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PREVALENCE AND PATHOLOGICAL INVESTIGATION OF FOWL TYPHOID IN COMMERCIAL POULTRY FARMS AT THE RAJSHAHI CITY CORPORATION AREA OF BANGLADESH

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ABSTRACT

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The present study was conducted to determine the prevalence of Fowl typhoid in apparently healthy, sick and dead birds at Rajshahi city corporation area of Bangladesh. A total of 500 birds (50 from each farm) and 30 different organs (liver from 10, ovary from 8, heart from 7, and caecal tonsils from 5) were randomly collected from different commercial poultry farms during the period from January 2018 to December 2018. The prevalence study was performed based on history, clinical signs, symptoms exhibited by the individual bird of a flock during the observation of farms, and illness of birds. The suspected birds were subjected to necropsy examination. During sample collection, clinical signs and gross necropsy changes were recorded very carefully. The collected tissues were fixed, processed, sectioned, stained, and studied light microscopically. The routine histopathological method was used for the detection of tissue-level alterations in Fowl typhoid infected cases. The prevalence of Fowl typhoid in apparently healthy birds of different poultry farms was 8.2%, and 23.33% of organs were involved. Grossly, the liver was enlarged, congested and revealed bronze discoloration with focal necrosis in the surface of the liver. Old raised hemorrhages were found in the caecal tonsils. Congested, deformed, and pedunculated ova were other important findings. Microscopically, the sections of the liver showing multifocal necrosis with infiltration of heterophils and reticulo-endothelial cells.

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INTRODUCTION

The poultry industry is an emerging agribusiness starting practically during 1980 in Bangladesh (Huque, 2001). Poultry is essential to the national economy and the welfare of human beings in Bangladesh. It is one of the most important sources of animal protein needed for all classes of people through supplying of meat and eggs (Khandokar et al., 1994). Poultry rearing plays a very important role in income generation and poverty reduction, particularly for the distressed women and unemployed youths in Bangladesh by means of self-employment (BBS, 2006). Almost every rural family usually keeps 10-20 chickens, ducks, or pigeons that are traditionally maintained by the female members of the family and fed on household waste and crop residues (Saleque, 2001; Rahman et al., 2003). Bangladesh livestock population statistics indicate poultry as the most important species of farm animal. A total of 98.15% of poultry are kept in rural area, and they are scavengers (BBS, 1987). Recently, poultry rearing has been developed as an industry in Bangladesh. At present, there are more than 150 hatcheries producing 7 million day-old-chicks per week and about 150,000 commercial broiler and layer farms supplying 570 million tonnes of poultry meat and 1552 crore table eggs per year (BBS, 2018). However, this sector is also facing a lot of constraints. Among the different constraints, bacterial infections are the major problem. Fowl typhoid is one of these (Haider et al., 2008; Islam et al., 2006). It is one of the most important bacterial diseases in the poultry industry, causing heavy economic losses through mortality and reduced production (Haque et al., 1997). A survey on both breeding flocks of commercial broiler and layer in major poultry raising belts in and around Dhaka and Gazipur District in Bangladesh was conducted by (Saleque et al., 2003) and reported 16.9% Salmonella infections among the infectious diseases in the breeding flock and 23.2% in layer farms. The disease is most significant because the causal agent of the disease is transmitted mainly vertically from parent to offspring. The disease is potentially responsible for various pathogenic processes in poultry (Freeman, 1985). It is distributed worldwide, and natural outbreaks occur in chicken, turkey, guinea fowl, peafowl, duckling, quail, and pheasant either in acute or chronic form.

The disease may cause a variety of clinical signs, from acute systemic disease and gastrointestinal disorders to the embryonic problem in the hatchery. It is cosmopolitan in distribution worldwide (Bhattacharjee et al., 1996; Shivaprasad, 1997). In recent years, poultry farming has been hampered by the outbreak of fatal infectious diseases caused by bacteria, viruses, mycoplasma, and other causal agents in Bangladesh (Ahmed and Hamid, 1991). The advancement of the poultry industry is being hampered seriously due to the outbreak of some bacterial diseases.

