higher moisture contents, allowing the fungal growth shown in Figure 2a and 2b.

Table 4. Incidence of avian aspergillosis in commercial broiler on the basis of litter used.

Litter	Total No. of farm observed	Total No. of birds observed	No. of infected birds	Incidence %
Saw dust	12	8295	638	7.69 ^a ±1.27
Rice husk	8	7900	274	3.46 ^b ±0.89
P value				0.042
Level of significant				*

* Significant ($p < 0.05$)



Figure 2a. Rice husk litter status of experimental flock



Figure 2b. Saw dust litter status of experimental flock

4.2 Clinical signs

The clinical signs observed in these birds were respiratory distress, dyspnea, gasping and accelerated breathing associated with loss of appetite, stunting growth, lethargy and increased thirst were clinically examined (Figure 3a and 3b).

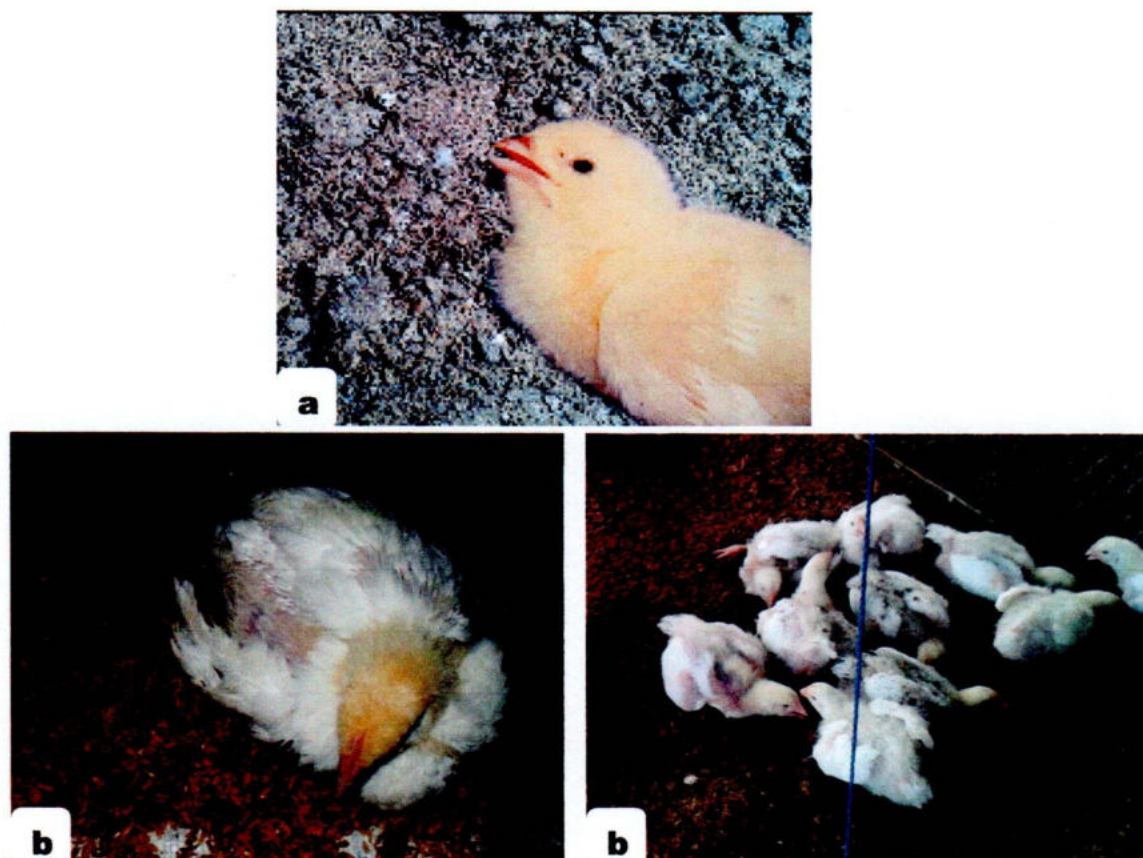


Figure 3. a. Bird showing gasping and **b.** Birds showing depression.

4.3 Pathological study of avian aspergillosis

4.3.1 Gross pathology

Pulmonary lesions are characterized by multiple hard creams to yellow colored, circumscribe plaques a few mm to several cm in diameter seen throughout the lungs surface, inside the lungs, scattered in ventral surface of sternum and air passages on gross examination (Figure 4a, 4b). The plaques also found in the syrinx, air sacs, liver and intestines. Lung parenchyma was consolidated and single or multiple necrotic areas are visible on cut surfaces of lungs.

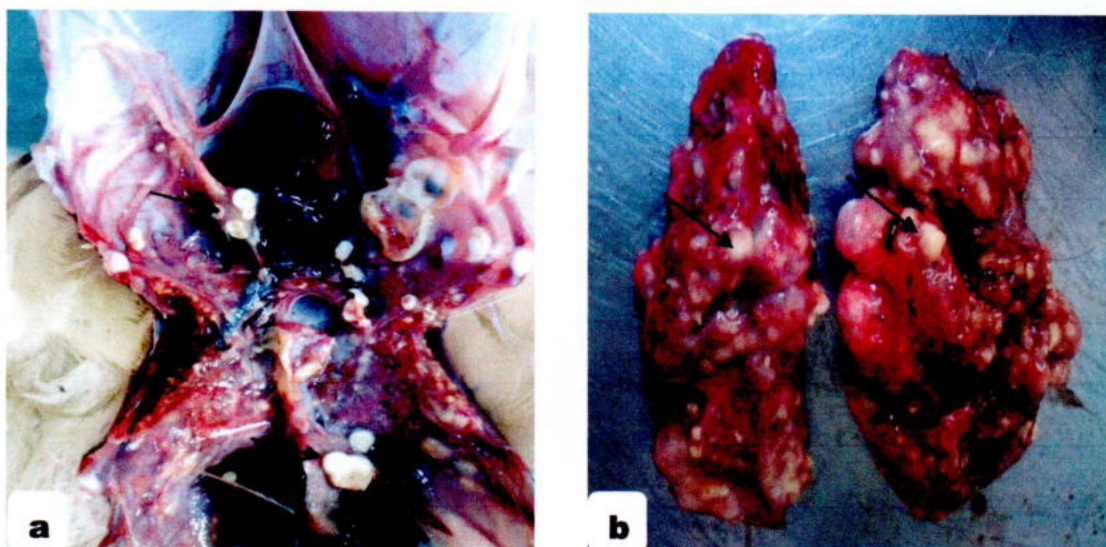


Figure 4. a. Lung shows the presence of cream color nodules in plural surface, air sacs (arrow). b. Creamy to yellow color nodules shows throughout the lung (arrow).

4.3.2 Histopathological examination

The microscopical examination showed congestion of pulmonary and perialveolar blood vessel and perivascular edema (Figure 5). The normal architecture of the lung and air sacs were replaced by disseminated granulomatous foci. The center of the granulomatous foci contained caseous necrosis and necrotic cellular debris surrounded by rims of heterophils, lymphocytes, macrophages and multinucleated giant cells was seen (Figure 6). The nodules consisted of coagulative necrotic center (Figure 7). A few, more severe, densification and inflammatory lesions were focally present on the pleura and the underlying pulmonary lobules (Figure 8).

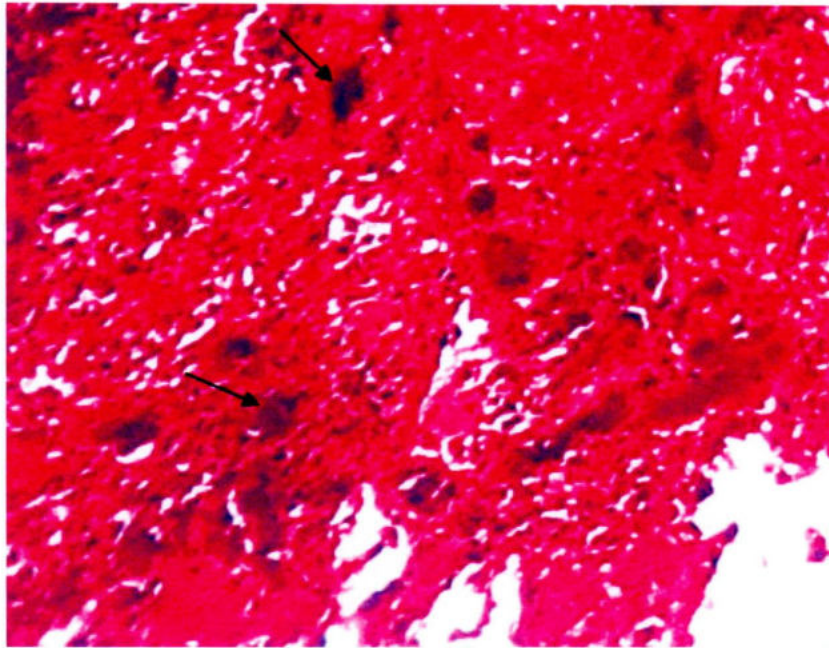


Figure 5. Showing congestion of pulmonary, perialveolar blood vessel and diffuse edema of pulmonary tissues (arrow) in Lung. H&E stain, 10 $\times$.

