Sero-Prevalence of Chicken Anaemia Virus (CAV) in Layer Poultry Flocks in Different Parts of India

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Abstract

Now-a-days, Chicken Infectious Anaemia (CIA) gained worldwide importance as it is an emerging viral disease of poultry industry. Mostly it is a disease of chicken but earlier study showed that it can affect all ages of birds. Investigation on sero-prevalence of Chicken Anaemia Virus (CAV) has been performed in India. The study regarding sero-prevalence of CAV was found very less and therefore study was carried out in the states where maximum poultry population exists. A sum of 422 serum samples was collected from Haryana, Karnataka, Madhya Pradesh, Maharashtra, Tamilnadu and Telangana. The samples were also categorized according to different age groups and then ELISA was performed to know the antibody titre against CAV. Overall sero-prevalence in India was found to be 39.33%. Results also showed significant effects (p<0.05) of age on occurrence of CIA that means the disease is dependent on the age of birds. The study indicates that disease is highly prevalent in birds aging between 30 to 50 weeks. The infection was found to be less in the birds aging up to 10 weeks. Out of six different states of India highest prevalence of CAV was observed in Telangana state while lower prevalence was observed in Maharashtra.

Keywords: Chicken Anaemia Virus, ELISA, Sero-Prevalence, India, Poultry.

1. Introduction

Chicken Infectious Anaemia (CIA) is a viral infection in poultry caused by Chicken Infectious Anaemia Virus (CIAV), a single-stranded DNA virus with icosahedral symmetry belonging to the family Circoviridae (Fenner et al., 1993). The virus is important because of its potential for inducing immune suppression alone or in combination with other infectious agents like Infectious Bursal Disease (Sachat, 2003). The disease is transmitted both horizontally (Bullow, 1991; Hoop et al., 1992; McNulty et al., 1988) and vertically (Chettle et al., 1989; Engstorm et al., 1988; Jorgensen, 1990; Jorgensen, 1991). CIA causes bone marrow aplasia with severe anemia, thymus, bursa, and spleen atrophy; and haemorrhages of the proventriculus and skeletal muscles and consequently, it adversely affects the production performance of chickens. It was first reported in Japan (Yuasa et al., 1979) and has since been observed in many other countries throughout the world. Serological data has suggested that CIAV appeared to be ubiquitous in all major chicken producing countries of the world. The virus was isolated from chickens in Australia, China, Japan, New Zealand, and South Africa (Sachat, 2003). Miller and Schat (2004) suggested that CIAV has evolved as a successful pathogen that can be maintained in successive generations of chickens without causing disease. The diagnosis of infection is made based on clinical signs and gross lesions in affected birds. However, a confirmatory diagnosis needs isolation and identification of the CIAV. On the basis of rapidity and suitability for testing large numbers of serum samples, enzyme-linked immunosorbent assay (ELISA) is the test of choice for investigating the epidemiology of CIAV infection in poultry (Todd et al., 1999; Schat, 2003; Dhama et al., 2008). However, in the modern high-stress environment of commercial poultry production it is a major pathogen that can cause significant economic problems (Sachat and Santen, 2008). Despite a large poultry industry, a few evidence of CIAV has been reported from India. The aim of this Sero-prevalence study was to investigate the presence of CIAV antibodies in commercial Layers poultry farms in India.
2. Materials and Methods

2.1 Sample Collection

Total 422 blood samples were collected from the different parts of India to screen the presence of CIAV antibodies. We collected serum samples from the different states of India. At the time of blood collection no clinical signs suggestive of CIA were observed in any of the flocks. 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 categorised into four different categories based on the age of the birds. Blood samples were centrifuged to separate the serum and then stored in to -20ºC until it was tested.

2.2 Competitive ELISA

All the 422 serum samples were screened for the presence of CIAV antibodies using commercial ELISA kit (IDEXX Laboratories, USA). The ELISA was performed after collection of all the blood samples and it was performed 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 1:100 with sample diluents provided by the manufacturer. 100 µl of undiluted negative control was added to wells A1 and B1 and 100µl of undiluted positive control was added to wells C1 and D1. Then 100µl of diluted samples were added into the appropriate wells and incubated at 18 to 26ºC for 60 minutes by covering the plate with lid. After that, content of the well was emptied and washed 3 to 5 times by the 350µl of the 1X wash solution using ELISA washer. Then 100µl of the conjugate was added to each well and incubated at 18 to 26ºC for 30 minutes. Following washing for 3 to 5 times with wash buffer, 100µl of TMB substrate reagent was added into each appropriate wells and incubated at 18 to 26ºC for 15 minutes. Finally, 100µl 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 650nm wavelength.

2.3 Statistical Analyses

The result from serology was entered in Microsoft Excel spreadsheet (Microsoft Corp., USA) before analyzing in Stata 13 software (Statacorp, LP College station, TX, USA). The dependent variables sero-status (sero positive (1) and sero negative (0)) were binary responses and resemble a binomial distribution. For analyses purpose, birds with different ages were categorized into birds aged 0 to 10 weeks, 10 to 30 weeks and 30 to 50 weeks and more than 50 weeks. A logistic model with ‘farm’ as a random effect, to adjust for clustering of birds within same farm, was used to model association, between sero status (positive or negative) and categorical risk factor: age effect. The above logistic model was used for both univariate and multivariate analyses.

3. Results and Discussion

Total six states of India were selected for the study viz Haryana, Karnataka, Madhya Pradesh, Maharashtra, Tamilnadu and Telangana. Out of total 422 samples 166 samples were found positive for CIAV antibodies. The overall prevalence of CIAV was found 39.3%. The study revealed that out of six states higher prevalence (75%) of CAV was found in Telangana states (27 samples were found positive out of 36) followed by Tamilnadu, Karnataka, Madhya Pradesh and Haryana while much lower prevalence (12.5%) was found in Maharashtra (4 samples were found positive out of 32) (Table 1).

