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Research Article

Pathological investigation of avian Aspergillosis in commercial broiler chicken at Chittagong district

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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%).

Keywords: *Aspergillus* **,** Incidence**,** Litter

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).

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 mm 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

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:

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.

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.

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.

Experimental layout

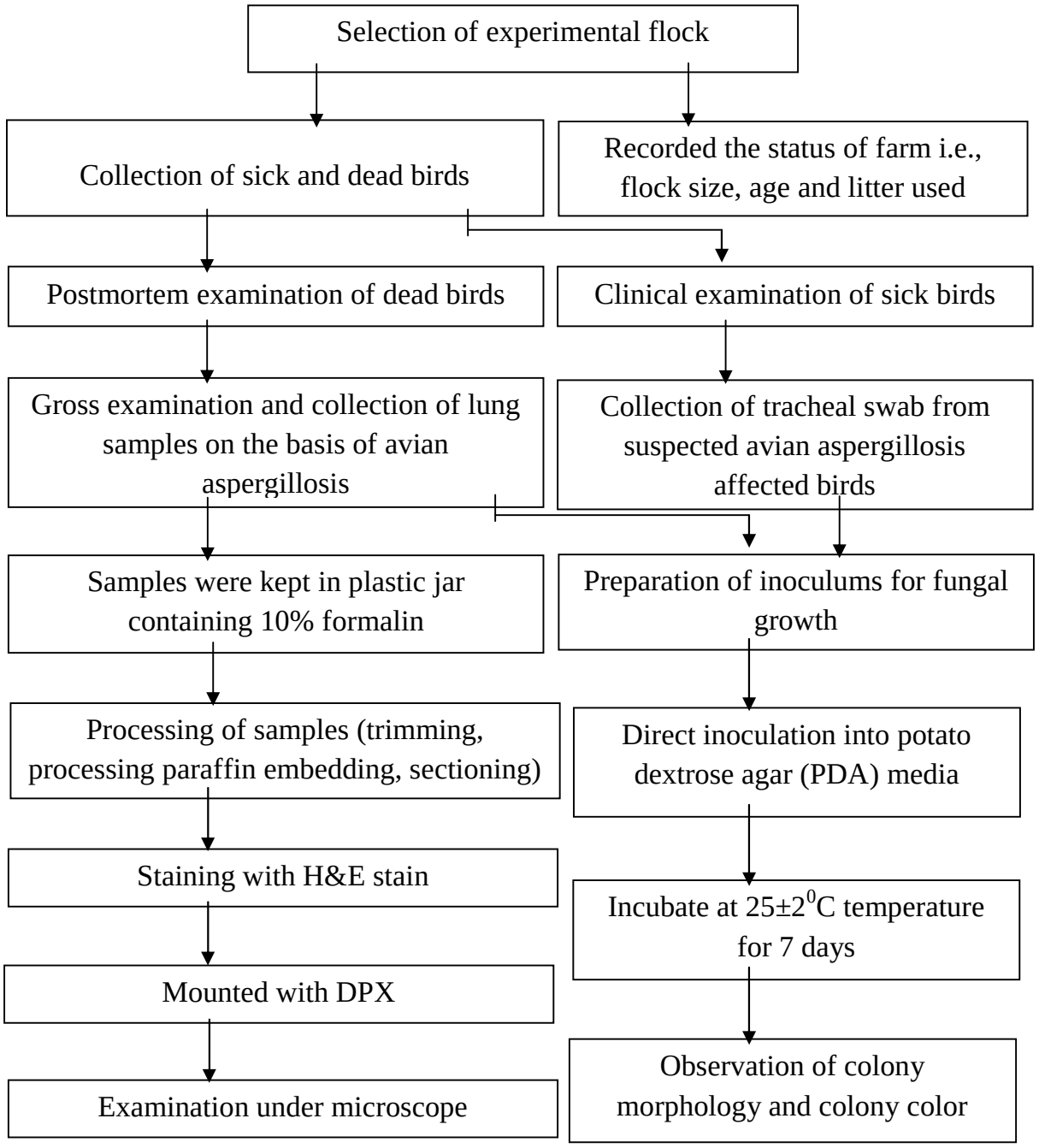

Figure 1. Schematic illustration of the experimental layout

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.

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.

Methods

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).

Mycological culture of samples

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 121^0C temperature for 15 minutes, 15Ibs/ cm^2 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 ± 2^0C temperature for 7 days. After 7 days, observed the colony morphology and colony color to identify the *Aspergillus* sp.

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.

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.

Results

Incidence study

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.

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.

Age wise incidence

Incidence of avian aspergillosis was analyzed on the basis of age of broiler chicken. The incidence was

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 ($p<0.01$)

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 ($p<0.05$)

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.