Village chickens can act as a reservoir of Salmonella, and thus a prophylactic campaign must be taken into account (Bouzoubaa et al., 1992). The epidemiology of Fowl typhoid in poultry, particularly with regard to transmission from one generation to the next, is known to be closely associated with infected eggs. Transmission is primarily through the egg but also occurs via direct or indirect contact with infected birds. Infection transmitted via egg or hatchery contamination usually results in death during the first few days of life up to 2-3 weeks of age (Wigley et al., 2001). The birds that survive from clinical disease when infected at a young stage may show few signs of infection but can become carriers (Berchieri et al., 2001).

Considering this fact, the present study was undertaken to know the prevalence of Fowl typhoid in selected poultry farms and the pathological lesions in Fowl typhoid infected birds at the Rajshahi city corporation area, which will certainly help to effective prevention and control measures for the expansion of poultry industry in Bangladesh.

MATERIALS AND METHODS

Study area and sample collection

Samples were collected from 10 different poultry farms of the Rajshahi city corporation area. A total of 500 birds from 10 poultry farms were collected for observation of Fowl typhoid infection, and 30 representative organs from sick and dead birds were collected from necropsy cases at different poultry farms during the study period from January 2018 to December 2018. These samples were collected in a plastic jar for histopathological examination containing 10 percent neutral buffered formalin.

Brief description of the experimental design

Samples were collected from 10 different poultry farms at the Rajshahi city corporation area, namely Grameen Poultry and Hatchery, Babu Broiler House, Ismail Store, Ashikur Layer House, Nur Islam Farm, Jony Farm, Sentu Farm, Milon Poultry Farm, Bablu Farm, and Shamim Farm. The prevalence study of Fowl typhoid was performed based on history, clinical signs, symptoms exhibited by an individual bird of a flock during illness were recorded in detail in a prescribed

form, as provided by the respective farm's owner or attendant. Detail histories including clinical signs, flock size, breed, age, sex of chicken, sources from which day-old birds were collected, rearing system, history of vaccination, date of the outbreak occurred, number of birds affected, number of birds died, previous outbreaks of the disease and control measures taken in any, were recorded.

Pathological studies

The pathological studies were carried out at specific places in and around the selected research area. The postmortem examination of all the cases was performed for the sick and dead birds. At necropsy, gross tissue changes were observed and recorded carefully by systemic dissection, and representative tissue samples containing lesions were fixed in 10% neutral buffered formalin for histopathological studies. The samples were brought to the Laboratory of the Department of Veterinary and Animal Sciences, University of Rajshahi. The formalin-fixed tissues were trimmed and placed in 10% neutral buffered formalin overnight and processed following standard procedure; the detailed methodology has been described elsewhere (Bondoc et al., 2016). Briefly, the tissues were dehydrated through ascending grades of alcohol, cleared in xylene, and embedded in paraffin. The tissue sections, cut at five microns in thickness by using a rotary microtome (Mosbi, China), were stained by Haematoxylin and Eosin (H&E) method (Bancroft and Gamble, 2007). The sections were examined under a light microscope at low and high magnification. Photographs from the selected sections were grabbed using a photographic microscope system (Camera model: LC-20, Labomed, Inc., USA fitted with microscope model: MBL-2100, Kruss, Germany).

RESULTS

Prevalence of fowl typhoid in apparently healthy chickens

The prevalence of Fowl typhoid was 8.2% in apparently healthy chickens (Table 1 & Fig. 1). The prevalence of Fowl typhoid infection was 23.33% in sick and dead birds. On an organ basis, 30% in liver, 20% in caecal tonsils, 12.5% in the ovary, and 28.57% in heart out of 30 sick and dead birds, and the infection was highest in the liver (Table 2 & Fig. 2).

