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Sero-prevalence of infectious bronchitis virus in Giriraja flocks at Puducherry, India

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Abstract
A study was conducted to evaluate the sero-prevalence of IBV (infectious bronchitis virus) in Giriraja birds that were supplied to the farmers in and around Puducherry from the breeder stock of the poultry unit of ILFC, RIVER. Serum from adult, healthy Giriraja birds, previously unvaccinated for IBV were tested for IBV antibodies using commercial IBV Enzyme-Linked Immunosorbent Assay (ELISA) kit. For comparison of the IBV antibody status, serum was sampled from healthy adult layers, vaccinated against IB, from various farms in Namakkal. All the serum samples collected from unvaccinated Giriraja birds in Puducherry (n=120) were negative for IBV antibody. Of the serum samples from vaccinated birds at Namakkal (n=184), 95% had antibodies against IBV. The findings indicated that IBV was not prevalent in Giriraja birds in Puducherry. Regular monitoring of sera from flocks for IB antibody titres may help to detect the exposure to the virus in unvaccinated flocks or indicate the level of vaccine response in vaccinated flocks.

Keywords: Infectious bronchitis virus, Elisa, giriraja birds, antibody titre

Introduction
Infectious bronchitis virus (IBV) is an enveloped coronavirus that contains an unsegmented, single-stranded, positive-sense RNA genome of 27–32 kb length. The virus has been classified underthe *Gamma coronavirus* genus in the family Coronaviridae, order Nidovirales. Infectious bronchitis virus infects primarily the respiratory system. However, some variants and several field isolates affect the reproductive, renal, and digestive systems of chickens. Disease pathogenesis differs according to the system involved, as well as the strain of the virus (Cavanagh, 2007) [5]. Chickens of all ages and breed types are susceptible to IBV infection, but the extent and severity of the disease is pronounced in young chicks, compared to adults. Similarly, resistance to infection was suggested to increase with increasing age. Infection of the respiratory system may result in clinical signs such as gasping, sneezing, tracheal rales, listlessness, and nasal discharges. Other signs may include weight loss and huddling of birds together under a common heat source (Cavanagh and Gelb, 2008) [3]. Other clinical outcomes associated with IB infection include frothy conjunctivitis, profuse lacrimation, oedema, and cellulitis of periorbital tissues. Infected birds may also appear lethargic, with evidence of dyspnoea and reluctance tomove (Terregino *et. al.*, 2008) [13]. Nephropathogenic IBV strains are most described in broiler-type chickens. Clinical signs include depression, wet droppings, and excessive water intake. Infection of reproductive tract is associated with lesions of the oviduct, leading to decreased egg production and quality. Eggs may appear misshapen, rough-shelled, or softwithwatery egg yolk. Unless effective measures are instituted, decline in egg production does not return to normal laying, thus contributing to high economic loss (Cavanagh, 2007; Winterfield *et. al.*, 1984) [5, 14]. The losses from production inefficiencies are usually of greater concern than losses from mortality (Cavanagh and Naqi, 2003) [4].
Infected chicks are the major source of virus excretion in the environment. Contaminated equipment and material are a potential source for indirect transmission over large distances. In a small number of chicks, latent infection is established with subsequent erratic shedding of virus for a prolonged period of time via both faeces and aerosol. Movement of live birds should be considered as a potential source for the introduction of IBV (Surendar *et al*., 2017) [12]. Despite regular vaccine use in certain parts of the country, IBV outbreaks occur frequently and owners of infected poultry farms suffer from tremendous economic losses. In the past, serological assays such as virus neutralization (VN) and haemagglutination inhibition (HI)were used widely for detecting and serotyping IBV strains.

These tests also have been used to measure flock protection following vaccination (King and Cavanagh, 1991; OIE, 2008) [8, 9]. Serotype-specific antibodies usually are detected using HI, even though the HI test isless reliable (OIE, 2008) [9]. On the other hand, ELISA assays being simple, rapid and sensitive can be utilized as a large-scale evaluation tool in IBV serological profiling (Praveen and Narasimha, 2016) [10] as well as for monitoring of both the immune status and virus infection in chicken flocks (Cardoso *et al.,* 2001, Emikpe *et al.,* 2010,; Hadipour *et al.,* 2011, Bayoumie and Hikal, 2015) [2, 6, 7, 1].

Giriraj birds are supplied to the farmers from the breeder stock of the poultry unit of the Instititutional Livestock Farm Complex (ILFC) Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Puducherry, India as part of a centrally sponsored scheme of the Department of Animal Husbandry and Dairying, New Delhi, Government of India. Perusal through the available literature revealed that there is scanty and unorganized information about the status of IBV disease among the poultry population in the Puducherry region of India. Hence, the aim of present study was to evaluate the IBV status of Giriraja birds that were supplied to the farmers in and around Puducherry, India from the breeder stock of the poultry unit of ILFC, RIVER, Puducherry, India using a commercial available enzyme-linked immunosorbent assay kits.