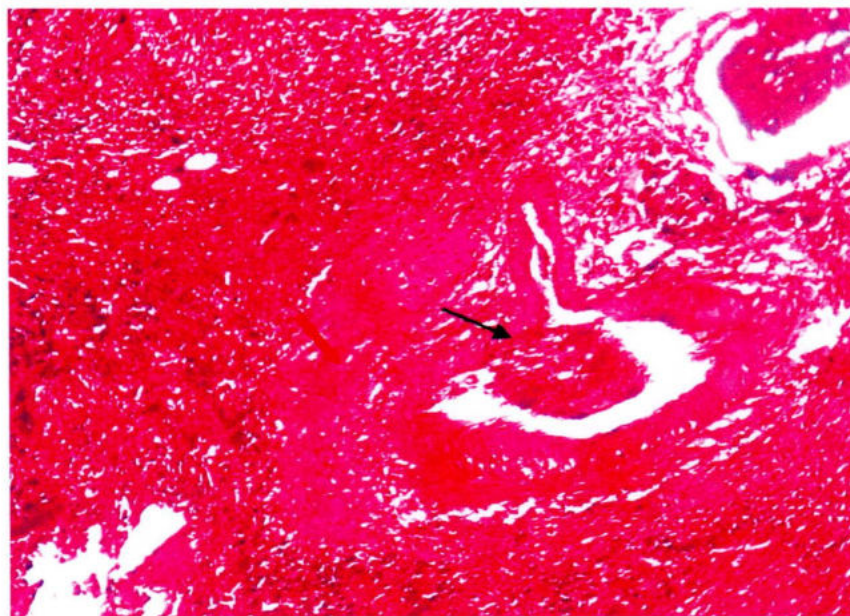


Figure 6 . Areas of caseous necrosis (black arrow) and cellular debris (red arrow) in lung. H&E stain, 10 $\times$.

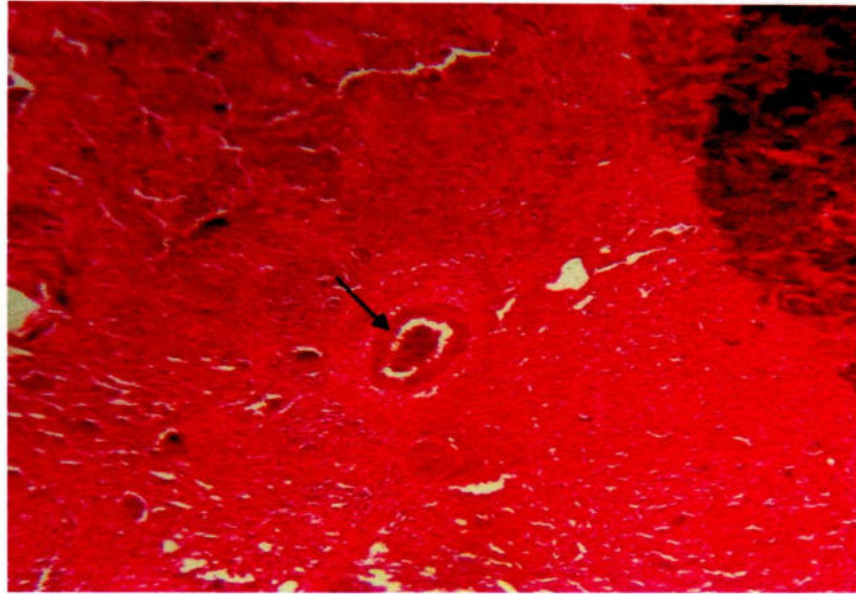


Figure 7. Aspergillosis showing granuloma formation with caseated center (arrow) in lung. H&E stain, 10 $\times$.

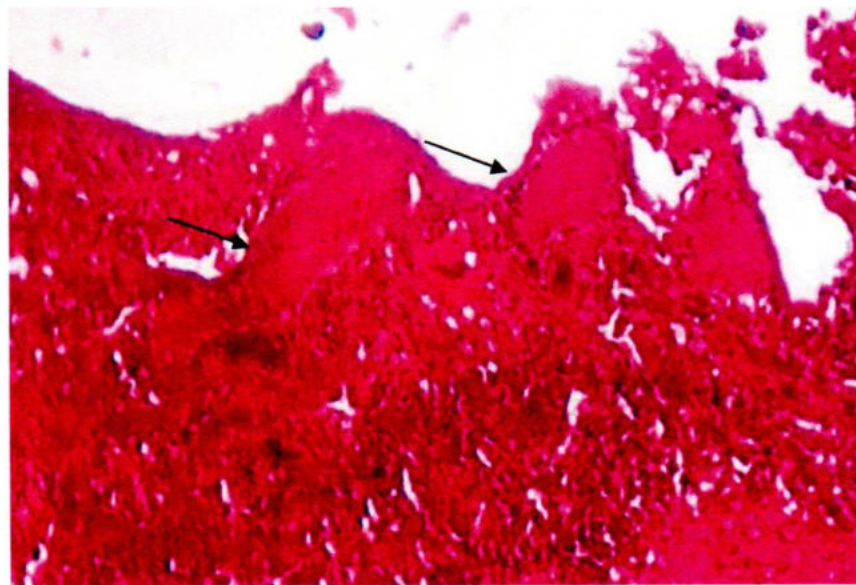


Figure 8. Diffuse densification of the pleural parenchyma by congestion and an inflammatory cellular infiltration (arrows) in lung. H&E stain, 10 $\times$.

4.4 Mycological examination

The suspected samples (tracheal swab, lung sample after postmortem) were inoculated on Potato Dextrose Agar (PDA) media for 7 days at $25\pm 2^{\circ}\text{C}$ temperature to detect characteristic colony of *Aspergillus* sp. Whitish color colony revealed by *Aspergillus fumigatus* (Figure 9a), parrot green color colony revealed by *Aspergillus flavus* and dark black color colony revealed by *Aspergillus niger* (Figure 9b).

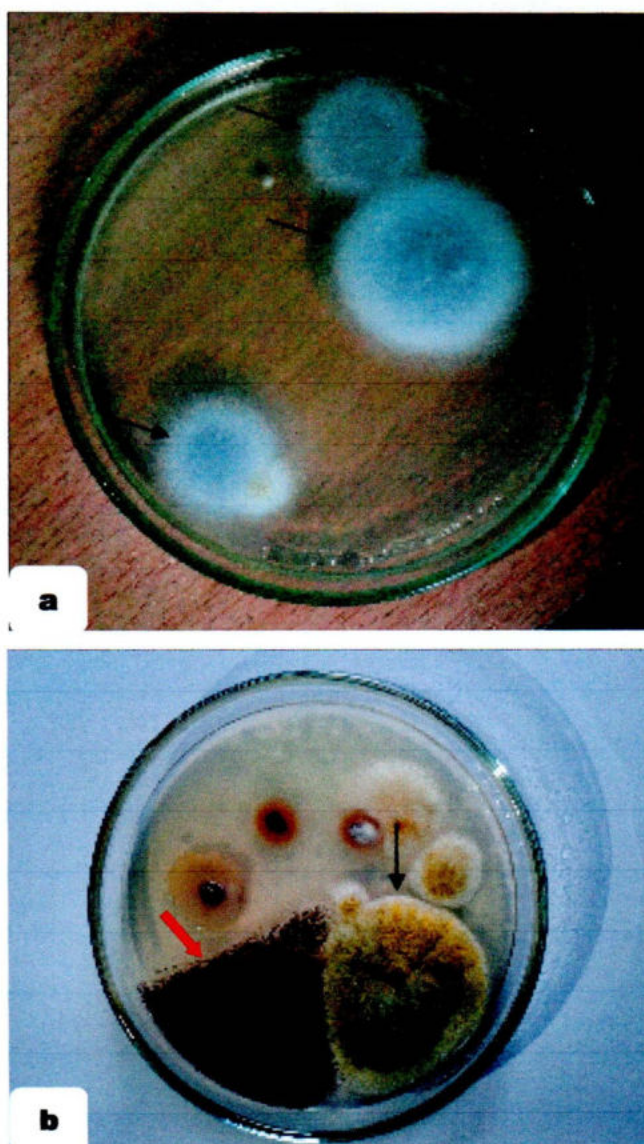


Figure 9. a. PDA media shows whitish color colony of *A. fumigatus* (arrows). b. PDA media shows parrot green color colony of *A. flavus* (black color arrow) and dark black color colony of *A. niger* (red color arrow)



