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Epidemiological Studies on Infectious Bursal Disease in Broiler Chickens in Haryana, India

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Abstract: The epidemiological data of Infectious Bursal Disease (IBD) in 483 broiler chicken flocks were analyzed from July, 2005 to June, 2008. Overall morbidity, cumulative mortality and case fatality rate due to the disease were recorded as 4.54, 2.34 and 51.69%, respectively. Of the total IBD-affected flocks, 192, 211, and 80 flocks were affected during the years 2005-06, 2006-07 and 2007-08, respectively. Dullness, depression, anorexia, ruffled feathers and inability to move were evident in almost all the IBD-affected flocks. The affected birds had yellowish white or greenish yellow diarrhoea. At necropsy, the gross lesions were observed mainly in bursa of Fabricius followed by changes in thigh and breast muscles. The disease was recorded throughout the year. Maximum cases (52.80%) were observed in birds of 21-30 days of age followed by 33.13% in the age group of 31-40 days. Although the disease was observed in both IBD-vaccinated (334) and unvaccinated (149) flocks, percent morbidity and cumulative mortality were higher in unvaccinated flocks than in the vaccinated flocks. Factors like improper vaccination, poor biosecurity measures and existence of very virulent strains of IBD virus could be the reasons for disease in the vaccinated flocks. Continuous surveillance may help in better understanding of the epidemiology of infectious bursal disease virus in broiler chickens in this region.

Key words: Infectious bursal disease, epidemiology, broiler chickens, Haryana

INTRODUCTION

Infectious Bursal Disease (IBD) is an acute, highly contagious and immunosuppressive viral disease of poultry caused by a birna virus. After its first outbreak in poultry in Southern Delaware in the United States in 1962 (Cosgrove, 1962), the disease has been recorded from all over the world (Muller *et al*., 2003). Before 1987, the IBD was essentially sub-clinical in most parts of the world and was satisfactorily controlled by vaccination using attenuated strains. Thereafter, the vaccination failures have been reported from different parts of the world. In the United States, new IBD strains caused a slight increase in mortality; whereas, in Europe and Asia, new virulent strains caused increased mortality in layers and broiler chickens (Chettle *et al*., 1989; Van Den Berg *et al*., 1991).

In India, the disease was first reported by Mohanty *et al*. (1971). It was observed in its classical form until early 1990's with mortality upto 30%. Unvaccinated chicken flocks experiencing heavy mortality in Gurgaon region of Haryana manifested appreciably high ELISA antibody titres for Infectious Bursal Disease Virus (IBDV) on seromonitoring (Asrani *et al*., 1993). Considerably high mortality upto the tune of 70% due to IBD was recorded in late 1990's with the emergence of Very Virulent (vv) strains of IBDV that resulted in heavy economic losses to the poultry industry in the country (Sah *et al*., 1995). According to OIE annual animal disease status, 281, 185, 167, 247, 260 and 177 outbreaks of IBD were recorded during the years 2002, 2003, 2004, 2005, 2006 and 2007, respectively in India (http://www.oie.int/wahis/public.php). Such data provide useful information about the prevalence of a disease in an area/country which may help in understanding the epidemiology of the disease in a better way. During past two decades, the state of Haryana, a north-western state of India, has made rapid strides in poultry farming resulting in phenomenal growth of poultry industry in the state with many small and marginal farmers opting for commercial broiler chicken farming for their livelihood. According to 18th livestock census of 2007, total poultry birds in the state were 2,98,69,402 out of which only 3,54,490 were backyard poultry birds and the remaining 2,95,14,912 were domestic poultry (http://pashudhanharyana.gov.in/html/dist_livestock_census.htm). In this paper, we present epidemiological data pertaining to IBD in commercial broiler chicken flocks from July, 2005 to June, 2008 in parts of Haryana. This work is in continuation to our previous report on epidemiological investigation of IBD in commercial chicken in this state (Jindal *et al*., 2004).

MATERIALS AND METHODS

Collection of data: The epidemiological data pertaining to IBD in commercial broiler chickens from July 2005 to June 2008 were collected from Hisar, Jind, Bhiwani,

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Sirsa and Fatehabad districts of Haryana state. The Department of Veterinary Public Health and Epidemiology provides diagnostic facilities to the livestock and poultry farmers of the state through a network of disease investigation laboratories (DI Labs). Live and/or dead birds are brought by the poultry farmers to these laboratories for disease investigation on routine basis. Detailed information such as total birds in a flock, number of birds affected, number of birds died, age of the affected birds, month of occurrence of the disease, vaccination status and type of IBD vaccines used was obtained from the affected flocks. Clinical findings and gross pathological changes in the IBD-affected birds from each flock were also recorded.