Materials and Methods

Sample collection: The study was conducted during the period from May to June, 2017 at Puducherry and Nammakal, Tamil Nadu, India. At Puducherry, India prior to sample collection, the farmers households where Giriiraja chicks had already been distributed were visited to obtain the willingness of owners to participate in the present study and also to ascertain the vaccination status of the birds, particulary against IBV (if any). For comparison of the IBV antibody status, selected layer farms vaccinated against IBV at Nammakal, Tamil Nadu, India were also visited. After obtaining the consent of the farmers, blood was drawn from the wing vein of healthy adult birds (n= 120 at Puducherry and n=184 at Namakkal, Tamil Nadu, India) with a disposable 2 ml syringe and needle and transferred into a 4 ml plain vacutainer tubes. The tubes were prelabelled and slanted on racks at an angle of about 45 degrees to facilitate clotting and separation of serum. Tubes were immediately placed on ice in a cool boxand were transported to the Department of Veterinary Pathology, RIVER, Puducherry, India. Vacutainers containing clotted blood were placed on the laboratory bench for at least 1 h for further serum separation. Vacutainers were centrifuged at 1500 rpm for 3 mins. Serum was collected into prelabelled 2 ml centrifuge tubes and stored at −20°C.

Each serum sample was singly tested for IBV antibodies using the enzyme-linked immunosorbent assay (ELISA) technique. A commercial IBV Antibody Test kit from IDEXX Laboratories Inc. (FlockChek ™, IDEXX, Iowa, USA) was used. Appropriate positive and negative controls included in the test kit were added to each plate run. Briefly, test serum and ELISA test reagents were bought to room temperature. Prior to being assayed, a 1:500 dilution of the samples was made with manufacturer's diluent in a 2-step process. 100 µl of each diluted sample was then pipetted into the appropriate well on the antigen-coated plate. One hundred microliters of undiluted positive and negative controls was added to their appropriate wells in duplicate. The plate was incubated for 30 mins at room temperature. Plates were then manually washed five times with deionised water and blotted dry on laboratory tissue paper after washing. Hundred microliters of conjugate was added to all wells and the plate was incubated at room temperature for 30 mins. Washing and blotting were repeated as described above. One hundred microliters of TMB substrate was added to all wells and incubated atroom temperature for 15mins. To stop the reaction, 100 µlof stop solution was added to all the wells. The optical density (OD)/absorbance value of each sample on the test plate was measured with Tecan Elisa reader at a wavelength of 650 nm.

Data Analysis: OD values were transferred onto an excel worksheet. The Positive Control Means (PCX) and Negative Control Means (NCX) for each test plate were calculated (Microsoft Excel, Microsoft Office 15). An assay was accepted to be valid when the NCX absorbance was less than or equal to 0.150 and the difference between PCX and NCX was greater than 0.075. The relative level of IBV antibody in the sample was determined by calculating the sample to positive (S/P) ratio using the following formula:

S/P= Sample (OD)- NCX (OD) / PCX (OD) -NCX (OD),

Where S/P is sample to positive ratio, Sample (OD) is OD of test serum, NCX (OD) is mean OD of negative control, and PCX (OD) is mean OD of positive control. Serum sample with S/P ratios less than or equal to 0.20 was considered negative; S/P ratio greater than 0.20 was considered positive. Data interpretation was as provided bythe manufacturer.

Data was exported and analysed using SPSS 17.0.1 (SPSS Inc.). The prevalence was calculated using the formula:

Prevalence= Number of positives detected /total number of samples analysed x 100 %

Results and Discussion

Serum samples collected from 120 adult Giriraja birds that were not vaccinated against IBVin Puducherry, India were found to be negative for infectious bronchitis virus antibody and the overall prevalence of IBV by serology was 0%. However, IBV antibodies were detected serologically in 175 out of 184 layer birds at Namakkal and the seroprevalence of IBV in the layer chickens was determined to be 95%. The findings indicated that IBV was not prevalent in Giriraja birds supplied from RIVER, Puducherry, India. There are no previous reports on detection of IBV in Puducherry, India. The detection of antibodies in 95% of layers in Namakkal vaccinated against IBV is an indication of the antibody status of the layers against IBV.

IBV has been isolated from apparently healthy and infected domestic chicken with respiratory disorders from in and around Namakkal, with an overall prevalence of (4/185) accounting to 2.16 per cent (Surendar *et al*, 2017) [12]. A seroprevalence of 0.11 per cent (7/66) has been recorded from suspected IBV field serum samples collected from different locations in Tirupati region of Andhra Pradesh (Praveen and Narasimha, 2016) [10]. A seroprevalence of 90 per cent for IBV has been reported in commercial layer chicken flocks with hydrosalpinx in the age group of 21 and 80 weeks using micro HI test against IB in Namakkal district. Various regimens are being employed in Namakkal for field vaccination programmes to confer protection in chickens against IBV. In layer flocks, vaccination with live virus against IBV is performed on 1st, 5th and 16th week of age. Hence detection of antibodies in serum against IB would arise

a question whether these antibodies are due to the vaccination or true infection (Srinivasan *et al*, 2019) [11]. The detection of, IBV antibodies in 95 % of samples from apparently healthy vaccinated birds from Nammakal, Tamil Nadu, India in the present study could be attributed to the vaccination status. Serological tests are important in epidemiological studies for IBV detection, diagnosis and evaluation of vaccination programme efficiency.

Conclusion

Although IBV was not prevalent in Giriraja birds at Puducherry, India in the present study, regular monitoring of serum from flocks for IBV antibody titres may help to detect the exposure to the virus in unvaccinated flocks or indicate the level of vaccine response in vaccinated flocks. It can serve as a tool for epidemiological monitoring of IBV in Puducherry, India.

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