Table 1. Prevalence of Fowl typhoid infection in apparently healthy birds at different poultry farms

No. of farms	No. of Birds randomly examined	No. of (+ve) cases	Farm-wise prevalence (%)	Total no. (+ve) of cases	Overall prevalence (%)
1	50	6	12%		
2	50	3	6%		
3	50	5	10%		
4	50	3	6%		
5	50	4	8%		
6	50	4	8%	41	8.2%
7	50	2	4%		
8	50	4	8%		
9	50	5	10%		
10	50	5	10%		

Table 2. Prevalence of Fowl typhoid infection from different organs of sick and dead chickens

Samples	No. of samples examined	No. of (+ve) cases	Organ-wise prevalence (%)	Total no. of positive cases	Overall prevalence (%)
Liver	10	3	30%		
Caecal tonsils	5	1	20%	7	23.33%
Ovary	8	1	12.5%		
Heart	7	2	28.57%		

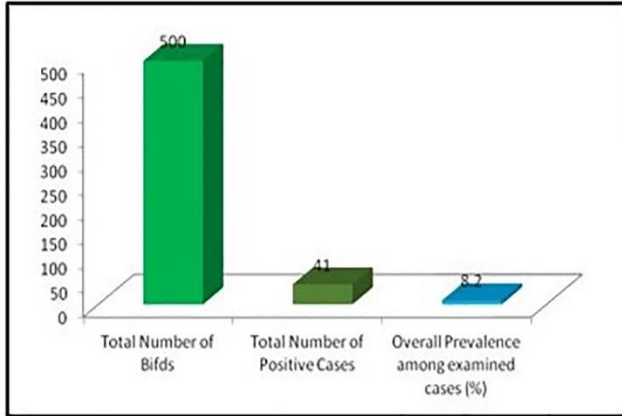


Figure 1. Prevalence of Fowl typhoid in apparently healthy birds at different poultry farms

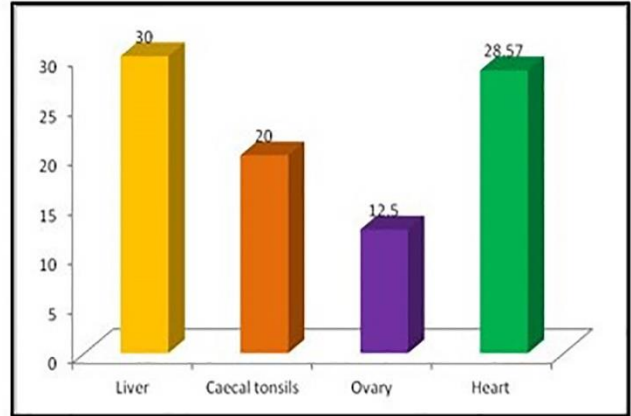


Figure 2. Prevalence of Fowl typhoid cases in different organs at study area

Pathological study

Gross necropsy findings

Necropsy findings showed the liver was enlarged and congested and, in few cases, revealed friable, bronze discoloration with white focal necrosis (Fig. 3). In the heart, there was the presence of inflammation in the pericardium, nodules in the myocardium (Fig. 4). Old raised hemorrhages were found in the caecal tonsil (Fig. 5). Congested, deformed, discolored, and pedunculated ova were other significant findings (Fig. 6).