Total four groups were made to check the effect of age on occurrence of CAV viz, 0 to 10 weeks, 10-30 weeks, 30 to 50 weeks, >50 weeks. The study showed higher prevalence (56.2%) of CAV in age group of 30 to 50 weeks followed by >50 weeks of age while lower prevalence (28.5%) was observed in age group of 0 to 10 weeks of age (Table 2).

The aim of this study was to know the infection of CAV in different poultry farms of six states. Cut-off antibody titre more than 1000 was considered to be positive for CAV. Out of 422 sera, 166 samples were found positive for the antibody against CAV. Out of six states higher sero-prevalence of CIAV was found in Telangana state (75%) which has the highest poultry population among India. In agreement with this study, the earlier Indian researchers who found slightly higher prevalence of CIAV of 86.66% in Northern India. Several researchers studied the sero-prevalence of CIAV in African and Asian countries and have observed high sero-prevalence rate in their Poultrie industries (Emikpe et al., 2005; Roussan, 2006; Farhoodi et al., 2007; Oluwayele, 2010) indicating CIAV as an emerging pathogen worldwide. Farmers do not vaccinate the birds against CAV, therefore the observed sero-prevalence was might be due to the virus infection. At the time of blood collection all the birds were found healthy and active and no clinical signs of CAV were observed and indicated that the disease was in sub-clinical form. Similar observations have been found by the Hoop et al. (1992). He found the higher sero-prevalence in apparently healthy bird flock and it is also possible that CIAV infection were not reported because they were not recognized as such but in any –
Table 1: State wise sero-prevalence of CAV

<table>
<thead>
<tr>
<th>State</th>
<th>No. of Samples</th>
<th>No. of Positive Samples</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haryana</td>
<td>154</td>
<td>37</td>
<td>24.0</td>
</tr>
<tr>
<td>Tamilnadu</td>
<td>100</td>
<td>52</td>
<td>52.0</td>
</tr>
<tr>
<td>Karnataka</td>
<td>58</td>
<td>29</td>
<td>50.0</td>
</tr>
<tr>
<td>Madhya Pradesh</td>
<td>42</td>
<td>17</td>
<td>40.0</td>
</tr>
<tr>
<td>Telangana</td>
<td>36</td>
<td>27</td>
<td>75.0</td>
</tr>
<tr>
<td>Maharashtra</td>
<td>32</td>
<td>04</td>
<td>12.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>422</strong></td>
<td><strong>166</strong></td>
<td><strong>39.3</strong></td>
</tr>
</tbody>
</table>

Table 2: Age wise sero-prevalence of CAV

<table>
<thead>
<tr>
<th>Age Group (Weeks)</th>
<th>No. of Samples</th>
<th>No. of Positive Samples</th>
<th>Prevalence (%)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>172</td>
<td>49</td>
<td>28.5</td>
<td></td>
</tr>
<tr>
<td>10-30</td>
<td>121</td>
<td>49</td>
<td>40.5</td>
<td></td>
</tr>
<tr>
<td>30-50</td>
<td>89</td>
<td>50</td>
<td>56.2</td>
<td>0.04</td>
</tr>
<tr>
<td>&gt;50</td>
<td>40</td>
<td>18</td>
<td>45.0</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>422</strong></td>
<td><strong>166</strong></td>
<td><strong>39.3</strong></td>
<td></td>
</tr>
</tbody>
</table>

* Significant if p<0.05

4. Conclusion

The present study indicated that overall 39.3% prevalence of CIAV in India that means, disease is present in subclinical form in poultry population which require good hygienic condition to control the disease. Poor nutrition also affects the health of the birds so it is advisable to provide highly nutritive feed to the birds. Therefore, for the prevention and control of the CIA there is a requirement of good hygienic measures and nutritive feed to the birds. Number of pathogens including CIAV is responsible for the rearing losses of poultry in India. Further studies and research are required to access the economic losses due to such pathogens. Furthermore, farmers need to be instructed to protect the about the signs, importance, economic losses and control measures of this economically important CIAV.

Acknowledgement

All the authors are highly thankful to the Mr. Rajiv Gandhi, CEO and Managing Director, Hester Biosciences Limited, for providing research area, financial support and other necessary facilities to carry out the study.

References


Emiêkpe BO, Oluwayelu DO, Ohore OG, Oladele OA and Oladokun AT (2005). Serological evidence of chicken case sub-clinical infection is known to have a negative impact on flock, with economic consequences (Goodwin et al., 1993; McNulty et al., 1991). This study indicated the presence of natural infection in the poultry flocks as there was no history of vaccination against CAV from the poultry farmers. The disease causes immune suppression in birds and makes them susceptible to other infections. The disease causes high economic losses either by the less productivity or the secondary bacterial infection to the poultry farmer.

Based on the different age groups, higher sero-prevalence was found in 30-50 weeks of age and statistical analyses showed that the disease is dependent on age as the P value of Chi square test was found less than 0.05 (Table II). In agreement with this study, Owode et al. (2004) found more than 80% sero-prevalence in birds aged more than 10 weeks of age. He stated that at this age the birds seem to have sero-converted without any obvious symptoms which was in agreement with our study. He also indicated that the disease is most likely related to the age of the bird and found >98% prevalence of CAV in 48 weeks of breeder birds. This indicated that birds were protected by the maternal antibodies against CAV during their early age.


