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***Corresponding author:**

Surajit Baksi;
Email: drsbaksi_vm@yahoo.com

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Sero-prevalence of Avian Reovirus in Broiler Breeders in Different Parts of India

Surajit Baksi*, Nirav Rao, Pravinsinh Chauhan, Ashish Chauhan

Hester Biosciences Limited, Ahmedabad, Gujarat, India.

Abstract

Avian Reovirus (ARV) has gained worldwide importance because it is an emerging viral disease of the poultry industry. Mostly it is a disease of young broiler chicken but various studies showed that it can affect all ages of birds. Investigation on seroprevalence of ARV was performed in past, but data was found very less and therefore study was carried out on the states of India, where maximum poultry population exists. A sum of 450 serum samples was collected from Haryana, Tamil Nadu, Karnataka, Telangana, and Maharashtra. The overall prevalence of ARV was found to be 8.67%. ARV was found highly prevalent in Tamil Nadu (11.76%), whereas lower prevalence in Maharashtra (7.63%). The samples were categorized according to different age groups. Results showed the occurrence of Avian Reovirus infection 11.79% in birds aging 10-20 weeks.

Keywords: Avian Reovirus, ELISA, Sero-prevalence, India, Poultry.



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INTRODUCTION

Avian reoviruses (ARV) are members of the Orthoreovirus genus (Kaleta *et al.*, 1996). There are many different reoviruses from pathogenic to apathogenic. Many strains like S1133, S1733, 2408 and ERS are considered most important in meat-type chicken. Increased mortality, arthritis/ tenosynovitis, disuniform flocks resulting in loss of performance and economic losses are the consequences of Reovirus infection. Reoviruses may enter the broken skin and established in hock joints (Al-Afaleq *et al.*, 1990). Avian reoviruses have been reported as a significant cause of intestinal symptoms of poultry. Poor feed conversions and poor weight gain were observed due to reovirus infections. Sporadic outbreaks of enteric disease are seen world-wide in commercial poultry and can vary widely in severity (Reynolds, 2003). Both vertical and horizontal transmission of avian reoviruses is recognized (Al-Mufarrej *et al.*, 1996; Eric *et al.*, 2017; Matthew *et al.*, 2018; Menendez *et al.*, 1975; Van der *et al.*, 1975). The causes of enteric disease have never been definitely established because they are complex and polymicrobial, and similar disease signs can likely be caused by different pathogens (Barnes and Guy, 2003; Baxendale and Mebatsion, 2004; McFerran, 2003; Reynolds, 2003).

Detection of avian viruses by molecular techniques has become routine in most diagnostic laboratories. ELISA detection tests are widely used in laboratories to quantify antibodies (Hess *et al.*, 1999; Pantin-Jackwood *et al.*, 2008; Sellers *et al.*, 2004; Spackman *et al.*, 2005; Tang *et al.*, 2005). The disease is widespread all over the poultry world, but relatively resistant outside the host. Maintaining freedom from infection in commercial chicken flocks is virtually impossible. Thus, the main approach to reovirus control has been vaccination, using live and killed vaccines. Although maintaining commercial flocks free of reovirus infection is difficult, good management and biosecurity procedures which minimize reovirus infection of very young chickens can be used in addition to vaccination to assist in the control of reovirus-associated disease (Jones, 2000). Worldwide prevalence of the infection and economic impact lead to undertake the present study, to find seroprevalence in India, which will help to prevent and control the infection.

MATERIALS AND METHODS

Sample collection

A total of 450 blood samples were collected from the several parts of India to screen the presence of ARV antibodies. No clinical signs of ARV infections were found during blood collection. Blood samples were collected from the Jugular vein and then samples were transferred to the Hester Biosciences Ltd, Anand for further investigation.

Blood samples were centrifuged to separate the serum and then stored in to -20 °C until it was tested.