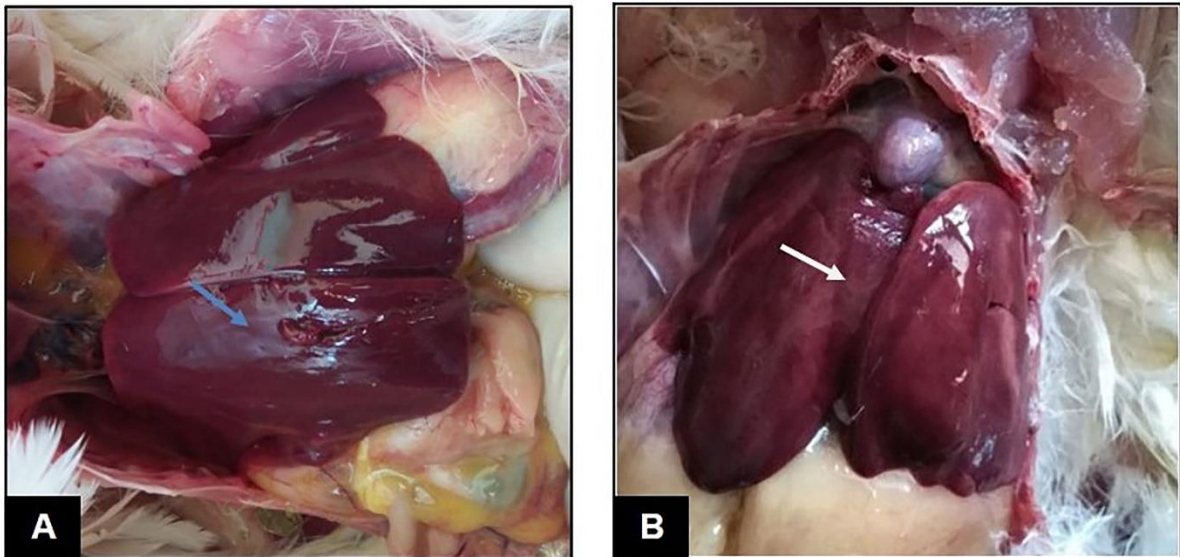


Figure 3. Liver of Fowl typhoid infected chickens showing congested, friable, bronze discoloration with focal necrosis (blue arrow A and white arrow B).

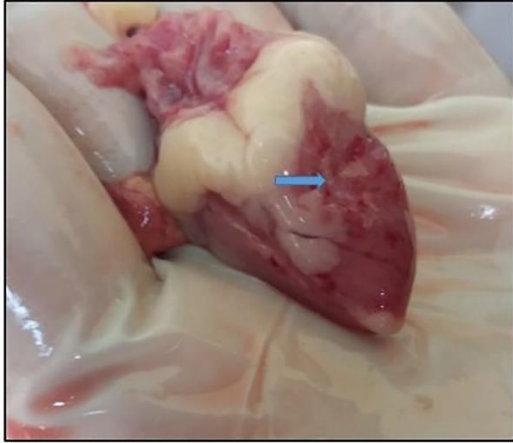


Figure 4. Fowl typhoid infected heart showing inflammation in the pericardium, and nodules in the myocardium.



Figure 5. Fowl typhoid affected chicken showing old raised hemorrhages in caecal tonsils.

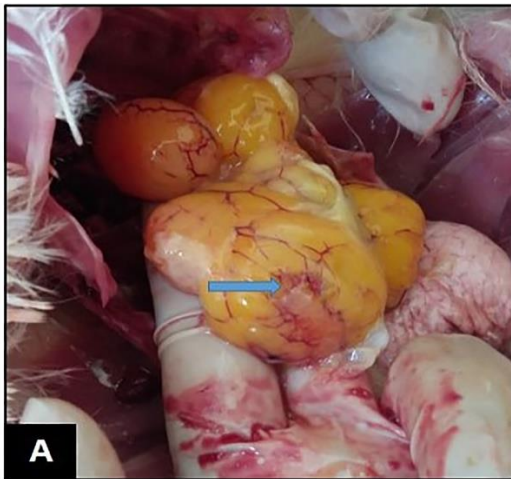


Figure 6. Fowl typhoid infected chickens showing congested, deformed, discolored (A) and pedunculated ova (B).

Histopathologic findings

The section of liver showing multifocal coagulation type of necrosis, accumulation of large number of inflammatory cells mainly lymphocytes, RE cells, and heterophils around the central vein (Fig. 7). Fowl typhoid affected the liver showing focal necrosis with the limited number of inflammatory cells around the central vein and infiltration of the huge amount of inflammatory cells in the focal area (Fig. 7).

DISCUSSION

The present study was conducted primarily to determine the prevalence of Fowl typhoid infection. The study was carried out in 30 commercial chickens subjected to post mortem examination based on clinical signs. During this study, the overall prevalence of Fowl typhoid was recorded as 8.2%, which supports the findings of Shahid et al. (2012); Uddin et al. (2010) and Bell et al. (1990), where they showed the prevalence as 8.8%; 7.68% and 6.2%, respectively.

But the findings of Robinson et al. (2000); Jha et al. (1995); Ghosh (1988), 18.4%, 21.3%, and 13.9%, respectively, were higher prevalence than the present study. Again, Mdegela et al. (2000) reported 2.6%, Lu et al. (1992) 2.0%

prevalence which was much lower than the present study. This difference may be due to the geographic location, farm management, or bio-security in the farm, age and breeds of the birds, and also for the resistance power of the commercial chickens.