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.

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).

Pathological study of avian aspergillosis

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.

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).

Mycological examination

The suspected samples (tracheal swab, lung sample after postmortem) were inoculated on Potato Dextrose Agar (PDA) media for 7 days at 25 ± 2^0C 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).

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 (%) ±SE
0-5	5	4900	178	4.11^{b} ±0.86
6-10	5	3350	276	8.27^{a} ±1.06
11-15	5	3420	235	6.85^{ab} ±1.01
16-20	5	4525	223	5.33^{ab} ±1.07
P value				0.050
Level of significant				*

*Significant ($p<0.05$)

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 ($p<0.05$)

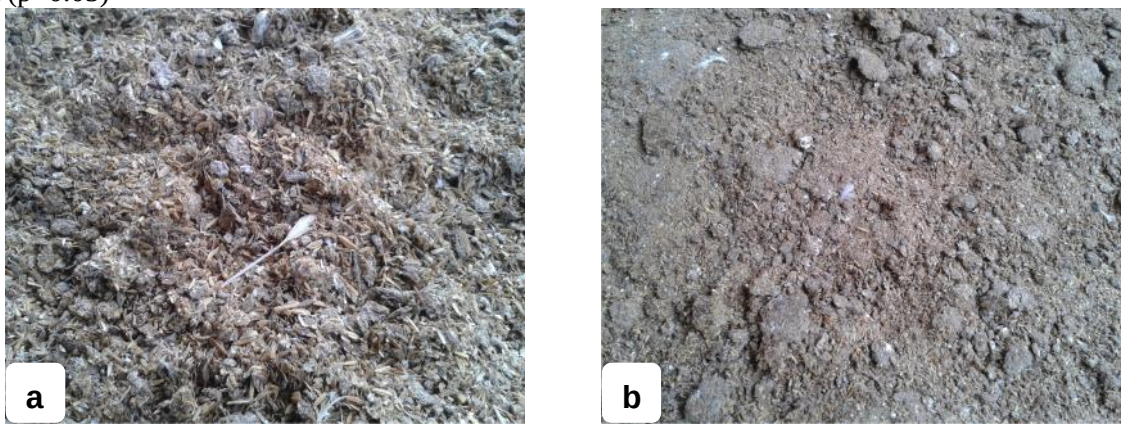

Figure 2.a. Rice husk litter status of experimental flock. **b.** Saw dust litter status of experimental flock

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

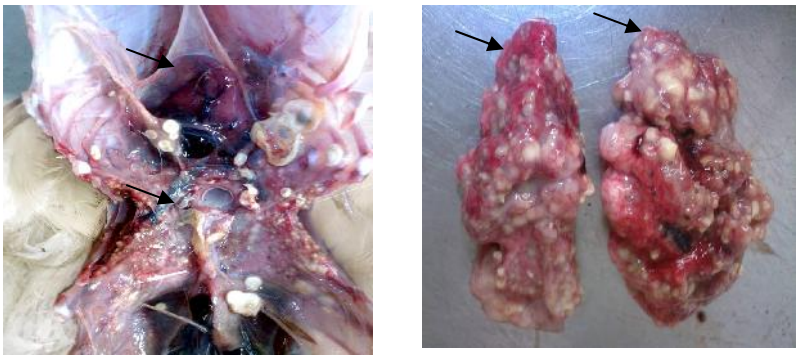

Figure 4. a. Lung shows the presence of cream color nodules in plural surface, air sacs (arrow). **b**. Creamy to yellow color nodules shows throughout the lung (arrow).

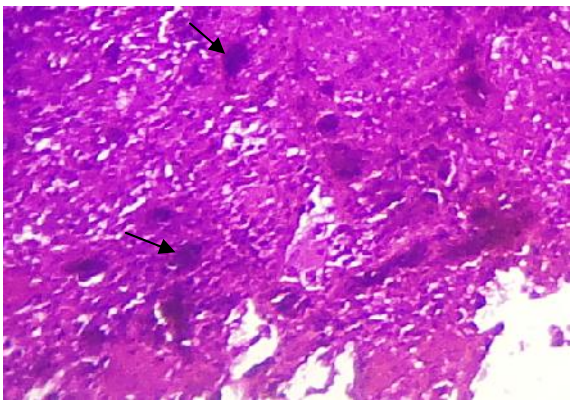

Figure 5. Showing congestion of pulmonary, perialveolar blood vessel and diffuse edema of pulmonary tissues (arrow) in Lung. H&E stain, 10×