CHAPTER 5

DISCUSSION

CHAPTER 5

DISCUSSION

In the present study, attempts were made to study the incidence of avian aspergillosis in commercial broiler chicken at Chittagong district of Bangladesh. The incidence of avian aspergillosis was observed on the basis of clinical signs, symptoms, postmortem findings, histopathological examination and fungal culture in 912 sick and dead chickens of 20 commercial broiler farms and overall incidence was 6.14%. Similar incidence have been described by Uddin *et al.*, (2010) reported 7.98% in Narsingdi, Talha *et al.*, (2001) reported 4.20% in Mymensingh, Uddin *et al.*, (2011) reported 1.54%, and Islam *et al.*, (2009) reported 1.60% in Gaibandha district of Bangladesh.

The present study was revealed that the avian aspergillosis is prevailing throughout the year but in rainy season were higher 8.22% might be due to high humid weather, followed by 5.16% in summer might be due to both strong sunlight and heavy rainfall and 3.16% in winter season might be due to low humid weather and statistically significant ($P < 0.05$) differences was found among seasons. The results of present study are in-agreement with the findings of Islam *et al.*, (2003) reported highest incidence of aspergillosis in rainy season (11.68%), than summer (5.33%) and winter (0.52%) season in Sylhet region of Bangladesh. The results also agreement with Sajid *et al.*, (2006) reported highest incidence of avian aspergillosis in summer season (54.84%) than winter (25.80%), autumn (12.9%) and spring (6.45%) seasons in Pakistan. The results also disagreement with the Uddin *et al.*, (2010) who reported highest incidence in winter season (3.95%) than rainy (3.31%) and summer (0.72%) seasons in Narsingdi of Bangladesh. This variation might be due to the different climatic factors such as humidity, temperature, rainfall etc. which influence the ecology of organism and host and geographic location of the experimental area.

The incidence of avian aspergillosis with respect to age was significantly ($p < 0.050$) higher (8.27%) in between 6-10 days of age than 11-15 days (6.85%), 16-20 days (5.33%) and lowest (4.11%) incidence shown in between 0-5 days of age. The findings are similar with Islam *et al.*, (2003) and Uddin *et al.*, (2010) who reported highest incidence of aspergillosis in between 0-7 days of age in Sylhet and Narsingdi region of Bangladesh. These findings also supported by Bennet (1988) and Sajid *et al.*, (2006) who reported that increased susceptibility of young chickens to aspergillosis might be due to immaturity of phagocytes or to environmental factors.

In the present study the incidence of avian aspergillosis was significantly ($p < 0.042$) higher in farms were being reared on saw dust (7.69%) and rice husk (3.46%) as litter. The present from results, it is obvious that litter is usually the source of infection, which is supported by Dyar *et al.*, (1984) and Rao *et al.*, (1982). The result also strongly agree with Sajid *et al.*, (2006) who reported incidence of aspergillosis increased when chicken reared on saw dust than rice husk as litter and incidence was 67.74% and 32.26% respectively might be due to high moisture content of saw dust allowing more fungal growth than rice husk.

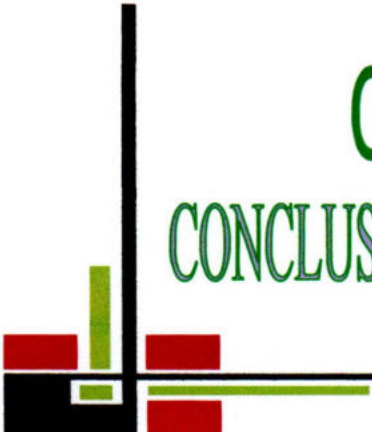
Dyspnea, gasping and accelerated breathing associated with loss of appetite, stunting growth, lethargy and increased thirst were clinically examined. The result also strongly agree with Sajid *et al.*, (2006) and Pascal *et al.*, (2011) who reported dyspnoea, gasping and nasal discharge occur in acute form of aspergillosis.

Postmortem examination revealed congestion of lungs, multiple hard creamy to yellow colored circumscribe plaques found throughout lung surface and air sacs. Similar findings were reported by Perelman and Kuttin (1992), Bhattacharya (2003) and Schmidt *et al.*, (2003) they observed gross lesions in postmortem findings of duckling, chicken, and ostrich respectively.

Congestion of pulmonary and perialveolar blood vessel and perivascular edema were found in the present study by histopathological examination that is similar with findings of Medani *et al.*, (2004). Microscopically, caseous necrosis and

necrotic cellular debris found within the granulomatous foci of lung tissues. The findings of present study was strongly agreement with the study of Akkoc *et al.*, (2009) and Badhy *et al.*, (2003) they reported the presence of caseous necrotic mass surrounded by fewer inflammatory cells in nodular lesions. Present study revealed the nodules consisted of coagulative necrotic center and densification of pleura with underlying pulmonary lobules. Similar result were demonstrated by Medani *et al.*, (2004) who reported presence of coagulative necrotic center in aspergillosis lung and Femenia *et al.*, (2007) who reported a few, more severe, inflammatory lesions were focally present on the pleura and the underlying pulmonary lobules of lungs experimentally infected with *Aspergillus fumigatus*.

In the present study, culture of *Aspergillus sp.* was carried from suspected samples on Potato Dextrose Agar (PDA) media for 7 days at $25\pm 2^{\circ}\text{C}$ temperature and identified the *Aspergillus sp.* according to the color of colony as whitish color colony for *Aspergillus fumigatus*, parrot green color colony for *Aspergillus flavus* and dark black color colony for *Aspergillus niger*. The findings of present study were in line with the findings of Reddy *et al.*, (2010) who reported the same result on rice grain in South Asia. Yokota *et al.*, (2004) who reported that white to green mold growth on the walls of caseous thickened air sacs when cultured yielded pure growth of *Aspergillus fumigatus*. Ustimenko (1982) also isolated *Aspergillus fumigatus* from lung tissue of dead chicken.



CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

From the results of present study it may be concluded that avian aspergillosis present in every farms in study area and overall incidence was 6.14% and among five Thana, Patenga shown higher (9.25%) incidence. The disease was prevailing throughout the year in Chittagong district and the incidence increases in rainy season and 6-10 days of age chicken. The saw dust litter is also has an important role in the incidence of aspergillosis in commercial broiler. The confirmatory diagnosis was done by histopathological examination and fungal culture on selective media for identification of fungal species. In the context of this study, it may be recommended that, infection with *Aspergillus* sp. in young chicks has been somewhat controlled by hatchery sanitation. Moldy litter or feed should be avoided to prevent outbreaks of aspergillosis. Examination of premises or materials used for feed or litter usually will reveal the source of infection. Areas around feed hoppers and watering places are fertile areas for growth of molds. Unless a permanent yard system is used, frequent moving of feed troughs and watering places is advisable. Drainage is advisable for areas where water is likely to stand after rains. Daily cleaning and disinfection of feed and water utensils will aid in eliminating infection. Spraying the ground around containers with chemical solutions may be advisable if it is impossible to change feeding areas frequently. In outbreaks, a 1:2000 aqueous solution of copper sulfate for all drinking water may be used to aid in preventing the spread. The mold count of contaminated litter and the mortalities due to aspergillosis can be reduced by treating the litter with nystatin and copper sulfate. Evidently, increased ventilation within poultry rearing houses reduces the airborne mycoflora suggesting that this could be used as a preventive measure in controlling aspergillosis.