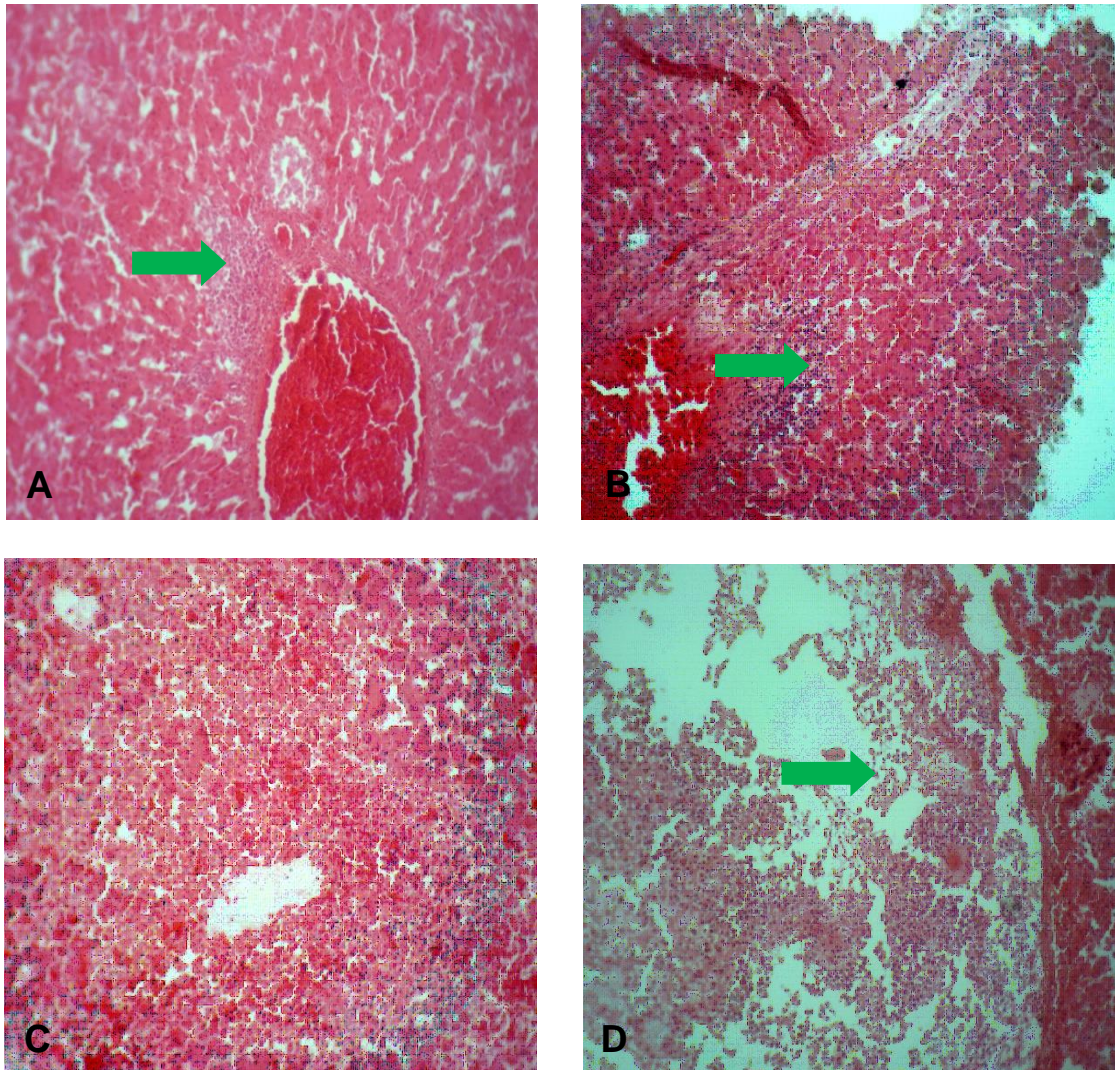


Figure 7. The section of the liver of Fowl typhoid affected birds at 33 weeks of age, showing (A) coagulation type of necrosis, (B) accumulation of a large number of inflammatory cells, mainly lymphocytes, RE cells, and heterophils around the central vein. Fowl typhoid affected liver of 11 weeks chicken showing (C) focal necrosis with a limited number of inflammatory cells around the central vein and (D) infiltration of the huge amount of inflammatory cells in the focal area.

