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# Temporo-Spatial Sero-epidemiology of Fowl Adenovirus (FAdV) Infection Causing Inclusion Body Hepatitis-Hydropericardium Syndrome (IBH-HPS) in Broiler Population of Assam

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## ABSTRACT

**Background:** *Aviadenoviruses* affect birds, particularly chickens, ducks, geese, turkeys and pheasants, which have total 12 serotypes. Inclusion body hepatitis (IBH) caused by aviadenovirus was has been reported in many countries worldwide. The diseases was first reported from Assam in 2017. Although there is increasing reports of the occurrence of IBH-HPS in the broiler population of North Eastern India, but its prevalence at different geoclimatic condition and at various seasons have not been carried out. Under these circumstances, the present study was envisaged to analyse the seroprevalence status of Fowl adenovirus infection in some broiler rearing districts of Assam.

**Methods:** For the present study, blood samples were collected from 12 different districts of Assam including all agroclimatic regions during the period from June, 2016 to May, 2017. Association of various factors like age, season and health status with the prevalence of the affected birds were also studied. Indirect ELISA was performed by using commercially available FAdV ELISA kit. The results obtained were analyzed by the Statistical Package for Social Sciences (SPSS) version 26.0.

**Result:** A total of 460 serum samples were screened, of which 213 were found positive for FAdV antibodies with a sero-positivity of 46.38 per cent. FAdV antibodies were observed in all age grouped birds. Highest (64.28%) positivity was recorded in the birds of 4$^{th}$ week of age, followed by above 4$^{th}$ week (63.47%), 3$^{rd}$ week (32.03%), 2$^{nd}$ week (27.36%) and 1$^{st}$ week (10.0%). Health status-wise 68.97 percent sero-positivity was recorded in the affected flock, where as 32.51 percent in apparently healthy flock. Season-wise, highest (61.53%) sero-positivity was recorded in post monsoon season followed by monsoon (49.00%), winter (32.49%) and pre-monsoon (32.39%).

**Key words:** Broiler, ELISA, Fowl adenovirus, Seroprevalence.

## INTRODUCTION

The avian adenovirus was first isolated from an outbreak of respiratory disease in quail (Olson, 1950). *Aviadenoviruses* affect birds, particularly chickens, ducks, geese, turkeys and pheasants. There are total 12 serotypes of Fowl adenovirus *viz*. FAdV-1, FAdV-2 FAdV-3, FAdV-4, FAdV-5, FAdV-6, FAdV-7, FAdV-8a, FAdV-8b, FAdV-9, FAdV-10 and FAdV-11which were classified on the basis of restriction fragment length polymorphism (RFLP) profile and sequencing data (Benko *et al*., 2000).Inclusion body hepatitis (IBH) caused by aviadenovirus was first described in 1963 from USA (Helmboldt and Frazier, 1963). Thereafter, the disease has been reported in many countries worldwide.

In late eighties, a new disease in broilers with some similarities to classical inclusionbody hepatitis (IBH) was reported from Angara Goth near Karachi, Pakistan. The disease was mainly characterized by accumulation of clear straw coloured fluid in the pericardial sac along with hepatitis (Jaffery, 1988) and named as Hydropericardium syndrome (HPS)/ Angara disease. In India, HPS was first noticed in the poultry belt of Jammu and Kashmir, Punjab and Delhi during April-July, 1994 (Gowda and Satyanarayana, 1994). After a few months, the disease spread to Terai of Uttarakhand in November, 1994 (Kumar *et al*., 1997). Then several outbreaks were recorded in Uttar Pradesh, Maharastra andhra Pradesh, Karnataka, Tamil Nadu, Kerala, Odisha, West Bengal, Chattisgarh and Mizoram resulting in huge economic losses to the poultry industry (Gupta *et al*., 2007, Asthana *et al.*, 2013, Suohu and Rajkhowa, 2021). The first outbreak of IBH-HPS in Assam was reported recently by Dutta *et al*. (2017).

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Various serological tests *viz*. AGID, counterimmune electrophoresis and various modifications of ELISA were used for studying the seroprevalence of fowl adenoviral infection in poultry (Kumar *et al*., 2003). The ELISA can be used for detecting both group and type specific antibodies against FAdV and proved to be more sensitive (Saifuddin *et al*., 1992). The indirect ELISA was found to be easier, accurate and appropriate for screening and monitoring large amount of samples (Junnu *et al*., 2014).

Although there is increasing reports of the occurrence of IBH-HPS in the broiler population of North Eastern India, but its prevalence at different geoclimatic condition and at various seasons have not been carried out. Under these circumstances, the present study was envisaged to analyse the seroprevalence status of Fowl adenovirus infection in some broiler rearing districts of Assam.

## MATERIALS AND METHODS

The present investigation was carried out in the Department of Veterinary Pathology, C.V.Sc, A.A.U., Khanapara, Guuwahati, Assam.

### Study area

Assam, the gateway of North Eastern states of India (78,438 km2 area, area rank 16$^{th}$of the country), with latitude and longitude of 26.244156 and 92.537842 respectively having gps coordinates of 26°14′38.9616″ and 92°32′16.2312″. The state shares international boarders with Bangladesh and the Kingdom of Bhutan. The state has 33 revenue districts and can be divided into 6 agro-climatic zones *viz*. Lower Brahamaputra Valley, North Bank plain zone, Central Barak Valley zone, Upper Brahamaputa Valley zone, Barak Valley zones and Hill Zones.

