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RESEARCH ARTICLE

Comparative Epidemiological Study of Infectious Bursal Disease of Commercial Broiler Birds in Bangladesh and China

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ABSTRACT

A systematic field study was undertaken on the outbreaks of infectious bursal disease (IBD) during the period from June 2010 to May 2011 in commercial broiler farms of Bangladesh (562) and China (89). Overall prevalence due to the disease was recorded as 12.1 and 7.1% for Bangladesh and China, respectively, and mortality rate was 5.3 and 2.4% for Bangladesh and China, respectively. Dullness, depression, anorexia, ruffled feathers, inability to move and yellowish white diarrhea were observed in almost all the IBD-affected flocks. At necropsy, the gross lesions were observed mainly in the bursa of Fabricius followed by changes in thigh and breast muscles. Outbreaks of the disease were recorded throughout the year. Seasonal influence of IBD showed a significantly ($P<0.05$) higher prevalence 13.5% and mortality rate 7.7% in winter season in Bangladesh. On the other hand, in China, significantly higher prevalence 6.5% and mortality rate 3.2% was found in summer season. Significantly higher prevalence and mortality rate was observed in the young birds (≤5 weeks of age) than older birds in both the countries. The outbreak of IBD was also found in vaccinated birds ($P<0.05$) of the two countries. The findings of the study indicated the difference of occurrence of IBD in Bangladesh and China, thereby would help to develop appropriate control strategies for both the countries

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INTRODUCTION

Infectious bursal disease (IBD), also known as Gumboro disease, is highly contagious and immuno-suppressive disease of young chickens caused by infectious bursal disease virus (IBDV) and is responsible for major economic losses in the poultry industry globally (Anjum *et al*., 1993; Okwor *et al*., 2012). The disease is characterized by the destruction of the lymphoid organs, in particular the bursa of Fabricius (BF), where IBDV infects the actively dividing and differentiating lymphocytes of the B-cells lineage (Nouën *et al*., 2012), resulting in lymphopenia (immune-suppression) and secondary infection of the infected birds (Mahgoub, 2012). Therefore, increases susceptibility to opportunistic pathogens such as Newcastle disease, Marek's disease and infectious bronchitis, and lowers responsiveness to vaccination (Homer *et al.,* 1992; Khan *et al.,* 2011). Chickens are most susceptible to clinical infection from 3-6 weeks of age (Ley *et al*., 1983).

The prevalence of diseases in a particular area depends on various factors like geo-climatic condition, management practices, immunization status, social awareness etc. The outbreaks of various diseases are directly or indirectly related to the management status or biosecurity of the farms. So, emphasis should be given to improve the management or biosecurity of the farm to check the mortality of chickens. A thorough knowledge about the epidemiology, pathogenesis and pathology of a particular disease is the first and fundamental factor for proper diagnosis of a malady, as well as, in the prevention, control and eradication of the disease. Therefore, the present study was undertaken to determine comparative epidemiology of IBD of Bangladesh and China.

MATERIALS AND METHODS

Study period and areas: The duration of the present study was 12 months from June 2010 until May 2011. Active surveillance was conducted in 8 locations of Bangladesh: Bera, Santhia, Shahjadpur, Sirajgong sadar, Narayangonj sadar, Gazipur sadar, Balaganj, Sylhet sadar of different districts (Fig. 1). In case of China, surveillance was confined in 8 locations of Guangdong provience: Xing Guang, Yi Keng, Huang Keng, Luo Bu, Guang Yan, Geng Lou, Jia Qing and Shui Tai of different districts (Fig. 2). The districts, locations and the farms were randomly selected by using multistage as well as simple random sampling technique.

Fig. 1: Locations of farms in Bangladesh where outbreak of infectious bursal disease occurred during June 2010-May 2011.

Fig. 2: Locations of farms in Guangdong province, China where outbreak of infectious bursal disease occurred during June 2010-May 2011.

Nature of the study and collection of information: In Bangladesh, the department of livestock services provides veterinary services through Upazila Livestock Offices (ULO). The list of poultry farms was collected from the ULO. In case of China, list of poultry farms was provided by WEN'S group (Guangdong Food Co. Ltd; the company provides services to those farms). The poultry farms were randomly selected from the list of firms. Minimum number of chickens required for each variable was 96 based on the formula $n=Z^2 PQ/L^2$ (Biswas *et al.*, 2005), where n is the sample size, P is the expected prevalence, $Q = 1 \square P$ and L is the required precision. As the prevalence was not known, 20% (P=0.20) was considered in this calculation; a precision of 5% was considered (L=0.05) with the confidence level 95% (i.e. Z=2). The occurrences of diseases were measured by two methods: prevalence of the disease and mortality rate of the disease. Prevalence of the disease was calculated by the number of affected bird as numerator and the number of total birds as denominator, and multiplied by 100. Mortality rate was calculated by the number of dead birds during the study period as numerator and the number of total birds as denominator, and multiplied by 100.