The present study was conducted to determine the prevalence of Fowl typhoid infection in commercial chickens which during postmortem examination. To characterize the diseases, the pathological study was done by necropsy and histopathological examination in apparently sick and dead birds.

The liver as a vital organ for Fowl typhoid infection produced gross necropsy findings in chickens. These include discoloration, enlargement, mottling, hemorrhages, nodulating abscesses, and necrotic foci Habib-ur-Rehman et al. (2003) which are close to the present study. Pathological lesions of Fowl typhoid in dead birds included enlargement with foci of necrosis on the liver Msoffe et al. (2006). Grey-white miliary foci observed in the liver Beyaz and Kutsal (2003); Goswami et al. (2003) which are similar to the present study.

The present study showed similar results that of experimental infection of Fowl typhoid in Fayoumi and Hyline layer chickens where they observed the most common gross necropsy changes were enlarged liver with necrotic foci. Some liver were dark red-colored, some were a bronze-colored shiny appearance on the surface of the liver Hossain et al. (2003). Hepatomegaly, bronze discoloration, mottling, congestion, whitish necrotic foci on hepatic parenchyma observed in another experiment Khan et al. (1998); Hafeji et al. (2001); Shivaprasad (2000); Kinde et al. (2000); Prasanna and Paliwal (2003).

The present study is similar to Fowl typhoid in poultry detected in caeca were severe congestion, mild hemorrhage, and mononuclear cell infiltration in mucosa and submucosa with degeneration and desquamation of lining epithelium Hafeji et al. (2001). In the ovarian follicles, fibrino-suppurative inflammation has been observed by Kinde et al. (2000).

In the pathological investigation, grossly, the liver was enlarged and congested with focal necrosis, darker ovary with stalk formation. Microscopically, the liver showed focal necrosis with infiltration of mononuclear cells. These types of lesions are supported Fowl typhoid infection by different investigations Rahman et al. (2004); Paul et al. (2014).

CONCLUSION

The findings of this study indicated that a huge amount of poultry is affected by Fowl typhoid organism and causing heavy economic losses through mortality and reduced production of that area. Congestion in the ovary with stalk formation, hemorrhage in caecal tonsils, friable, bronze discoloration in the liver, and microscopically multifocal necrosis with infiltration of mononuclear cells could indicate the presence of Fowl typhoid infection. This is the first time in the study area. This study will help in the prevention and control of Fowl typhoid infection. However, further investigation should be focused on the determination of immunogenic variation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES

1. Ahmed S and MA Hamid, 1991. Status of poultry production and development strategy in Bangladesh. Workshop in Livestock Development of Bangladesh, BLRI, Dhaka, Bangladesh.
2. BBS (Bangladesh Bureau of Statistics), 2018. Bangladesh Bureau of Statistics, Sher-e-Bangla Nagar, Dhaka, Bangladesh.
3. BBS (Bangladesh Bureau of Statistics), 1987. Bangladesh Bureau of Statistics. The Bangladesh Census of Agriculture and Livestock, 1983-84.
4. Bell JG, M Kane and C Lejan, 1990. An investigation on diseases status of village poultry in Mauritania. *Veterinary Medicine*, 8: 291-294.
5. Berchieri A, P Wigley, KL Page, AL Smith and PA Barrow, 2001. *Salmonella enterica* serovar Pullorum persists in splenic macrophages and in the reproductive tract during persistent infection. *Diseases free carriage in chickens. Infectious Immunology*, 69: 591-592.
6. Beyaz L and O Kutsal, 2003. Pathological and immunohistochemical studies in experimental *Salmonella gallinarum* infection (Fowl typhoid) in chickens. *Ankara Universitesi Veteriner Fakultesi Dergisi*, 50: 219-227.