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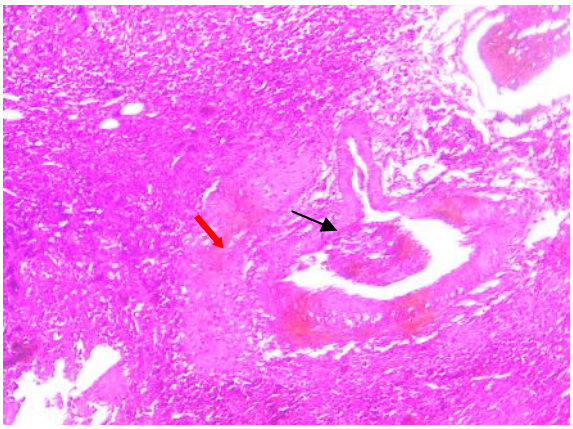

Figure 6 . Areas of caseous necrosis (black arrow) and cellular debris (red arrow) in lung. H&E stain, 10×.

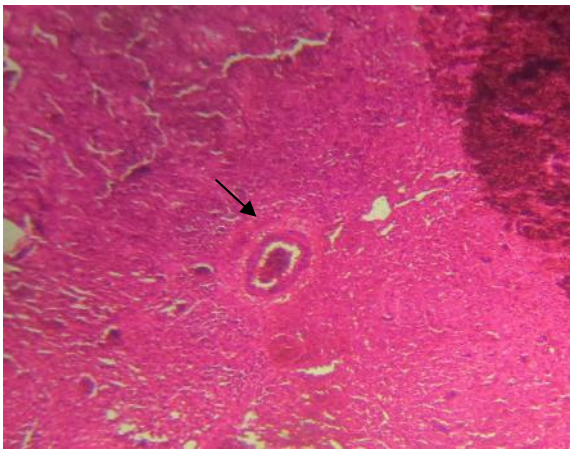

Figure 7. Aspergillosis showing granuloma formation with caseated center (arrow) in lung. H&E stain, 10×.

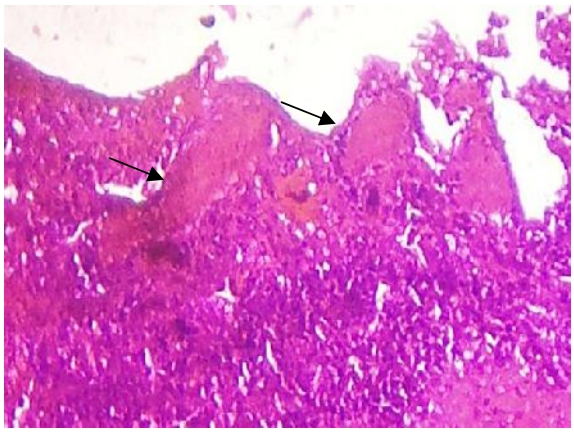

Figure 8. Diffuse densification of the pleural parenchyma by congestion and an inflammatory cellular infiltration (arrows) in lung. H&E stain, 10×.

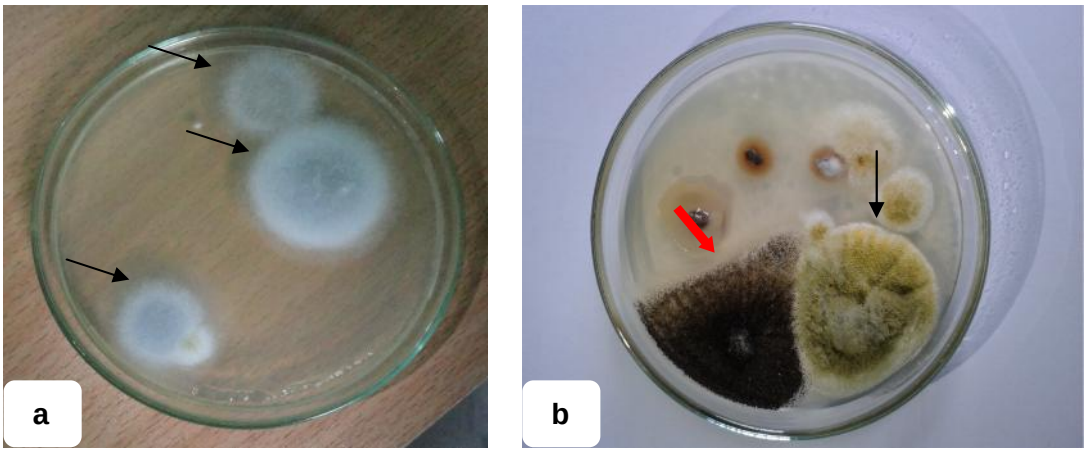

Figure 9. a. PDA media shows whitish color colony of *A. fumigatus* (arrows). **b.** PDA media shows parrot green color colony of *A. flavus* (black color arrow) and dark black color colony of *A. niger* (red color arrow)

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 ± 2^0C 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.

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