In Bangladesh, 8 field technicians were recruited (one for each location) for collection of epidemiological data and tissues (bursa of Fabricius) from dead birds. All tissues collected were frozen at the ULO, and then sent to Department of Medicine and Surgery, Sylhet Agricultural University by the technicians for further investigation. In case of China, WENS group's field technicians collected epidemiological data and tissues (bursa of Fabricius) from dead birds and sent to the their laboratory for investigation.

Diagnosis of disease: The disease was diagnosed by following the criteria in the OIE manual (OIE, 2009) and on the basis of history, clinical findings, post-mortem changes and RT-PCR techniques. The agar gel precipitation test was used for confirmation of the infectious bursal disease virus (IBDV), where bursal homogenate was used as a crude source of antigen. The methodology used for the identification of IBDV was previously described by Ren *et al.* (2009). Briefly, RNA was extracted from the bursal tissues by TRIzol method and the extracted RNA was subjected to RT-PCR using VP2 gene specific primers. The amplification products were detected by gel electrophoresis (Bio Rad, Hemel Hempstead, U.K.) in 1% agarose gel.

Data analysis: All statistical analyses were conducted using SPSS (SPSS, USA), the descriptive statistics was expressed as proportion with 95% confidence interval (CI). For Chi-square test, the difference between parameters was regarded as significant when the P value was less than 0.05.

RESULTS

Occurrence of the disease: A total of 562 flocks in Bangladesh and 89 flocks in China were affected with IBD of the two countries during the 12 months study period. The overall prevalence of IBD was 12.13% (CI, 12.05-12.21) in Bangladesh and 7.11% (CI, 7.05-7.17) in China. The mortality rate of IBD was 5.25 % (CI, 5.20-5.25) in Bangladesh and 2.42% (CI, 2.38-2.46) in China (Fig. 3).

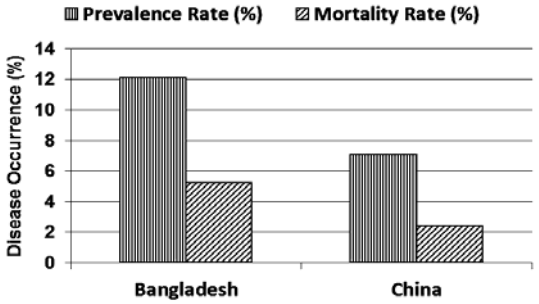

Fig. 3: Occurrence of IBD in broiler birds in Bangladesh and China (June 2010-May 2011).

Effect of age: The details of IBD in different age groups of broiler chicks are presented in Table 1 and 2. In both the countries, age specific prevalence and mortality rate revealed that chicks below 5 weeks of age were significantly higher ($P<0.05$) susceptible to IBD than chicks over 5 weeks of age. The disease was not recorded in chicks of less than 10 days of age in any of the country.

Temporal and topographical distribution: IBD was found in all seasons in the study area. Significantly ($P<0.05$) higher prevalence (13.47%) and mortality rate (7.73%) was recorded in the winter season followed in order by rainy, autumn and summer in Bangladesh (Table 1), on the other hand, in case of China, significantly higher prevalence (6.53%) and mortality rate (3.16%) was found in summer season and the lowest prevalence (4.25%) and mortality rate (1.94%) was found in winter season (Table 2). Topographical study revealed that IBD was consistently prevalent in all the locations of the study area of both the countries. Location of the farms have significant effect on the prevalence and mortality of IBD ($P<0.05$) (Table 1 and 2).

Effect of vaccine: Of the 562 IBD-affected flocks of Bangladesh, 176 flocks were administered booster dose of IBD vaccine while the remaining 386 flocks were administered single dose of IBD vaccine. Prevalence and mortality rate were relatively higher in the one dose vaccinated flocks 13.17 and 7.66%, respectively than in the two dose vaccinated flocks 9.81 and 5.87%, respectively; the difference was statistically significant ($P<0.05$) (Table 1). Of the 562 IBD vaccinated flocks, the correct information regarding the type of vaccine used was provided by the owners of the 427 flocks only; live, intermediate and intermediate plus strains of vaccine were used in the study area. All the flocks of China were vaccinated with VAXXITEK® HVT+IBD vaccine against IBD according to the manufacturer's recommendation.