REFERENCES

REFERENCES

- Abrams, G. A., Paul-Murphy, J., Ramer, J. C. and Murphy, C. J. (2001). *Aspergillus blepharitis and dermatitis in a peregrine falcon-gyr Falcon hybrid (Falco peregrinus x Falco rusticolus)*. *Journal of Avian Medicine and Surgery*, 15:114-120.
- Akan, M., Haziroğlu, R., İlhan, Z., Sareyyüpoğlu, B. and Tunca, R. (2002). A case of aspergillosis in a broiler breeder flock, *Avian Dis.* 46(2): 497-501.
- Akkoc. A., Yilmaz, R., Cangul, I. T., Ozyigit, O. M. (2009). Pulmonary Aspergillosis and Amyloid Accumulation in an Ostrich (*Struthio camelus*). *Turk. J. Vet. Anim. Sci.* 33(2): 157-160.
- Alvarez-Perez, S., Mateos, A., Dominguez, L., Martinez- Nevado, V., Blanco, J. L. and Garcia, M. E. (2010). Polyclonal *Aspergillus fumigatus* infection in captive penguins. *Veterinary Microbiology*, 144(3-4): 444-449.
- Anonymous (1997). National Poultry Improvement Plan and Auxiliary Provisions. United States Department of Agriculture. Animal and Health Inspection Services, APHIS, 91: 55-138.
- Arca-Ruibal, B., Wernery, U., Zachariah, R., Bailey, T. A., Di Somma, A., Silvanose, C. and McKinney, P. (2006). Assessment of a commercial sandwich ELISA in the diagnosis of aspergillosis in falcons. *The Veterinary Record*, 158: 442-444.
- Atasever, A. and Gumussoy, K. S. (2004). Pathological, clinical and mycological findings in experimental aspergillosis infections of starlings. *J. Vet. Med. A. Physiol. Pathol. Clin. Med.* 51(1): 19-22.
- Atkinson, R. and Brojer, C. (1998). Unusual presentations of aspergillosis in wild birds. *Proc. Assoc. Avian. Vet.* 177-181.

- Bacon, C. W. and Burdick, D. (1977). Growth of fungi in broiler houses. *Poultry science*, 56(2): 653-661.
- Badhy, S. C., Amin, K. M. R., Kabir, S. M. L., Paul, B. K. and Das, S. K. (2003). Brooders pneumonia: histopathological changes in the lungs of broiler chickens. *Int. J. BioRes.* 1(1): 55-58.
- Balachandran, C. and Ramakrishnan, R. (1987). An experimental study on the pathology of aflatoxicosis in broiler chicken. *Indian Veterinary Journal*, 64: 911-916.
- Balseiro, A., Espí, A., Márquez, I., Pérez, V., Ferreras, M. C., Marín, J. F. and Prieto, J. M. (2005). Pathological features in marine birds affected by the prestige's oil spill in the North of Spain. *Journal of Wildlife Diseases*, 41(2): 371-378.
- Bandera, A., Trabattoni, D., Ferrario, G., Cesari, M., Franzetti, F., Clerici, M. and Gori, A. (2008). Interferon-gamma and granulocyte-macrophage colony stimulating factor therapy in three patients with pulmonary aspergillosis. *Infection*, 36: 368-373.
- Bardana, E. J. (1980). The clinical spectrum of Aspergillosis Part-1 epidemiology, pathogenicity, infection in animals and immunology of *Aspergillus* CRC Crit. Rev. Clin. Lab. Sci., 13:21-83.
- Barnes, A. J. and Denning, D. W. (1993) Aspergilli-Significance as pathogens. *Rev. Med. Microbiol.*, 4:176-180.
- Barton, J. T., Daft, B. M., Read, D. H., Kinde, H. and Bickford, A. A. (1992). Tracheal aspergillosis in 6 1/2-week-old chickens caused by *Aspergillus flavus*. *Avian Diseases*, 36: 1081-1085.
- Bauck, L., Hillyer, A. and Hoefer, H. (1992). Rhinitis: case reports. Proceedings of the Annual Conference of the Association of Avian Veterinarians. New Orleans, USA. p. 134.

- Beckman, B. J., Howe, C. W., Trampel, D. W., DeBey, M. C., Richard, J. L. and Niyo, Y. (1994). *Aspergillus fumigatus* keratitis with intraocular invasion in 15-day-old chicks. *Avian Diseases*, 38: 660-665.
- Beernaert, L. A., Pasmans F, Van, W. L., Haesebrouck, F., Martel, A. (2010). *Aspergillus* infections in birds: a review. *Avian Pathol.* 39(5): 325-331.
- Bennett, J. E. (1988). Role of the phagocyte in host defense against Aspergillosis. In: *Aspergillus and aspergillosis*. Bassche, H. V., Mackenzie, D. W. and Cauwenbergh, G. Third Eds. Plenum press, New York. PP.115-119.
- Beytut, E. (2007). Immunohistochemical diagnosis of aspergillosis in adult turkeys. *Turkish Journal of Veterinary and Animal Sciences*, 31(2): 99-104.
- Beytut, E., Zcan, K. O. and Erginsoy, S. (2004). Immunohistochemical detection of fungal elements in the tissues of goslings with pulmonary and systemic aspergillosis. *Acta Veterinaria Hungarica*, 52(1): 71-84.
- Bhattacharya, A. (2003). *Aspergillus fumigatus* infection in Khaki Campbell ducks in an organized duck farm in Tripura. *Indian Vet. J.* 80(11): 1178-1179.
- Bizard and Pommay. (1887). Mycose de l'autruche. *Bull. Soc. Cent. Med. Vet.* 41: 296-302.
- Brown, P. A. and Redig, P. T. (1994). *Aspergillus* ELISA: a tool for detection and management. Main conference. In M.J. Kornelsen (Ed.). Proceedings of the Annual Conference of the Association of Avian Veterinarians. Reno, NV, USA. p. 295.
- Cacciuttolo, E., Rossi, G., Nardoni, S., Legrottaglie, R. and Mani, P. (2009). Anatomopathological aspects of avian aspergillosis. *Veterinary Research Communications*, 33(6): 521-527.

- Campbell, C. K. (1970). Electron microscopy of aspergillosis in fowl chicks. *Sabouraudia*, 8(2): 133-140.
- Carrasco, L., Lima, Jr, J. S., Halfen, D. C., Salguero, F. J., Sanchez- Cordon, P. and Becker, G. (2001). Systemic Aspergillosis in an oiled Magallanic penguin (*Spheniscus magellanicus*). *J. Vet. Med. B. Infect. Dis. Vet. Public Health*. 48(7): 551-554.
- Cary, J. W., Ehrlich, K. C., Beltz, S. B., Harris-Coward, P. and Klich, M. A. (2005). Characterization of the *Aspergillus ochraceoroseus* aflatoxin/sterigmatocystin biosynthetic gene cluster. *Mycologia*. 101(3): 352-362.
- Ceolin, L. V., Flores, F., Oliveira, I. M. C., Lovato, M., Galiza, G. J. N., Kommers, G. D., Risso, N. and Santurio, J. M. (2012). Macroscopic and Microscopic Diagnosis of Aspergillosis in Poultry. *Acta Scient. Vet.* 40(3): 1061.
- Charlton, B. R., Chin, R. P. and Barnes, H. J. (2008). Fungal infections. In: Saif Y.M., Fadly, A. M., Glisson, J. R., McDougald, L. R., Nolan, L. K. and Swayne, D. E. (Eds), *Diseases of Poultry*. Blackwell Publishing. Ames, Iowa. pp. 989- 1001.
- Chute, H. L., O'Meara, D. C. and Barden. (1962). A bibliography of avian mycosis (partially annotated). *Maine. Agr. Expt. Sta. Bull.* 5: 19-22.
- Clark, D. S., Jones, E. E., Cowl, W. B. and Ross, K. F. (1954). Aspergillosis in newly hatched chicks. *J. Am. Vet. Med. Ass.* 124: 116-117.
- Copetti, M. V., Segabinazi, S. D., Flores, M. L., Alves, S. H. and Santurio, J. M. (2004). Pulmonary aspergillosis outbreak in *Rhea americana* in Southern Brazil. *Mycopathologia*, 157(3): 269-271.
- Corde, A. C. (1997). *Icones fungorum hucusque cognitorum* Vols 1–VI, J.G. Calve (1–IV), Fr. Ehrlich (V–VI), Prague (1837-1854).