In order to study the temporal distribution of the disease, the year was divided into four quarters viz. July-September (A), October-December (B), January- March (C) and April-June (D). Quarters B and C comprised of the winter season; D, the summer season and A, the rainy season. The data so collected were analyzed statistically using Z test to draw the inferences following the procedure of Snedecor and Cochran (1980).

Confirmation by reverse transcription-polymerase chain reaction (RT-PCR): In most cases, the disease was diagnosed on the basis of history, clinical findings and post-mortem changes. Bursa samples of three to four birds from a flock were collected in 50% buffered glycerine. Such samples were collected from 40 IBD-affected flocks. The samples were tested for the presence of IBDV nucleic acid by RT-PCR. The methodology used for the detection of IBDV was essentially the same as described by Mittal *et al*. (2005). Briefly, total RNA was extracted from the samples using TRIzol method and the extracted RNA was subjected to RT-PCR using VP2 gene specific primers. The amplified PCR products were gel electrophoresed and a band of 643 bp was observed in positive cases.

RESULTS

Occurrence of the disease: A total of 483 flocks were affected with IBD in the five districts of Haryana during the specified period of three years. During the year 2005-06, 8.75% of the total flocks which were brought to the DI Labs had IBD. These figures were 6.29 and 2.27% for the years 2006-07 and 2007-08, respectively. Analysis of the data revealed that 192, 211 and 80 flocks were affected during the years 2005-06, 2006-07 and 2007-08, respectively. A total of 1,10,278 birds (4.5%) were affected during three year period with a cumulative mortality of 56,998 (2.3%) birds. Percent morbidity varied from 4.1-4.9% while percent cumulative mortality varied between 2.0-2.5% during the period under study. Case Fatality Rate (CFR) due to the disease was 51.7% (41.3-59.7% range) (Table 1). The CFR was significantly higher during 2006-07 as compared to that in 2005-06. Though the CFR during 2006-07 was higher than that in 2007-08, the difference was not statistically significant (Table 1).

Temporal distribution: The disease was recorded in all quarters with maximum number of IBD-affected flocks (n = 145) in quarter A followed by quarters D (n = 137), B (n = 101) and C (n = 100) (Table 2). Percent morbidity varied from 3.3-5.6%. It was significantly higher in quarter B than that in quarter C. Though percent morbidity in quarter B was higher than that in quarters A and D; the difference was not statistically significant. Cumulative mortality in all the four quarters varied from 1.7-2.9%; it was comparable in quarters A, B and D (2.2-2.9%) and the lowest in quarter C (1.7%). Though the CFR in quarter A was higher than that in other quarters, however, the difference was significant only in comparison to quarter B (Table 2).

Effect of age: The details of IBD in different age groups of broiler chicks are presented in Table 3. The disease was not recorded in chicks of less than 10 days of age. Of the total IBD-affected flocks, 255 (52.8%) flocks had birds of 21-30 days of age and 160 (33.1%) had birds of 31-40 days of age. Remaining flocks had birds of other age groups (less than 20 days of age and more than 40 days of age). Though percent morbidity due to the disease was higher in broiler chicks of 31-40 days of age than the other age groups, however, the difference was significant when compared to that in birds of 41-50 days of age. Cumulative mortality varied from 1.8-2.7% in all age groups. Though the cumulative mortality was higher in birds of 21-30 and 31-40 days of age as compared to other age groups; the difference was not statistically significant. The CFR was maximum (68.0%) in birds of 41-50 days of age while it varied from 46.5-56.4% in birds of other age groups. The CFR in birds of 41-50 days of age was significantly higher than that in birds of 11-20 and 31-40 days of age (Table 3).

Effect of vaccination: Of the 483 IBD-affected flocks, 334 flocks were vaccinated against IBD while the remaining 149 were not vaccinated against IBD (Table 4). Morbidity and cumulative mortality were relatively higher in the unvaccinated flocks (5.8 and 2.6%, respectively) than in the vaccinated flocks (4.1 and 2.3%, respectively). However, CFR was comparatively higher in the vaccinated flocks (55.1%) than in the unvaccinated flocks (44.2%); the difference was not statistically significant (Table 4).