7. Bancroft JD and M Gamble, 2007. Theory and practice of histological techniques. 6 edition. Churchill livingstone, London, UK, Pp. 744.
8. Bondoc A, C Katou-Ichikawa, HM Golbar, M Tanaka, T Izawa, M Kuwamura and J Yamate, 2016. Establishment and characterization of a transplantable tumor line (RMM) and cell line (RMM-C) from a malignant amelanotic melanoma in the F344 rat, with particular reference to galectin-3 expression in vivo and intro. *Histology and Histopathology*, 31: 1195-1207.
9. Bhattacharjee PS, RL Kundu, RK Biswas, JU Mazumder, E Hossain and AH Miah, 1996. A retrospective analysis of chicken diseases diagnosed at Central Disease Investigation Laboratory, Dhaka. *The Bangladesh Veterinarian*, 130: 105-113.
10. Bouzoubaa K, K Lemainguer and JG Bell, 1992. Village chickens as a reservoir of *Salmonella pullorum* and *Salmonella gallinarum* in Morocco. *Preventive Veterinary Medicine*, 12: 95-100.
11. Bangladesh Bureau of Statistics (BBS), 2006. Bangladesh Census of Agriculture (Rural) 1996. Vol. 1. Bangladesh Bureau of Statistics, Ministry of Planning, Government of the People's Republic of Bangladesh.
12. Freeman BA, 1985. Burrow's Text Book of Microbiology. 22nd edn. W. R. Saunders Company, London, UK, 63: 372-472.
13. Ghosh SS, 1988. Incidence of pullorum disease in Nagaland. *Veterinary Microbiology*, 65: 949-951.
14. Goswami P, A Chakraborti, AK Hui, R Das, P Sarkar and TL Som, 2003. Isolation and identification of *Salmonella gallinarum* form field cases and their antibiogram. *Veterinary Microbiology*, 80: 184-185.
15. Habib-ur-Rehman S, HK Sirzanin, K Saleem, A Nazir and WM Bhatti, 2003. Incidence and gross pathology of Salmonellosis in chicken in Hyderabad. *Veterinary Advances*, 2: 581-584.
16. Hafeji YA, DH Shah, BP Joshi, A Roy and KS Prajapati, 2001. Experimental Pathology of field isolates of *Salmonella gallinarum* in chickens. *The Indian Journal of Medical Research*, 36: 338-340.
17. Haider MG, EH Chowdhury, AHNA Khan, MT Hossain, MS Rahman, HJ Song and MM Hossain, 2008. Experimental Pathogenesis of Pullorum Disease with the Local Isolate of *Salmonella enterica* serovar. enterica subspecies Pullorum in Pullets in Bangladesh. *Korean Journal of Poultry Science*, 35(4): 341-344.
18. Haque ME, MA Hamid, MAR Howleder and QME Huque, 1997. Performance of native chicks and hens reared together or separately under rural condition in Bangladesh. *Bangladesh Veterinary Journal*, 8: 11-13.
19. Huque QME, 2001. Poultry industry in Bangladesh and strategies for its improvement. In: Proceedings of 2nd international poultry show and seminar, February 2001, held in IBD Bhaban, Dhaka, Bangladesh.
20. Hossain MA, B Aalbaek, P Christensen, H Elisabeth, MA Islam and K Pankaj, 2003. Observations on experimental infection of *Salmonella gallinarum* in Fayoumi and byline layer chickens in Bangladesh. *Journal of Poultry Science*, 14: 85-89.
21. Islam MM, MM Hossain, MG Haider, EH Chowdhury and M Kamruzzaman, 2006. Seroprevalence and pathological study of Salmonella infections in layer chickens and isolation of causal agents: In Proceedings of the 5th International Poultry show and seminar from 01-03 march 2007, held in Bangladesh China Friendship Conference Centre (BCFCC), Sher-e-Bangla Nagar, Dhaka, Bangladesh, 9: 15-65.
22. Jha VC, RP Thakur, TK Chand and JN Yadav, 1995. Prevalence of Salmonellosis in chickens in the Eastern Nepal. *Veterinary Bulletin*, 65: 7.
23. Khan AHNA, ASM Bari, MR Islam, PM Das and MY Au, 1998. Pullorum disease in semi mature chickens and its experimental pathology. *The Bangladesh Veterinary Journal*, 32: 124-128.
24. Khandoker MSA, MSR Khan, AJ Sarker, MM Rhaman, PM Das and MM Amin, 1994. Physicochemical and serological studies of a fowl pox virus vaccine. *Bangladesh Journal of Microbiology*, 17: 121-126.
25. Kinde I, HL Shivaprasad, BM Daft, DH Read, A Ardans, R Breitmeyer, G Rajashekara, KV Nagaraja and IA Gardner, 2000. Pathologic and bacteriologic findings in 27-week-old commercial laying hens experimentally infected with *Salmonella enteritidis*, phage type 4. *Avian Diseases*, 44: 239-48.
26. Lu YS, LH Lee, YK Liao, CS Tseng, DF Lin, HK Shieh, ML Chien, HT Sung, YL Lee and HJ Ysai, 1992. Serological survey on antibodies against Newcastle disease, infectious bursal disease, chronic respiratory disease and pullorum disease in chicken breeder flocks in Taiwan. *International Journal of the Chinese Society of Veterinary Science*, 18: 53-58.