- Corkish, J. D. (1982). Mycotic tracheitis in chickens. *Avian Pathology*, 11: 627-629.
- Cortes, P. L., Shivaprasad, H. L., Kiupel, M. and Sent'ies- Cu'e, G. (2005). Omphalitis associated with *Aspergillus fumigatus* in poult. *Avian Diseases*, 49(2): 304-308.
- Cruickshank, R. H, and Pitt, J. I. (1990). Isoenzyme patterns in *Aspergillus flavus* and closely related species. In: Modern Concepts in *Penicillium* and *Aspergillus* Classification. Eds: Samson, R. A, Pitt, J. I, NATO ASI Ser, 102, pp. 259-268.
- Cui, C. B., Kakeya, H. and Osada, H. (1997). Novel mammalian cell cycle inhibitors, cyclotryprostatins A-D, produced by *Aspergillus fumigatus*, which inhibit mammalian cell cycle at G2/M phase. *Tetrahedron*. 53:59-72.
- Dahlhausen, B., Abbott. R. and VanOverloop, P. (2004). Rapid detection of pathogenic *Aspergillus* species in avian samples by real-time PCR assay: a preliminary report. In E. Bergman (Ed.). Proceedings of the 25th Annual Conference & Expo of the Association of Avian Veterinarians. New Orleans, LA, USA. p. 37.
- De Bary, A. (1854). Ueber die entwicklung und den zusammenhang von *Aspergillus glaucus* und *Eurotium*. *Botan Ztg* pp.12, 425-434; 441-451; 465-471.
- De Herdt, P. (1996). Aspergillose bij papegaaien. *Vlaams Diergeneeskundig Tijdschrift*, 65: 343-344.
- Debeaupuis, J. P., Sarfati, J., Goris, A., Stynen, D., Diaquim, M. and Latge, J. P. (1990). Extracellular polysaccharides form *Aspergillus fumigatus* and related taxa. In: Modern Concepts in *Penicillium* and *Aspergillus* Classification. Eds: Samson R. A, Pitt, J. I, NATO ASI Ser 102. Pp. 209-223.

- Debey, M. C., Trampel, D. W., Richard, J. L. Bundy, D. S., Hoffman, L. J., Meyer, V. M. and Cox, D. F. (1995). Effect of environmental variables in turkey confinement houses on airborne *Aspergillus* and mycoflora composition. *Poultry science*, 74(3): 463-471.
- Demet, Ö., Oğuz, H., Çelik, I. and Nizamlioğlu, F. (1995). Production of aflatoxin on rice. *Veteriner Bilimleri Dergisi*, 11: 19-23.
- Dennis, C. and Gee, J. M. (1973). The microbial flora of broiler house litter and dust. *Journal of General Microbiology*, 78(1): 101-107.
- Dorrestein, G. M. (1992). De benauwde papegaai. *Tijdschrift voor Diergeneeskunde*, 117: 55-57.
- Dsa, S. R., Singh, S., Satyendra, S. and Mathews, P. (2013). Case report of *Aspergillus* osteomyelitis of the ribs in an immunocompetent patient. *J. Glob. Infect. Dis.* 5(3):118-120.
- Dyar, P. M., Fletcher, O. J. and Page, R. K. (1984). Aspergillosis in turkeys associated with use of contaminated litter. *Avian Diseases*, 28(1): 250-255.
- Dykstra, M. J., Loomis, M., Reininger, K., Zombeck, D. and Faucette, T. (1997). A comparison of sampling methods for airborne fungal spores during an outbreak of aspergillosis in the forest aviary of the North Carolina Zoological Park. *J. Zoo. Wildl. Med.* 28(4): 454-463.
- Eggert, M. J. and Barnhart, J. V. (1953). A case of egg-borne aspergillosis. *J. Am. Vet. Med. Ass.* 86: 781-784.
- Eidam, E. (1883). Zur Kenntniss der Entwicklung bei den Ascomyceten. III. *Sterigmatocystis nidulans* n. sp. In F. S. Cohn, Beitr. *Bio Pflanz.* 3: 392-411.
- Fate, M. A.; Skeeles, J. K., Beasley, J. N., Slavik, M. F., Lapp, N. A. and Shriver, J. W. (1987). Efficacy of thiabendazole (Mertect 340-F) in controlling mold in turkey confinement housing. *Avian Diseases*, 31(1): 145-148.

- Fedde, M. R. (1998). Relationship of structure and function of the avian respiratory system to disease susceptibility. *Poultry Science*, 77(8): 1130-1138.
- Femenia, F., Fontaine, J. J., Fulleringer, S. L., Berkova, N., Huet, D., Towanou, N., Rakotovao, F., Granet, O. I., Le Loc'h, G., Arné, P. and Guillot, J. (2007). Clinical, mycological and pathological findings in turkeys experimentally infected by *Aspergillus fumigatus*. *Avian Pathology*, 36(3): 213-219.
- Flammer, K. and Orosz, S. (2008). Avian mycoses: managing these difficult diseases. In E. Bergman (Ed.). Proceedings of the 29th Annual Conference & Expo of the Association of the Avian Veterinarians with the Association of the European College of Avian Medicine and Surgery. Savannah, GA, USA. p. 153.
- Forbes, N. A. (1991). Aspergillosis in raptors. *Vet. Rec.* 128 (11): 263.
- Forbes, N. A. (1992). Diagnosis of avian aspergillosis and treatment with itraconazole. *The Veterinary Record*, 130: 519-520.
- Forbes, N. A. (1999). Rapaces. In: Beynon PH, Cooper JE, editors. Manual de animals exóticos. España: Harcourt Brace, 1999.
- Forbes, N. A. and Rapaces. (1999). In: Beynon, P. H., Cooper, J. E, editors. Manual de animals exóticos. España: Harcourt Brace.
- Frame, R., and Carlton, W. W. (1988). Acute toxicity of gliotoxin in hamsters. *Toxicol. Lett.* 40: 269-273.
- Friend, M. and Franson, J. (1999). editors. Field manual of wildlife diseases: general field procedures and diseases of birds. US Geological Survey.

- Frisvad, J. C. and Samson, R. A. (1990). Chemotaxonomy and morphology of *Aspergillus fumigatus* and related taxa, In R. A. Samson and J. I. Pitt (ed.), Modern concepts in *Penicillium* and *Aspergillus* classification. Plenum Press, New York, N.Y. pp. 201-208.
- Frisvad, J. C., Rank, C., Nielsen, K. F. and Larsen, T. O. (2009). Metabolomics of *Aspergillus fumigatus*. *Med Mycol.* 47(1): 53-71.
- Fuhrmann, B., Roquebert, M. F., Lebreton, V. and van Hoegaerden, M. (1990). Immunological differentiation between *Penicillium* and *Aspergillus*. In: Modern Concepts in *Penicillium* and *Aspergillus* Classification. Eds: Samson, R. A, Pitt, J. I, NATO ASI Ser 102, pp. 423-432.
- German, A. C., Shankland, G. S., Edwards, J. and Flach, E. J. (2002). Development of an Indirect ELISA for the detection of serum antibodies to *Aspergillus fumigatus* in captive penguins. *Vet. Rec.* 150(16): 513-518.
- Giambrone, J. J., Diener, U. L., Davis, N. D., Panangala, V. S. and Hoerr, F. J. (1985). Effects of aflatoxin on young turkeys and broiler chickens. *Poultry Science*, 64: 1678-1684.
- Giasuddin, M., Sil, B. K., Alam, J., Koike, I., Islam, M. R. and Rahman, M. M. (2002). Prevalence of poultry diseases in Bangladesh. *Online Journal of Biological Sciences*, 2 (4): 212-213.
- Gigli, A. C. S., Baracho, M. S., N`a`as, I. A., Silva, R. A., Zago, R. and Dall'Anese, F. P. (2005). Diagnosis and evaluation of fungi presence in the air of two different ventilation systems for broiler houses. *Brazilian Journal of Poultry Science*, 7(4): 205-208.
- Gugnani, H. C. 2003. Ecology and taxonomy of pathogenic aspergilli. *Front Biosci.* 8: 346-357.
- Hamet, N. (1990). Prophylaxie de l'aspergillose dans les ´elevages industriels de volailles. *Le Point V´et´erinaire*, 22: 23-31.

- Heidenreich, M. 1997. Birds of prey: medicine and management. Malden MA: Blackwell Science. pp. 123-125.
- Hoog, de. G. S, Guarro, J., Gene, J. and Figueras, M. J. (2000). Atlas of Clinical Fungi. Centraalbureau voor Schimmelcultures, Baarn, and Delft, The Netherlands/Universitat Rovira I Virgil, Reus, Spain, pp. 442-545.
- Hoppes, S., Gurfield, N., Flammer, K., Colitz, C. and Fisher, P. (2000). Mycotic keratitis in a blue-fronted Amazon parrot (*Amazona aestiva*). *Journal of Avian Medicine and Surgery*, 14: 185-189.
- Hudson, C. B. (1947). *Aspergillus fumigatus* infection in the eyes of baby chicks. *Poult. Sci.* 26: 192-193.
- Islam, A. A., Das, M. T. and Amin, M. R. (2009). Retrospective study of some poultry diseases at Gaibandha district in Bangladesh. *Bangl. J. Vet. Med.* 7(1): 239-247.
- Islam, M. R., Bas, B. C. and Hossain, K. (2003). Study on the occurrence of poultry diseases in Sylhet region of Bangladesh. *International Journal of Poultry Science*, 2(5): 354-356.
- Ivey, E. S. (2000). Serologic and plasma protein electrophoretic findings in 7 psittacine birds with aspergillosis. *Journal of Avian Medicine and Surgery*, 14: 103-106.
- Jenkins, J. (1991). Aspergillosis. In Proceedings of the Annual Conference of the Association of Avian Veterinarians. Chicago, IL, USA. p. 328.
- Jensen, H. E., Christensen, J. P., Bisgaard, M. and Nielsen, O. L. (1997). Immunohistochemistry for the diagnosis of aspergillosis in turkey poult. *Avian Pathology*, 26: 5-18.
- Jones, M. P. and Orosz, S. E. (2000). The diagnosis of aspergillosis in birds. *Seminars in Avian and Exotic Pet Medicine*, 9: 52-58.

