SEROPREVALENCE OF AVIAN INFECTIOUS BRONCHITIS VIRUS IN SRI LANKA

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ABSTRACT: *Infectious bronchitis virus (IBV) is one of most important emerging viral diseases among poultry industry in Sri Lanka. The present study was aimed to detect and identify the IBV in commercial layers, broilers and village chicken in many districts of Sri Lanka by using ELISA test. IBV suspected poultry farms were investigated from December 2016 to January 2018. An indirect enzyme–linked immunosorbent assay (ELISA) was developed for screening of antibody to avian infectious bronchitis virus (IBV). ELISA test was used and found very useful tool in diagnosis of infected chickens. The results of determining the antibody concentration of the sera of 413 chickens by the ELISA. In the present study, all birds 191/413 (46.24%) were serologically positive as determined by ELISA. Most of the suspected flocks showed high level of antibody titers to IBV by ELISA technique, broilers 42/147 (28.57 %), layers 112/215 (50.09%) and village chickens 37/51 (72.54 %) were positive, which was expected finding due to the highly infectious nature of the disease. It proves that IBV is prevalent in Batticaloa, Jaffna and Kurunegala districts. While all farms visited in Kurunegala district vaccinate their birds against IBV, Farms visited in Jaffna, Trincomalee and Batticaloa districts do not vaccinate their birds for IBV. As the results of our investigation showed that vaccinated and non-vaccinated birds get infection of IBV, it is essential to evaluate the efficacy of the current vaccines used in the country.*

Key word: infectious bronchitis, ELISA, antibody, Sri Lanka

INTRODUCTION

Infectious bronchitis (IB) caused by the IB virus (IBV) which belongs to the genus Coronavirus in the family Coronaviridae causes major economic losses in poultry industry. Infectious bronchitis (IB) is an acute and highly contagious viral disease affecting chickens of all ages, causing an acute respiratory signs including gasping, coughing, sneezing, tracheal rales, nasal discharge and reduction in egg production and quality and increasing mortality rate (Cavanagh, 2007; Wit, 2000).

The virus is enveloped which has a positive sense, single-stranded, approximately 27 kb ribosomal nucleic acid (RNA) genome encodes four major structural proteins: the spike (S) glycoprotein, the membrane glycoprotein (M), the envelope protein (E) and the phosphorylated nucleocapsid (N) protein (Stern and Sefton, 1982).The S and N proteins are the major inducers of immune response in chicken where IBV exists as scores of serotypes/ genotypes (Cavanagh, 2001). IBV is highly adaptable and evolves rapidly by mutation and recombination (Lee &Jackwood, 2000), leading to the continuous occurrence of new genetic and antigenic variants which are found worldwide in large and a small scale farms (Wunderwald& Hoop, 2002). In most of the laboratories in the world, the common methods to diagnose infectious bronchitis (IB) are based on immunological identification of S and N protein of the virus (Ignjatovic&Sapats, 2005). This procedure is time consuming and requires the use of specific polyclonal or monoclonal antibodies. IBV infections can be diagnosed by detection of IBV virus itself or the specific antibody response. IBV infections are

detected serologically by demonstrating a seroconversion, using paired serum sets or the demonstration of IBV-specific immunoglobulin M (IgM) (Wit, 2000). The levels of IBV antibodies in serum, as well as the immune status of entire flocks can be monitored using ELISA (Mierset al,. 1987; Snyder et al, .1984). The enzyme-linked immunosorbent assay (ELISA), in contrast, cannot discriminate between different serotypes, as the cross-reactive antibody interferes with the detecting signal, particularly when the coating antigen is derived from whole virions (Lyn & Chen, 2017). ELISA technique is a sensitive serological method and gives earlier reactions and higher antibody titres than other tests (Bronzoni et al, 2005; Mockett&Darbyshire, 1981).The techniques used for detection of IBV-specific antigen all use IBV-specific antibodies. These antibodies are either in the form of antisera or monoclonal antibodies (Mabs) (Chen et al., 2011; Karaca&Naqi, 1993). Antisera are from an animal that was infected with IBV or injected with certain parts of the virus and consequently may contain antibodies against different parts of the virus. Standardization of antisera is hampered by the in vivo biological variation of the infected animal and virus. Since a Mab only reacts with one or a small number of epitope(s) of the IBV antigen, it provides a well-defined, reproducible and specific product (Koch et al., 1986).

One of the main issue of the poultry farmers in Sri Lanka is not having sufficient facilities for quick confirmatory diagnosis for the emerging diseases like IB. The two districts namely Kurunegala and Gampaha, are called 'Poultry belt' have more intensive farms (DAPH, 2016). However, major poultry operation in Jaffna, Trincomalee and Batticaloa district are the small scale farms including village chicken. The present study was aimed to find prevalence of the IBV in commercial layers, broilers and village chicken in non-vaccinated flocks by using serological test in Sri Lanka.

METHODOLOGY

Sample collection

Suspected IBV chickens were selected based on the clinical signs such as Sneezing, coughing, diarrhea, nasal discharges, pasted vent, loss of appetite and low egg quality and production, and postmortem lesions such as hemorrhages in the kidney and nephritis, hemorrhagic trachaeitis, caseous or catarrhal exudates in the nasal passages.

Blood collection

Blood samples were collected from 276 non–vaccinated chickens including broilers, layers and village chickens from poultry farms in Jaffna, Trincomalee, Kurunegala and Batticaloa districts of Sri Lanka during the period of December, 2016 to September 2018. Two milliliters of blood was collected from each birds from wing vein puncture. Serum samples were separated from each blood samples and stored at -20^0 C for further use.