Of the 334 IBD vaccinated flocks, the exact information regarding the type of vaccine used was provided by the owners of 267 flocks only; Intermediate (I), intermediate plus (I^+), Georgia (G) or MB strains were the commonly used vaccines in broiler flocks in this region. The vaccination was generally carried out at 12-16 days of age via drinking water.

Table 1: Year wise distribution of infectious bursal disease in broiler flocks during July 2005-June 2008

Year	No. of flocks	Flock size	Morbidity (%)	Mortality (%)	CFR* (%)
2005-06	192	8,24,680	40,449 (4.9^{a})	16,701 (2.0^{a})	41.3^{b}
2006-07	211	11,27,510	46,318 (4.1^{a})	27,634 (2.4^{a})	59.7^{a}
2007-08	80	4,78,800	23,511 (4.9^{a})	12,013 (2.5^{a})	51.1^{ab}
Total	483	24,30,990	1,10,278 (4.5)	56,998 (2.3)	51.7

*Case fatality rate. Different superscripts within a column indicate significant difference at 5% level of probability

Table 2: Temporal distribution of infectious bursal disease in broiler flocks during July 2005-June 2008

Quarter	No. of flocks	Flock size	Morbidity (%)	Mortality (%)	CFR* (%)
July-September (A)	145	6,68,830	23,609 (3.5^{ab})	14,582 (2.2^{a})	61.8^{a}
October-December (B)	101	5,51,450	31,180 (5.6^{a})	12,317 (2.2^{a})	39.5^{b}
January-March (C)	100	4,39,650	14,568 (3.3^{b})	7,549 (1.7^{a})	51.8^{ab}
April-June (D)	137	7,71,060	40,921 (5.3^{ab})	22,500 (2.9^{a})	55.0^{ab}
Total	483	24,30,990	1,10,278 (4.5)	56,998 (2.3)	51.7

*Case fatality rate. Different superscripts within a column indicate significant difference at 5% level of probability

Table 3: Distribution of infectious bursal disease in different age groups of broiler flocks during July 2005-June 2008

Age group (days)	No. of flocks (%)	Flock size	Morbidity (%)	Mortality (%)	CFR* (%)
11-20	49 (10.2)	2,71,800	10,444 (3.8^{ab})	4,870 (1.8^{a})	46.6^{b}
21-30	255 (52.8)	12,89,340	52,305 (4.1^{ab})	29,496 (2.3^{a})	56.4^{ab}
31-40	160 (33.1)	7,89,350	45,014 (5.7^{a})	20,921 (2.7^{a})	46.5^{b}
41-50	19 (3.9)	80,500	2,515 (3.1^{b})	1,711 (2.1^{a})	68.0^{a}
Total	483	24,30,990	1,10,278 (4.5)	56,998 (2.3)	51.7

*Case fatality rate. Different superscripts within a column indicate significant difference at 5% level of probability

Table 4: Vaccination status in infectious bursal disease affected broiler flocks during July 2005-June 2008

Vaccination status	No. of flocks (%)	Flock size	Morbidity (%)	Cumulative mortality (%)	CFR (%)
Vaccinated	334 (69.2)	18,29,040	75,480 (4.1^{a})	41,618 (2.3^{a})	55.1^{a}
Unvaccinated	149 (30.8)	6,01,950	34,798 (5.8^{a})	15,380 (2.6^{a})	44.2^{a}
Total	483	24,30,990	1,10,278	56,998	

*Case fatality rate. Different superscripts within a column indicate significant difference at 5% level of probability

Clinical findings: In almost all IBD-affected flocks, the symptoms of dullness, depression, anorexia, ruffled feathers and inability to move were observed. There was drastic decrease in feed and water intakes of the affected birds. The affected birds had yellowish white or greenish yellow diarrhoea. The mortality increased with the progression of the disease, peaked at third and fourth day and then started declining. The severity of the clinical disease increased as the disease progressed. Flock to flock variation in severity of the disease was, however, observed.