- Jones, M. P., Orosz, S. E., Cox, S. K. and Frazier, D. L. (2000). Pharmacokinetic disposition of itraconazole in red-tailed hawks (*Buteo jamaicensis*). *J. Avian Med. Surg.* 14(1): 15-22.
- Jordan, F. T. and Pattison, M. 1997. Poultry diseases. 4th ed. London: Saunders. pp. 332-345.
- Joseph, V. (2000). Aspergillosis in raptors. *Seminars in Avian and Exotic Pet Medicine*, 9, 66-74.
- Julian, R. J. and Goryo, M. (1990). Pulmonary aspergillosis causing right ventricular failure and ascites in meat-type chickens. *Avian Pathology*, 9(4): 643-654.
- Jungherr, E. and Gifford, R. (1944). Three hitherto unreported turkey diseases in Connecticut. Erysipelas - hexamitiasis - mycotic encephalomalacia. *Cornell Vet.* 34: 214-226.
- Kaufman, L., Standard, P. G., Jalbert, M. and Kraft, D. E. 1997. Immunohistologic identification of *Aspergillus* spp. and other hyaline fungi by using polyclonal fluorescent antibodies. *Journal of Clinical Microbiology*, 35: 2206-2209.
- Kelly, T. R., Vennen, K. M., Duncan, R. and Sleeman, J. M. (2004). Lymphoproliferative disorder in a great horned owl (*Bubo virginianus*). *Journal of Avian Medicine and Surgery*, 18: 263-268.
- Kew, M. C. (2013). Aflatoxins as a cause of hepatocellular carcinoma. *J. Gastrointestin Liver Dis.* 22(3): 305-310.
- Kunkle, R. A. (2003). Aspergillosis, in *Diseases of Poultry*, Saif, Y. M., Barnes, H. J., Glisson J. R. , Eds., Iowa State University Press, Ames, Iowa, USA, 11th edition. pp. 883-895.

- Kuriahs, H., Itoh, M., Tsuzaki, N., Katayama, Y., Yokoyama, T. and Sugiyama, J. (1990). The ubiquinone system as a taxonomic aid in *Aspergillus* and its teleomorphs. In: Modern Concepts in *Penicillium* and *Aspergillus* Classification. Eds: Samson, R. A, Pitt, J. I, NATO ASI Ser 102, pp. 407-421.
- Lair-Fuller, S., Seguin, D., Warin, S., Bezille, A., Desterque, C., Arné, P., Chermette, R., Bretagne, S., Guillot, J. (2006). Evolution of the environmental contamination by thermophilic fungi in a turkey confinement house in France. *Poultry Science*, 85(11): 1875-1880.
- Lillehoj, E. B. and Milburn, M. S. (1973). Viriditoxin production by *Aspergillus viridi-nutans* and related species. *Appl. Microbiol.* 26(2): 202-205.
- López, R., Méndez, L. J., Hernández, F. and Castañón, R. (1995). Micología médica: procedimientos para el diagnóstico de laboratorio. México: Trillas. Pp. 85-88.
- Low, M., Berggen, A., Morgan, K. J. and Alley, M. R. (2005). Aspergillosis in a North Island robin (*Petroica longipes*). *New Zealand Veterinary Journal*, 53: 462-464.
- Lugauskas, A., Krikštonis, A. and Sveistyte, L. (2004). Airborne fungi in industrial environments—potential agents of respiratory diseases. *Annals of Agriculture and Environmental Medicine*, 11(1): 1925.
- Luna, L. G. (1968). Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd edn., Mc Graw Hill Book Co., New York.
- Lupo, C., Bouquin, S. L., Allain, V., Petetin, I., Colin, P. and Chauvin, C. (2010). Risk and indicators of condemnation of male turkey broilers in western France, February-July 2006. *Preventive Veterinary Medicine*, 94(3-4): 240-250.

- Mahajan, N. K., Jindal, N. and Kulshreshtha, R. C. (1994). Major broiler diseases in some parts of Haryana. *Indian Journal of Animal Science*, 64: 1118-1122.
- Maina, J. N. (2002). Some recent advances on the study and understanding of the functional design of the avian lung: morphological and morphometric perspectives. *Biological Reviews of the Cambridge Philosophical Society*, 77: 97-152.
- Malir, F., Ostry, V. and Novotna, E. (2013). Toxicity of the mycotoxin ochratoxin A (OTA) in the light of recent data. *Toxin Rev.* 32: 19-33.
- Marks, S. L., Stauber, E. H. and Ernststrom, S. B. (1994). Aspergillosis in an ostrich. *Journal of the American Veterinary Medical Association*, 204: 784-785.
- Martin, M. P., Bouck, K. P., Helm, J., Dykstra, M. J., Wages, D. P. and Barnes, H. J. (2007). Disseminated *Aspergillus flavus* infection in broiler breeder pullets. *Avian Diseases*, 51(2): 626-631.
- Martins, H. M., Almeida, I., Camacho, C., Costa, J. M. and Bernardo, F. A. (2012). Survey on the occurrence of ochratoxin, A in feeds for swine and laying hens. *Mycotoxin Res.* 28(2): 107-110.
- McMillan, M. C. and Petrak, M. L. (1989). Retrospective study of aspergillosis in pet birds. *Journal of the Association of Avian Veterinarians*, 211-215.
- Medani, G. G., Desouki, A. and Sobhy, N. M. (2004). Bacteriological, mycological and histopathological studies on zoo birds suffering from respiratory manifestations. *Benha. Vet. Med. J.* 15(2): 172-192.
- Meredith, A. (1997). Prophylactic administration of itraconazole for the control of aspergillosis in gentoo penguins (*Pygoscelis papua*). In The 4th Conference of the European Committee of the Association of Avian Veterinarians. London, UK. p. 227.

- Micheli, P. A. (1729). *Nova plantarum genera. Juxta tournefortii methodum disposita*. Florence.
- Moore, E. N. (1953). *Aspergillus fumigatus* as a cause of ophthalmitis in turkeys. *Poult. Sci.* 32: 796-799.
- Moretti, L.A., Dias, R. A., Telles, E. O. and Balian, S. C. (2010). Time series evaluation of traumatic lesions and airsacculitis at one poultry abattoir in the state of S~ao Paulo, Brazil (1996–2005). *Preventive Veterinary Medicine*, 94(3-4): 231-239.
- Mu"llbacher, A. and Eichner, R. D. (1984). Immunosuppression *in vitro* by a metabolite of a human pathogenic fungus. *Proc. Natl. Acad. Sci.* 81: 3835-3837.
- Mu"llbacher, A., Waring, P. and Eichner, R. D. (1985). Identification of an agent in cultures of *Aspergillus fumigatus* displaying anti-phagocytic and immunomodulating activity *in vitro*. *J. Gen. Microbiol.* 131: 1251-1258.
- Mukaratirwa, S. (2006). Outbreak of disseminated zygomycosis and concomitant pulmonary aspergillosis in breeder broiler cockerels. *J. Vet. Med.* 53: 51-53.
- Mycoses, B. L. (1997). In: Ritchie BW, Harrison GJ, Harrison LR, editors. *Avian medicine: principles and application*. Lake Worth: Wingers Publishing.
- Nardoni, S., Ceccherelli, R., Rossi, G. and Mancianti, F. (2006). Aspergillosis in *Larus cachinnans* micaellis: survey of eight cases. *Mycopathologia*, 161: 317-321.
- Nichita, I., Marcu, A., Seres, M., Tirziu, E., Mot, D. and Gros, R. V. (2010). Evaluation of fungi presence in the air of two broiler houses with different ventilation systems. *Animal Science and Biotechnologies*, 43(1): 415-418.