27. Mdegela RH, MGS Yongolo, UM Minga and JE Olsen, 2000. Molecular epidemiology of *Salmonella gallinarum* in chickens in Tanzania. *Avian Pathology*, 29: 457-463.
28. Msoffe PLM, UM Minga, MMA Mtambo, PS Gwakisa and JE Olsen, 2006. Differences in resistance to *Salmonella enterica* serovar Gallinarum infection among indigenous local chicken ecotypes in Tanzania. *Avian Pathology*, 35: 270-276.
29. Paul PK, MG Haider, R Khaton, SK Ghosh, PM Das and MM Hossain, 2014. Pathological investigation of Fowl typhoid in chickens in Mymensingh. *Annals of Bangladesh Agriculture*, 18: 33-41.
30. Prasanna K and OP Paliwal, 2003. Experimental fowl typhoid and pullorum disease in chickens, clinical and pathomorphological studies. *International Journal of Veterinary Pathology*, 26: 27-29.
31. Rahman MH, MA Saleque and MI Hossain, 2003. Seasonal variation in the prevalence of poultry disease in Bangladesh. 9th BSVR Annual Scientific Conference held at BAU, Mymensingh. BSVR Publication, 24: 23-24.
32. Rahman KMS, K Hamayun, A Nazir and WM Bhatii, 2004. Incidence and gross pathology of *Salmonella gallinarum* infection in chicken. *Journal of Animal and Veterinary Advances*, 3: 175-178.
33. Robinson F, MGS Mdegela, U Yongolo, M Minga and E Johin, 2000. Molecular epidemiology of *Salmonella gallinarum* in chickens in Tanzania. *Avian Pathology*, 29: 457-463.
34. Saleque MA, MH Rahman and MI Hossain, 2003. Seasonal variation in the prevalence of poultry disease in Bangladesh. 9th BSVR Annual Scientific Conference held at BAU, Mymensingh. BSVR Publication, 24: 23-24
35. Saleque MA, 2001. Poultry as a tool in poverty alleviation: a special program for the rural poor in Bangladesh. Proceedings of the 2nd International Poultry show and seminar, Dhaka, Bangladesh. The Worlds Poultry Science Association, Bangladesh Branch, pp. 66-67.
36. Shahid N, SA Kamil and M Shahnawaz, 2012. Prevalence and Seasonality of Fowl Typhoid in Kashmir Valley. *Journal of Pure and Applied Microbiology*, 6(1): 472-475.
37. Shivaprasad HJ, 1997. Pullorum disease and Fowl typhoid. In: Calnek, B.W., Jarnes, H.J., Beard, C.W., McDougald, L.R., and Saif, Y.M. [Editors]. *Diseases of Poultry*, 10th (eds), Ames, IA: Iowa State University Press, USA, 82-96 pp.
38. Shivaprasad IL, 2000. Fowl typhoid and pullorum disease. *Revolution Standard Technology*, 19: 405-424.
39. Uddin MZ, MA Samad and SML Kabir, 2010. Mortality and disease status in Hi-line and Isa brown strains of layer chickens reared in cage system in Bangladesh. *Bangladesh Journal of Veterinary Medicine*, 9(1): 1-16.
40. Wigley P, A Berchieri, KL Page, AL Smith and PA Barrow, 2001. *Salmonella enterica* serovar Pullorum persists in splenic macrophages and in the reproductive tract during persistent infection. Diseases free carriage in chickens. *Infectious Immunology*, 69: 591-592.