Enzyme-linked immunosorbent assay (ELISA)

The Infectious Bronchitis Virus Antibody Test Kit was used for detection of antibody to IBV in the serum samples according to the manufactures recommendations. Sample

diluent (4X) was diluted by mixing 1 part with 3 parts of deionized water. Samples were diluted 1:400 ratio with sample diluent. Hundred microliters of negative and positive samples were dispensed to duplicate wells. Hundred microliters of diluted test samples were dispensed to each well of a 96-well micro ELISA plate and incubated at room temperature for 30 minutes. Wash solution (20X) was diluted by mixing 1 part with 19 parts of deionized water. After incubation ELISA plate was washed 3 times using wash solution (300 µl) per well. Hundred microliters of conjugate was dispensed per well. ELISA plate was incubated at room temperature for 30 minutes. ELISA plate was washed 3 times using wash solution (300 µl) per well. Hundred microliters of substrate was dispensed per well and incubated at room temperature for 30 minutes. After incubation 100 µl of stop solution was added per well. ELISA plate was read using microplate reader (BIORAD, Japan) at 405 nm and 630 nm.

RESULTS AND DISCUSSION

The present study found the presence of IBV in four districts namely, Jaffna, Trincomalee, Batticaloa and Kurunegala.

Table 1: Prevalence of IBV with relevance to poultry in Jaffna, Batticaloa, Trincomalee and Kurunegala districts

District	Flocks tested	Total no. of chickens		No. of positive samples by ELISA	Prevalence (%) by ELISA	Prevalence (%)
Jaffna	Broiler	110	50	10	20	55.25
	Village chicken		1	1	100	
	Layer		59	27	45.76	
Kurunegala	Broiler	107	50	3	6	53.87
	Village chicken		-	-	-	
	Layer		57	29	50.87	
Trincomalee	Broiler	103	16	2	12.5	33.83
	Village chicken		-	-	-	
	Layer		87	48	55.17	
Batticaloa	Broiler	93	31	27	87.09	75.25
	Village chicken		50	36	72	
	Layer		12	8	66.67	

Table 2: Results of ELISA test in Jaffna, Trincomalee, Kurunegala and Batticaloa Districts

Flocks tested	Total no. of chickens	No. of (%) positive samples	No. of (%) negative samples
Broilers	147	28.57	71.43
Village chicken	51	72.54	27.45
Layers	215	50.09	49.90

When comparing the non-vaccinated flocks 107 samples fromKurunegala district were collected out of 5,5000 birds whereas 110 samples from Jaffna were collected out of 3500 birds, 103 samples from Trincomalee and 93 samples from Batticaloa districts . Therefore, the prevalence of IBV in Jaffna is significantly higher than that of Kurunegala.

Diagnosis of infectious bronchitis (IB) is commonly based on virus isolation in embryonated eggs, following by immunological identification of the isolates (Wit, 2000) and by molecular identifications by PCR (Bijlengaet al., 2004). The infectious bronchitis infections are moderately prevalence in tropical countries of poultry (Fellahiet al., 2015).

The ELISA is comparatively a very sensitive test, especially when drawing the base line as stated, and this is indicated by its ability to detect early IgG (Mockett and Derbyshire, 1981). The ELISA assay was a convenient method widely used to detect antibody response to IBV infection in chicken flock (Wing et al, 2002; Chen et al., 2003). The results of determining the antibody concentration of the sera of 413 chickens by the ELISA. In the present study, all birds 191/413 (46.24%) were serologically positive as determined by ELISA. Most of the suspected flocks showed high level of antibody titers to IBV by ELISA technique, broilers 42/147 (28.57 %), layers 112/215 (50.09%) and village chickens 37/51 (72.54 %) were positive, which was expected finding due to the highly infectious nature of the disease. In Pakistan a survey conducted in the commercial poultry revealed 88% of the flocks were seropositive (Ahmed et al., 2007). In Jordan overall 92.9% of the flocks free from respiratory disease were seropositive for antibodies (Roussan et al. 2009). A high seropervalence of 82.7% was reported in chickens in south western Nigeria (Emikpe et al., 2010). Previous studies reported in Grenada 18.02% of non-vaccinated broiler chickens were positive for IBV-antibodies which is lower than the result obtained by present study (Sabrarinath et al., 2011). Mahzounieh et al. (2006) have reported that 85.3% of the village chickens in Iran had high titers of anti-IBV antibody without any clinical signs. IBV antibodies titers in broiler flocks in Iran, seroprevalence were reported 82.43% (Hadipour et al, 2011). Prevalence rate of disease has also been reported through ELISA by Das et al. (2009), Abraham et al. (2014), Shirvan and Mardani (2014) and Mungadi et al. (2015) as 100% in Gajipur of Bangladesh, 44.2% in Kerala, 12.5%, and 89% in Sokoto State, Nigeria respectively.

191/413 (46.24 %) samples from flocks which were positive and Another 222/413 (53.75) samples were negative by ELISA. Overall 191/413 (46.24%) samples

symptomatic group were found positive by ELISA, which clearly demonstrates the presences of IBV in flocks.

CONCLUSION

ELISA provides a quick diagnosis of IB infection in poultry farms. The present study find out the presence of the infectious bronchitis virus in Jaffna and Kurunegala district, mainly in village chicken and commercial layers respectively. In all cases, the birds less than five months old get more infection than old birds. It was also found that vaccinated flocks were less affected than non-vaccinated birds. As there were vaccinated birds also affected, theefficacy of the current vaccine used in the country should be assessed.

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