- Oglesbee, B. L. (1997). Mycotic diseases. In R.B. Altman (Ed.). *Avian Medicine and Surgery* 1st edn. Philadelphia, PA: W.B. Saunders Company. pp. 323-361.
- Olias, P., Hauck, R., Windhaus, H., van der Grinten, E., Gruber, A. D. and Hafez, H. M. (2010). Articular aspergillosis of hip joints in turkeys. *Avian Diseases*, 54(3): 1098-1101.
- Olson, L. D. (1969). Case report—aspergillosis in Japanese quail. *Avian Diseases*, 13(1): 225-227.
- O'Meara, D. C. and Chute, H. L. (1959). Aspergillosis experimentally produced in hatching chicks. *Avian Diseases*, 3: 404-406.
- Ostry, V., Malir, F. and Ruprich, J. (2013). Producers and important dietary sources of ochratoxin A and citrinin. *Toxins (Basel)* 5(9):1574-1586.
- Pascal, A., Thierry, S., Wang, D., Deville, M., Le Loc'h, G., Desoutter, A., F'ém'enia, F., Nieguitsila, A., Huang, W., Chermette, R. and Guillot, J. (2011). *Aspergillus fumigatus* in Poultry. *International Journal of Microbiology*, Vol. 2011 (2011), Article ID 746356.pp. 1-14.
- Peden, W. M. and Rhoades, K. R. (1992). Pathogenicity differences of multiple isolates of *Aspergillus fumigatus* in turkeys. *Avian Diseases*, 36: 537-542.
- Perelman, B. (1993). Evaluation of azole anti-mycotic agents using an experimental model of aspergillosis in turkey poults. In *Proceedings of the European Conference on Avian Medicine and Surgery*. Utrecht, the Netherlands. p.120.
- Perelman, B. and Kuttin, E. S. (1992). Aspergillosis in ostriches. *Avian Pathology*, 21(1): 159-163.
- Pérez, J. and Carrasco, L. (2000). Diagnóstico histopatológico de micosis en patología veterinaria. *Rev. Iberoam. Micol.* 17: 18-22.

- Phalen, D. N. (2000). Respiratory medicine of cage and aviary birds. *Veterinary Clinics of North America: Exotic Animal Practice*, 3: 423-452.
- Pinello, C. B., Richard, J. B. and Tiffany, L. H. (1977). Mycoflora of a turkey confinement brooder house. *Poultry Science*, 56: 1920-1926.
- Planel, R., Herault, L., Gavaret, T. and Plassiart, G. (2001). Granulomes d'origine mycosique sur des canes pékinoises futures reproductrices. *Bulletin des Groupements Techniques Vétérinaires*, 10: 247-249.
- Polonelli, L., Conti, S., Campani, L. and Fanti, F. (1990). Biotyping of *Aspergillus fumigatus* and related taxa by the killer yeast system. In: *Modern Concepts in Penicillium and Aspergillus Classification*. Eds: Samson, R. A, Pitt, J. I, NATO ASI Ser 102, pp. 225-233.
- Powell, K. A, Renwick, A. and Peberdy, J. F. (1994). (5th eds.): *The Genus Aspergillus* Plenum Press, New York.
- Rahman, M. A. and Samad, M. A. (2003). Pattern of occurrence of single and concurrent diseases associated with mortality in commercial chickens in Bangladesh. *Bangladesh Journal of Veterinary Medicine*, 1: 15-20.
- Rahman, M. M., Das, M. P., Bar, A. S. M., Islam, M. R. and Rahman, M. M. (2003). Pathological investigation of diseases of broiler in some farms of Mymensingh. *Journal of Animal and Veterinary Advances*, 2(12): 660-665.
- Raines, T. V., Kuzdas, C. D., Winkel, F. H. and Johnson, B. S. (1956). Encephalitic aspergillosis in turkeys - a case report. *J. Amer. Vet. Med. Ass.* 129: 435-436.
- Ramírez, L. J., Chávez, S. L. and Velasco, G. (2002). Reporte de un caso de aspergilosis en un ave búho cornudo (*Pseudocops clamator*) en el zoológico Miguel Alvarez del Toro, Tuxtla Gutiérrez, Chiapas. *Memorias del XIX Simposio Sobre Fauna Silvestre*, Gral. MV Manuel Cabrera Valtierra. México.

- Rao, P. N., Lakshmanachar and Rao, S. V. (1982). Pulmonary Aspergillosis in chicks. *Poultry Abstracts*. 8(4):138.
- Rashid, M. H., Xue, C., Islam, M. R., Islam, M. T. and Cao, Y. (2013). A longitudinal study on the incidence of mortality of infectious diseases of commercial broiler birds in Bangladesh. *Prev. Vet. Med.* 109(3-4): 354-358.
- Reddy, K. R. N., Farhana, N. I., Wardah, A. R., and Sallsh, B. (2010). Morphological identification of food borne pathogens colonizing rice grains in South Asia. *Pak. J. Biol. Sci.* 13(16): 794-801.
- Redig, P. (2005). Mycotic infections in birds I: aspergillosis, in *The North American Veterinary Conference Proceedings*, Eastern States Veterinary Association. pp. 1192-1194.
- Redig, P. T. (1994). Diagnosis of avian aspergillosis. In Kornelsen, M. J. (Ed.). Main Conference Proceedings of the Annual Conference of the Association of Avian Veterinarians. Reno, NV, USAp-355.
- Reese, S., Dalamani, G. and Kaspers, B. (2006). The avian lung associated immune system: a review. *Veterinary Research*, 37(3): 311-324.
- Reidarson, T. H. and McBain, J. (1995). Serum protein electrophoresis and *Aspergillus* antibody titers as an aid to diagnosis of aspergillosis in penguins. In M.J. Kornelsen (Ed.). Proceedings of the Annual Conference & Expo of the Association of Avian Veterinarians. Philadelphia, PA, USA. p-61.
- Rementeria, A., López-Molina, N., Ludwig, A., Vivanco, A. B., Bikandi, J., Pontón, J. and Garaizar, J. (2005). Genes and molecules involved in *Aspergillus fumigatus* virulence. *Rev. Iberoam. Micol.* 22(1): 1-23.
- Richard, J. L. (1997). Aspergillosis. In: Calnek BW, editor. Diseases of poultry. 10th ed. Iowa State University Press. Pp- 345-349.

- Richard, J. L. and Thurston, J. R. (1983). Rapid hematogenous dissemination of *Aspergillus fumigatus* and *A. flavus* spores in turkey poult following aerosol exposure. *Avian Diseases*, 27(4): 1025-1033.
- Richard, J. L., Dvorak, T. J. and Ross, P. F. (1996). Natural occurrence of gliotoxin in turkeys infected with *Aspergillus fumigatus*, Fresenius. *Mycopathologia*, 134: 167-170.
- Richard, J. L., Peden, W. M. and Sacks, J. M. (1991). Effects of adjuvant augmented germing vaccines in turkey poult challenged with *Aspergillus fumigatus*. *Avian Diseases*, 35: 93-99.
- Richard, J. L., Peden, W. M. and Williams, P. P. (1994). Gliotoxin inhibits transformation and is cytotoxic to turkey peripheral blood lymphocytes. *Mycopathologia*, 126: 109-114.
- Richard, J. L., (1991) *Aspergillosis* In: Disease of Poultry. 9th ed. Calnek, B. W., Barnes, H. J., Beard, C. W., Reid, W. M. and Yoder, H. W. Jr., eds. Iowa State University Press. Ames, Iowa, PP. 326-334.
- Richardson, M. D. (1998). *Aspergillus* and *Penicillium*. In: Topley & Wisons's Microbiology and Microbial Infections Medical Mycology. Eds: Ajello L, Hay R.J, Arnold, London, IV, pp. 282-312.
- Roskopf, W. and Woerpel, R. (1997). Diseases of cage and aviary birds. 3rd ed. Baltimore: Williams and Wilkins.
- Ryan, K. J. (2004). *Candida*, *Aspergillus*, and other opportunistic fungi. In Ryan, K. J. and Ray, C. G. (Ed.), *Sherris Medical Microbiology* (4th ed., pp. 659-668). USA: McGraw-Hill.
- Sajid, M. A., Khan, I. A. and Rauf, U. (2006). *Aspergillus fumigatus* in commercial poultry flocks, a serious threat to poultry industry in Pakistan. *J. Anim. Pl. Sci.* 16(3-4): 79-81.