RT-PCR: RT-PCR was done for all the bursal tissues of both the countries. Of the bursal tissues from 651 flocks, 597 were positive for RT-PCR. In positive cases, a band of 1339-bp length of VP2 gene was observed (Fig. 4).

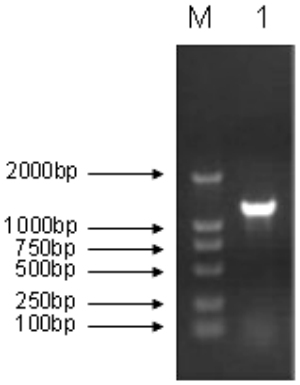

Fig. 4: cDNA of the VP2 gene synthesized by RT-PCR from Bangladesh and Chinese isolates (M: 2000bp DNA Marker; 1: VP2 gene of IBDV).

DISCUSSION

The results of the present study help us to understand the differential epidemiology of IBD of the two countries and design prevention and control measures of the disease. The overall prevalence of IBD (12.13%) in Bangladesh was consistent with the earlier study of Hossain *et al.* (2010). The mortality rate of IBD (5.25%) in this study was similar with previous report of Shil *et al.* (2003). On the other hand, in China, overall prevalence (7.11%) and mortality rate (2.42%) was similar with earlier study of Zhang *et al.* (2009) and it was lower than Bangladesh due to better prevention and control strategies of the country. The clinical findings and post-mortem lesions recorded in this study both in China and Bangladesh are identical with previous study (Zelek *et al*., 2005). Differences in severity of the disease occurred due to clinical and necropsy findings. The differences in

Table 1: Prevalence and mortality rate of IBD in broiler birds in Bangladesh (June 2010- May 2011)

Variables	Category	No of Flocks	Flock size	Prevalence		P value	Mortality		P value
				No.	%		No.	%	
	Gazipur	65	75653	9551	12.6		5456	7.2	
	Sirajgong	71	82661	10009	12.1		5679	6.9	
	Bera	78	91386	10578	11.6		6662	7.3	
Location	Shahjadpur	63	73256	8649	11.8	<0.05	5413	7.3	<0.05
	Santhia	87	102289	10513	10.3		7027	6.9	
	Narayangang	62	72457	9252	12.8		5369	7.4	
	Balagang	70	82563	11658	14.1		6035	7.3	
	Sylhet	66	76524	9462	12.4		5057	6.6	
	Winter (Dec to Feb)	175	198980	26805	13.5		15381	7.7	
	Summer (March to May)	132	156982	162369	10.3		10329	6.6	
Season	Rainy (June to August)	74	82572	10565	12.8	<0.05	5951	7.2	<0.05
	Autumn(Sep to Nov)	181	218255	26066	11.9		15037	6.9	
Age	<5 week	410	482740	58943	12.2	<0.05	35481	7.4	
	>5 week	152	174049	20729	11.9		11217	6.4	
Immunization	Booster dose vaccination	176	203855	19989	9.8	<0.05	11966	5.9	<0.05
	one dose vaccination	386	453134	59683	13.2		34732	7.7	

Table 2: Prevalence and mortality rate of IBD in broiler birds in China (June 2010-May 2011)

Variables	Category	No of Flocks	Flock size	Prevalence		P value	Mortality		P value
				No.	%		No.	%	
	Xing Guang	11	78494	3981	5.1		1554	2.0	
	Yi Keng	12	80951	4379	5.4		1951	2.4	
	Huang Keng	13	93967	5651	6.0		3195	3.4	
Location	Luo Bu	14	105123	5186	4.9	<0.05	2292	2.2	<0.05
	Guang Yan	10	76109	3709	4.9		2093	2.8	
	Geng Lou	10	76634	4107	5.4		1832	2.4	
	Jia Qing	10	83076	4636	5.6		1487	1.8	
	Shui Tai	9	69721	3232	4.6		1667	2.4	
	Winter (Dec to Feb)	16	156788	6663	4.3		3042	1.9	
	Summer (March to May)	13	72052	4703	6.5		2278	3.2	
Season	Rainy (June to August)	32	218680	13033	6.0	<0.05	5576	2.6	<0.05
	Autumn(Sep to Nov)	28	216555	10482	4.8		5175	2.4	
Age	<5 week	68	498056	27642	5.6		13746	2.8	
	>5 week	21	166019	7238	4.4		3320	2.0	
Immunization	Booster dose vaccination	89	664075	34881	5.2		16071	2.4	<0.05
	One dose vaccination [a]								

[a]In China all the flocks were vaccinated with booster dose vaccine against IBD, there were no one dose vaccinated flocks.

occurrence might have due to the differences in management practices and biosecurity measures in two countries. As observed in this study, traffic control and hygienic management of the farms were not strictly maintained in Bangladesh. The keeping of used litter adjacent to farms was the common practice in Bangladesh. Moreover, movement of infected material via contaminated vehicles, clothing and shoes, and/or wild chickens were important factors of disease spreading (Boon *et al*., 2007). On the other hand, opposite scenario was found in China, the farmers are well aware of hygienic management of the farms, follows biosecurity, prevention and control measures of the disease. However, other factors such as age, health and nutritional condition of the birds, season, type of virus, concomitant infection, vaccination status etc might have effect on the occurrence of IBD (Sunil *et al*., 2010).