Competitive ELISA

Commercial ELISA kit (IDEXX Laboratories, USA) was used to screen presence of ARV antibodies. The ELISA was run after collection of all the blood samples and it was run as per the manufacturer's protocol and instruction. Before use, all the samples and reagents were allowed to room temperature and homogenized by gentle mixing. All the samples were diluted at 1:500 with sample diluents provided by the manufacturer. 100 µl of the negative control was added to wells A1 & B1 and 100 µl of the positive control was added to wells C1 & D1. Then 100 µl of diluted samples were added into the appropriate wells and incubated at 18 to 26 °C for 30 minutes by covering the plate with lid. After that, the content of the well was emptied and washed 3 to 5 times by the 350 µl of the wash solution using ELISA washer. Then 100 µl of the conjugate was added to each well and incubated at 18 to 26 °C for 30 minutes. Following washing for 3 to 5 times with wash buffer, 100 µl of TMB substrate reagent was added into appropriate each wells and incubated at 18 to 26 °C for 15 minutes. Finally, 100 µl of stop solution was added to the each well to stop the reaction. Then the microtitre ELISA plate was placed in the ELISA reader and the intensity of the color produced from the ELISA test was measured photometrically at 650 nm wavelength.

Statistical analysis

The result from serology was entered in Microsoft Excel spreadsheet (Microsoft Corp., USA) before analyzing with one way ANOVA (Microsoft Corp., USA). Statistical analysis was performed with the data of different states' seroprevalence. Furthermore, birds with different ages were categorized into age 0 to 10 weeks and 10 to 20 weeks. The data were considered as significant between groups if p-value was <0.05.

RESULTS

The aim of this study was to know the infection of ARV in different poultry farms of five states i.e., Haryana, Tamil Nadu, Karnataka, Telangana, and Maharashtra. Cut-off antibody titer more than 1000 was considered to be positive for ARV. Out of the total 450 samples, 39 samples were found positive for ARV antibodies. The overall prevalence of ARV was found 8.67% in the present study. The study revealed that out of five states higher prevalence (11.76%) of ARV was found in Tamil Nadu state (6 samples were found positive out of 51), while the lowest prevalence (7.63%) was found in Maharashtra (10 samples were found positive out of 131). 8.39%, 8.86%, and 8.69% samples were found positive in Telangana, Haryana and Karnataka respectively (Table 1). At the time of blood collection, all the birds were found healthy and active and

no clinical signs of ARV were observed and indicated that the disease was in sub-clinical form.

Blood samples were also divided according to two age groups: 0-10 weeks and 10-20 weeks. Higher prevalence

was found in birds of more than 10 weeks (11.79%), while 5.42% birds of less than 10 weeks age were found positive for ARV antibodies (Table 2).

Table 1. State wise sero-prevalence of ARV

State	No. of Samples tested	No. of samples found positive	Prevalence (%)
Haryana	79	07	8.86
Tamil Nadu	51	06	11.76*
Karnataka	46	04	8.69
Telangana	143	12	8.39
Maharashtra	131	10	7.63
Total	450	39	8.67

*p<0.05, Significantly different from Maharashtra

Table 2. Age wise sero-prevalence of ARV

Age group (Weeks)	No. of samples tested	No. of samples found positive	Prevalence (%)
0-10	221	12	5.42
10-20	229	27	11.79*
Total	450	39	8.67

*p<0.05, Significantly different from age group of 0-10 weeks

DISCUSSION

Nural et al. (2012) reported 95.83% positive samples for ARV antibodies in broiler breeders in Western Provinces of Turkey, while Cordia et al. (2002) reported 98.5 % positive samples of ARV in Swiss poultry flocks, which is much higher than the present study. ARV prevalence was also found 98.3% in Tehran province of Iran, which indicates the significant role of ARV vaccination in poultry flocks (Saied *et al.*, 2006). The high amount of seroprevalence may be due to poor vaccination programmes in the flocks and reovirus infection in the early age of birds. This study indicated the presence of natural infection in the poultry flocks as there was no history of vaccination against ARV from the poultry farmers. High prevalence of ARV antibodies in the flocks may be the result of improper biosecurity and management practices in the farms. However, Several Variables (Age of flock at shipment, flock exposure to Infection Bursal Disease Virus and other infections) may be associated with immune exposure and viral shedding of ARV.

CONCLUSION

To control the ARV infection in broiler breeder poultry flocks, it is necessary to vaccinate the birds against reovirus vaccine and even booster dose is required to transfer the antibodies vertically in their progenies. Further research may be needed to explore the probable factors for infection. Through this study, we concluded that there is a need for improvement in hygiene and nutrition.

CONFLICT OF INTEREST

The authors declare that no there is no conflict of interest for this study.

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