- Saleque, M. A., Rahman, M. H. and Hossain, M. I. (2003). A retrospective analysis of chicken diseases diagnosed at the BRAC Poultry Disease Diagnostic Centre of Gazipur. *Bangladesh Journal of Veterinary Medicine*, 1: 29-31.
- Samson, R. A. and Pitt, J. I. (2000). (eds.) Integration of Modern Taxonomic Methods of *Penicillium* and *Aspergillus* Classification. Harwood, Amsterdam, The Netherlands.
- Santos, R. M. D. B., Firmino, A. A. P., de S'a, C. M. and Felix, C. R. (1996). Keratinolytic activity of *Aspergillus fumigatus* Fresenius. *Current Microbiology*, 33(6): 364-370.
- Sauter, E. A., Petersen, C. F., Steele, E. E. and Parkinson, J. F. (1981). The airborne microflora of poultry houses. *Poultry science*, 60(3): 569-574.
- Schmidt, A. (2002). Animal models of aspergillosis also useful for vaccination strategies. *Mycoses*, 45: 38-40.
- Schmidt, R. E., Schmidt, D. R. and Phalen, D. N. (2003). Pathology of pet and aviary birds. 1st Edn. Blackwell Publishing Professional. 2121 State Avenue, Ames, Iowa 50014. Pp. 23-25.
- Singh, S., Borah, M. K., Sharma, D. K. Joshi, G. D. and Gogoi, R. (2009). Aspergillosis in turkey poult. *Indian Journal of Veterinary Pathology*, 33(2): 220-221.
- Spanemberg, A., Machado, G., Casagrande, R. A., Sales, G. M., Fraga, C. F., Corbellini, L. G., Driemeier, D. and Ferreira, L. (2013). *Aspergillus fumigatus* from normal and condemned carcasses with airsacculitis in commercial poultry. *Pesq. Vet. Bras.* 33(9): 1071-1075.
- Steinlage, S. J. T., Sander, J. E., Brown, T. P., Lobsinger, C. M., Thayer, S. G. and Martinez, A. (2003). Disseminated mycosis in broiler cockerels and pullets. *Avian Diseases*, 47(1): 229-233.

- Stone, W. B. and Okoniewski, J. C. (2001). Necropsy findings and environmental contaminants in common loons from New York. *J. Wildl. Dis.* 37(1): 178-184.
- Suedmeyer, W. K., Bermudez, A. J. and Fales, W. H. (2002). Treatment of epidermal cysts associated with *Aspergillus fumigatus* and *Alternaria* species in a silky bantam chicken. *Journal of Avian Medicine and Surgery*, 16: 133-137.
- Talha, A. F. S. M., Hossain, M. M, Chowdhury, E. H., Bari, A. S. M., Islam, M. R. and Das, P. M. (2001). Poultry diseases occurring in Mymensingh district of Bangladesh. *The Bangladesh Veterinarian*. 18: 20-23.
- Taylor, M. (1993). A new endoscopic system for the collection of diagnostic specimens in the bird. In G. Jackson (Ed.). *Proceedings of the Annual Conference of the Association of Avian Veterinarians*. Nashville, TN, USA. p-83.
- Tell, L. A. (2005). Aspergillosis in mammals and birds: impact on veterinary medicine. *Medical Mycology Supplement*, 1: S7-S73.
- Tessari, E. N. C., Cardoso, A. L. S. P., Castro, A. G. M., Kanashiro, A. M. I. and Zanatta, G. F. (2004). Prevalência de aspergilose pulmonar em pintos de um dia de idade. *Arqs Inst. Biológico, São Paulo*, 71:75-77.
- Thiso, D. W., Dick, J. W., Holleman, K. A. and Labovsky Jr. P. (1978). Mold spore populations in bark residues used as broiler litter. *Poultry Science*, 57: 870-874.
- Thom, C. and Raper, K. B. (1954). *A Manual of Aspergilli*. Williams & Wilkins, Baltimore.
- Throne, S. J., Steinlage, J. E., Sander, T. P., Brown, C. M., Lobsinger, Thayer, S. G. and Martinez, A. (2003). Disseminated mycosis in broiler cockerels and pullets,” *Avian Diseases*, 47(1): 229-233.

- Tokarzewski, S., Zio'łkowska, G., Łopuszyn' ski, W. and Nozdryn-Plotnick, Z. (2007). *Aspergillus fumigatus* infection in a pigeon flock. *Bulletin of the Veterinary Institute in Pulawy*, 51(4): 563-567.
- Tortora, G. J., Funke, B. R. and Case, C. L. (2010). *Microbiology an Introduction*. 10th ed. Benjamin Cummings, New York. pp. 329-339.
- Toth, T. E. (2000). Nonspecific cellular defense of the avian respiratory system: a review. *Developmental and Comparative Immunology*, 24: 121-139.
- Toth, T. E. and Siegel, P. B. (1986). Cellular defense of the avian respiratory tract: paucity of free-residing macrophages in the normal chicken. *Avian Diseases*, 30: 67-75.
- Tsai, S. S., Park, J. H., Hirai, K. and Itakura, C. (1992). Aspergillosis and candidiasis in psittacine and passeriforme birds with particular reference to nasal lesions. *Avian Pathology*, 21: 699-709.
- Turner, W. B. and Aldridge, D. C. (1983). *Fungal metabolites (II)*. Academic Press, Ltd., London, United Kingdom. pp. 54-78.
- Uddin, M. B., Ahmed, S. S. U., Hassan, M. M., Khan, S. A. and Mamun, M. A. (2010). Prevalence of poultry diseases at Narsingdi, Bangladesh. *Int. J. BioRes.* 1(6): 09-13.
- Uddin, M. Z., Samad, M. A. and Kabir, S. M. L. (2011). Mortality and disease status in Hy-line and ISA-Brown strains of broiler chickens reared in cage system in Bangladesh. *Bangl. J. Vet. Med.* 9(1): 1-16.
- Ustimenko, A. N., (1982). Aspergillosis of fowls and sanitary condition of incubators in U.S.S.R. *Poultry Abstract.* 8(4): 138.
- Vanderheyden, N. (1993). Aspergillosis in psittacine chicks. In G. Jackson (Ed.). *Proceedings of the Annual Conference of the Association of Avian Veterinarians*. Nashville, TN, USA. p. 207.

- Verstappen, F. A. L. M. and Dorrestein, G. M. (2005). Aspergillosis in Amazon parrots after corticosteroid therapy for smoke-inhalation injury. *Journal of Avian Medicine and Surgery*, 19: 138-141.
- Verweij, P. E. and Brandt, M. E. (2007). *Aspergillus*, *Fusarium* and other opportunistic moniliaceous fungi. In P. R. Murray (Ed.), (9th ed.), Washington D.C. ASM Press. pp. 1802-1838.
- Watkins, C. V. (1940). Some remarks on two diseases affecting the lungs and airsacs in poultry. *Vet. J.* 96: 207-210.
- Wehmer, C. (1991). Entstehung und physiologische Bedeutung der Oxalsäure im Stoffwechsel einiger Pilze. *Botan Ztg.* 49: 233-246.
- Wehmer, C. (1992). Die Pilzgattung *Aspergillus* morphologischer, physiologischer und systematischer Beziehung. *Mem. Soc. Phys. Hist. Nat.* 33: 1-57.
- Williams, C. J. and Brake, J. (2000). Evaluation of application methods for control of *Aspergillus fumigatus* proliferation on the air cell membrane of in ovo injected broiler eggs. *Poult. Sci.* 79: 1531-1535.
- Xavier, M. O., Soares, M. P., Meinerz, A. R. M. Nobre, M. O., Osório, L. G., Filho, R. P. S. and Meireles, M. C. A. (2007). Aspergillosis: a limiting factor during recovery of captive magellanic penguins. *Brazilian Journal of Microbiology*, 38(3): 480-484.
- Yokota, T., Shibahara, T., Wada, Y., Hiraki, R., Ishikawa, Y. and Kadota, K. (2004). *Aspergillus fumigatus* infection in ostrich (*Struthio camelus*). *J. Vet. Med. Sci.* 66(2): 201-204.
- Young, E. A., Cornish, T. E. and Little, S. E. (1998). Concomitant mycotic and verminous pneumonia in a blue jay from Georgia. *J. Wildl Dis.* 34(3): 625-628.



Zafra, R., Pérez, J., Pérez-Ecija, R. A., Borge, C., Bustamante, R., Carbonero, A. and Tarradas, C. (2008). Concurrent aspergillosis and ascites with high mortality in a farm of growing broiler chickens. *Avian Diseases*, 52(4): 711-713.