Post mortem findings: Gross pathological lesions in almost all IBD-affected birds were recorded in bursa of Fabricius. The changes included: edematous and turgid bursa, presence of gelatinous exudate around bursa and bursal haemorrhages. In addition, haemorrhages on thigh and pectoral muscles were also observed. These changes were observed only in acute form of the disease. However, in chronic form, the bursal changes comprised of atrophy and/or presence of cheesy core inside the bursa. The haemorrhages on thigh and pectoral muscles were of milder degree in subacute form and were mild or absent in chronic form. Of the 483 IBD-affected flocks, 176 flocks had hemorrhages at the junction of proventriculus and gizzard. Swollen kidneys and enlargement of liver were also noticed in some cases; however, these findings were not consistent.

RT-PCR: Of the bursal samples from 40 flocks, 38 were positive for IBDV by RT-PCR. In positive cases, a band of 643 bp was observed.

DISCUSSION

The present study was carried out to analyze three years retrospective data in respect of IBD in broiler chickens. The data analysis may help us to understand the epidemiology of the disease better in the given geographical area which would ultimately be helpful in designing suitable prevention and control strategies at the farm level. This study is based on data obtained from DI Labs for the past three years (2005-2008). During this period, 5.32% broiler chicken flocks were found affected with IBD. Since all the IBD-affected flocks were not brought to the DI Labs for disease investigation, the actual number of flocks affected with IBD is not known, but may be higher. The IBD has been reported in broiler chicks from different countries (Anjum *et al*., 1993; Khurshid *et al*., 1993; Bekhit, 1997; Qureshi, 1999; Farooq *et al*., 2000; Tran *et al*., 2002; Anku, 2003).

In this study, overall cumulative mortality was lower as compared to already published reports from India and

abroad (Anjum *et al*., 1993; Sami and Baruah, 1997; Tran *et al*., 2002; Anku, 2003; Jaisankar *et al*., 2003). Such variation could be due to the fact that the collection of data was done at one time point only without a follow-up. As such, the mortality (%) in this study may not reflect actual mortality due to the IBD and as a matter of fact it could be on higher side. Follow up studies are therefore, needed to determine the exact morbidity and/or mortality rates. Higher CFR (51.7%) in this study may be due to acute form of the disease. The results of the present study are consistent with the previous study in the Haryana state by Jindal *et al*. (2004) in which a total of 8.89% flocks were affected with IBD during a nine year period with morbidity, cumulative mortality and case fatality rate of 5.9, 3.6 and 61.43%, respectively.

The clinical findings and post-mortem lesions observed in this study are consistent with those reported by earlier workers (Cosgrove, 1962; Mohanty *et al*., 1971; Rajeswar and Mohan, 1992; Sah *et al*., 1995; Qureshi, 1999; Tran *et al*., 2002; Zeleke *et al*., 2005). We observed differences in severity of the disease based on the clinical and necropsy findings and CFR. Such differences could be attributed to a number of factors such as type of virus involved, age of the birds, vaccination status, concurrent infections, health status of birds, nutritional status, season etc. We observed that in some of the affected flocks there was history of IBD in previous flock. It seems that majority of the poultry farmers are small and marginal and are not fully aware of the role of biosecurity in disease prevention and control. Improper cleaning of the sheds, keeping used litter near farms and frequent movement of personnel might have helped in spread of the disease from flock to flock and farm to farm.

Though the disease was recorded throughout the year, however, the occurrence was more in winter season followed by rainy and summer season. These findings have the support of Qureshi (1999) who on the basis of four year data reported higher occurrence of disease in parts of Pakistan during winter months compared to summer months. Though the occurrence of disease in this study was found on higher side in winter season, yet morbidity and cumulative mortality were comparatively higher in summer months. The exact reason for higher occurrence in winter season is not fully understood. However, it may be due to adoption of inadequate biosecurity measures including the inadequate and improper disinfection of poultry sheds due to harsh cold conditions during winter season and keeping of sheds air tight so as to maintain the temperature as most of the commercial broiler farms in this region are not environmentally controlled. This could result in aerosol generation of IBDV due to close contact thereby leading to maintenance of large amount of the virus at the farm premises. In the present study, the cumulative mortality was also higher in hot and humid season. High mortality in summer season is expected because of heat as day temperature shoots up to 46-47°C during the months of May and June. At such high temperatures, higher mortality would be obvious due to combined effects of heat and IBDV. Likewise, immediately after rains, there is a high humidity in the environment which may also substantially increase the mortality rate. However, more comprehensive data are required for substantiating seasonal occurrence of the disease and/or mortality. Contrary to our observations, Farooq *et al*. (2003) in Kashmir area of Pakistan reported higher losses in winter than in spring. Jaisankar *et al*. (2003) found maximum incidence in winter (43.93%) followed by summer (39.74%) and monsoon (16.74%).