Season had significant effect on prevalence and mortality of IBD (P<0.05). Though the disease was found throughout the year in both the countries, in Bangladesh, the occurrence was higher (13.47%) in winter season. Higher mortality in winter season was also described by earlier researchers (Farooq *et al*., 2003; Biswas *et al*., 2005). The practice of inadequate and inappropriate disinfection of poultry sheds due to cold conditions, and keeping of sheds air tight by some polythene sheets in order to maintain the required temperature inside the poultry house as most of the broiler farms in Bangladesh are not environmentally controlled might be the possible explanation behind this. Air tight condition of poultry sheds result in airborne generation of IBDV because of close contact of birds (Sunil *et al*., 2010).

Significantly (P<0.05) higher prevalence and mortality was observed in summer in case of China. The reasons were higher temperature (36-39ºC) in southern China during summer. Therefore, combined effects of high temperatures and IBDV infection caused higher mortality. At the same time, heavy rainfall caused high humidity in the environment which also significantly increases the mortality of chicks. Similar findings were also previously reported (Jindal *et al*., 2004; Yi *et al.,* 2009).

Age of the bird had a significant relationship on prevalence and mortality of the disease. IBD was recorded with a significantly higher proportion (P<0.05) in chicks below 5 weeks of age in both the countries. Jindal *et al*. (2004) and Saif *et al*. (2000) also reported that broiler chicks of age 3-6 weeks were more susceptible to the disease. MDA protect chicks from IBD for first two weeks, proper vaccination protect birds from IBD in later stage of life. Topographic study showed significant relationship with occurrence of the disease but the cause was not understood.

Vaccination practice had a significant relationship with the occurrence of the disease. Prevalence and Mortality was significantly higher (P<0.05) in chicks administered one dose vaccine than in those vaccinated booster dose vaccine according to the recommended schedule. Properly vaccinated birds developed more resistance and better immunity against IBDV than others (Farooq *et al*., 2002). Outbreak of IBD in vaccinated flocks have also been described previously by Anku (2003) in Southern Ghana, Islam and Samad (2003) in Bangladesh, Jindal *et al.* (2004) in India, Wang *et al*. (2009) in China. They opined that factors like improper vaccination, poor biosecurity measures and existence of very virulent strains of IBD virus contributed to the occurrence of IBD in the vaccinated flocks.

Modified live vaccines ("mild", "intermediate", "intermediate plus", and "hot") of different manufacturers are generally used in Bangladesh and China. Mild and intermediate vaccines are safer, in that they cause less bursal damage than "hot" vaccines, but have a poor efficacy in the presence of MDA and against vvIBDV. On the other hand, "intermediate plus" and "hot" vaccines can overcome higher levels of MDA, but they may cause more severe lesions in the follicles of bursa Fabricius, resulting in immunosuppression (Van den Berg, 2000; Müller *et al*., 2003). There are some predisposing factors such as overcrowding of the birds, improper constructed brooder house, poor ventilation in the farm were responsible for disease to occur in the vaccinated flocks. Beside those factors, there are some factors in agreement with the findings of Islam and Samad (2003) and Sunil *et al*. (2010), i.e., improper vaccination practices, mistreatment of the vaccine, break in cooling chain during transport and at the farm, use of chlorinated water during vaccination, exposing vaccine virus to outside environment for a longer period to time, mixed infections with other pathogens are the persuasive factors for the occurrence of disease in the vaccinated flocks.

Conclusion: The prevalence and mortality rate of IBD is lower in China than Bangladesh due to many reasons; among them most important is the biosecurity and preventive measures are strictly followed by the Chinese farmers. IBD was prevalent throughout the year but frequency of IBDV infections increased with weather change where winter season was found most vulnerable in Bangladesh and summer season was most vulnerable in China. There were occurrences of IBD in vaccinated flocks in the study areas. IBD is strongly associated with age of the birds. Young birds were more susceptible to IBD in the study areas. It is necessary to follow biosecurity measures and vaccinate birds regularly with appropriate strain of vaccine. Furthermore, regular surveillance and identifications of field strains would help in making effective control strategies.

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