The occurrence of the disease was more in the birds of 21-40 days of age indicating that birds in this age group are more susceptible to IBD. Bekhit (1997) and Anku (2003) also reported that birds of 3-6 weeks of age are more susceptible to the disease. Khurshid *et al*. (1993) observed that incidence of IBD in broiler chicks was maximum at 4th week of age followed by 5th, 3rd and 6th weeks. Similar observations with regards to occurrence of disease were made by Sami and Baruah (1997), Jaisankar *et al*. (2003), Jindal *et al*. (2004) and Zeleke *et al*. (2005). The occurrence of the disease in older birds may be correlated well with maternal antibody titers and vaccination status of birds. Sufficient maternal antibodies may protect birds from the disease for first two weeks. If birds receive appropriate vaccination during this period, chances of outbreaks during growing period would be reduced. However, improper vaccination would make birds susceptible to IBD at a later stage.

To study the impact of vaccination on disease occurrence, we analyzed the data with regards to disease and vaccination in flocks. Of the total IBD-affected flocks, about 70% flocks were those in which prophylactic IBD vaccination was carried out and the remaining 30% were unvaccinated flocks. The IBD outbreaks in vaccinated flocks have also been recorded by Anku (2003) in Southern Ghana. Previously in Haryana, Jindal *et al*. (2004) reported that about 59% of the flocks were vaccinated in which IBD was recorded. Gupta *et al*. (2006) have also reported outbreaks in vaccinated flocks in Punjab and Haryana states. Morbidity and cumulative mortality due to IBD in this study were comparatively higher in unvaccinated flocks than in the vaccinated ones. Anjum *et al*. (1993) and Farooq *et al*. (2000) also reported that the severity of disease was more in unvaccinated broiler flocks.

Intermediate or intermediate plus vaccines (both live vaccines) of different manufacturers are generally used for vaccination against IBD in broiler chicks in this region. The vaccination is generally carried out at 12-16 days of age via drinking water. These vaccines though

elicit protection against IBD but appear mildly immunosuppressive particularly the intermediate plus vaccine. A number of predisposing factors such as overcrowding, poorly constructed brooder house and poor ventilation in the farm may be responsible for disease to occur in the vaccinated flocks. Poor vaccination practices, improper handling of the vaccine, break in cold chain during transport and at the farm, use of chlorinated water during vaccination, exposing vaccine virus to outside environment for a longer duration, concurrent infection(s) and no or low maternal antibody levels in chicks are other potent factors that may contribute to the occurrence of disease in the vaccinated flocks. Most of the poultry farmers in this region are not fully aware about proper handling of vaccines. More importantly, the role of Very Virulent (vv) strains of IBDV in causing the disease inspite of vaccination cannot be ruled out. The vv strains emerged during the year 1992 in India were capable of breaking the maternal antibodies barrier. In such a situation, the disease can be observed even in a flock that has been vaccinated using good vaccination practices. The vv strains of IBD have been reported from many countries including India (Ramadass *et al*., 2003; Shamsara *et al*., 2006; Martin *et al*., 2007; Sreedevi and Jackwood, 2007; Juneja *et al*., 2008; Fernandes *et al*., 2009). We have previously reported the existence of vv strains of IBD in commercial broiler chickens in Haryana that were distinct from vvIBDVs from other parts of India based on VP2 gene phylogeny (Mittal *et al*., 2006). Recently, we have also characterized some more IBDVs and found them to be very virulent in nature (Mor, unpublished data). Prevalence of v v strains of IBDV in this region also warrants the need for strict biosecurity measures at the farm level.

Thus, it can be concluded from this study that infectious bursal disease is prevalent throughout the year in commercial broiler chicken flocks in the state of Haryana both in unvaccinated as well as vaccinated flocks resulting in huge economic losses to the farmers. There is a need to educate farmers to adopt strict biosecurity measures and vaccinate their broiler chicken flocks regularly and properly against this disease to minimize the losses. Further, regular surveillance and characterization of field strains would help in chalking out and re-evaluating control strategies from time to time.

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