For the present study, blood samples were collected from 12 different districts of Assamn *viz* Kamrup (M), Karrup (R), Nalbari, Goalpara, Morigaon, Nagaon, Sonitpur, Lakhimpur, Dhemaji,Karbi-Anglong, Golaghat and Jorhat during the period from June, 2016 to May, 2017, to determine the sero-prevalence of Fowl adenovirus (Fig 1).

### Study design and sampling method

A structured questionnaire covering the age, health status, vaccination status,previous occurrence of any other diseases *etc*. of birds were prepared to collect epidemiological data.

The sample size required for the study was framed according to the formula given by Thrustfield (1995) considering the 50% prevalence as expected prevalence 95% confidence interval and 5% absolute precision, the number of birds to be studied were 384 as calculated below and in order to improve the precision, the sample size was increased upto 460.

$n = 1.962 \times Pexp (1-Pexp) / d2$

$n = 1.962 \times 0.5\ \% (1-0.5)/0.052$ 1.96 - Z value of 95% CI

n = 384

n= Required sample size.

Pexp= Expected prevalence.

d2 = Desired absolute precision.

### Temporal and spatial distribution

**Factor associated with Sero-prevalence of Fowl adenovirus**

**Season:** To study the effect of season, a calendar year was divided into four (4) seasons viz. pre-monsoon (March - May), monsoon (June - September), post-monsoon (October-November) and winter (December -February). During the period of study, the meteorological data were collected from the Regional Meterological Centre, Barjhar, Guwahati, Assam.

**Age:** To study the effect of age on seroprevalence, the birds were divided into 5 groups as follows.

| Age (Week) | Group |
|---|---|
| 1$^{st}$ | I |
| 2$^{nd}$ | II |
| 3$^{rd}$ | III |
| 4$^{th}$ | IV |
| ≥ 4$^{th}$ | V |

### Health status

To study the effect of health-status on prevalence, the blood samples were collected from both from apparently healthy as well as diseased flock.

### Sample collection

The sampling procedure was approved by institutional animal ethics committee. About 2 ml of blood was collected from each from the medial metatarsal vein in clot activator vial. The serum was separated from blood by centrifuging at 3000 rpm for 10 min. The separated serum was collected in a screw capped plastic vial and stored at -20°C until they were tested.

### ELISA test

The seroprevalenceof fowl adenovirus was studied by using FAdV ELISA kit manufactured by BioCheck, UK (Xia *et al*., 2017). Briefly, 5µl of test sample were taken into dilution plate and added 245 µl of sample diluents to make 1:50 dilution. 100µl of negative control was added to the wells A1 and B1 and 100 µl of positive control was added to the wells C1 and D1. Then 50 µl of sample diluents were added to the rest of the well and then add 50 µl of 1:50 dilution test sample in the appropriate well to obtain 1:100 sample dilution. The plates were then incubated at room temperature for 30 minutes. Then plate was washed four times with wash buffer (350µl each well). The plate was inverted and tap firmly on absorbent paper until no moisture was visible. The 100µl of conjugate reagent was added into the appropriate well and incubated at room temperature for 30 minutes. After incubation, the plate was again washed four times with wash buffer (350µl each well) and 100 µl of substrate reagent was added and incubated at room temperature for 15 minutes. The reaction was stopped after 15 minutes by adding 50 µl of stop solution. The plate was observed for

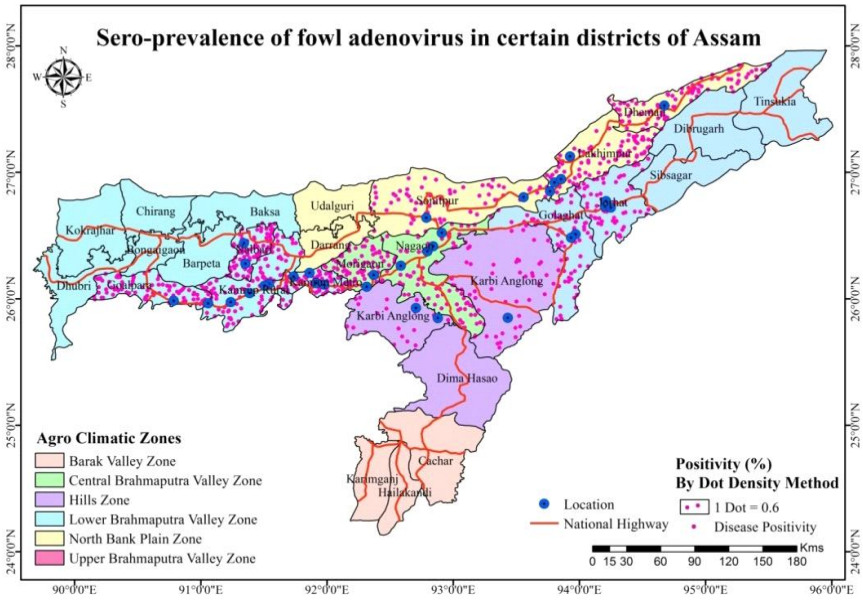


**Fig 1:** Map of Assam, showing the districts from where samples were collected.

colour development and the reaction was read optically by using an ELISA reader (BioRad) at 405 nm wavelength.

The results are expressed as antibody units which were calculated as subtract the optical density of negative control serum NC (ODNC) from the optical density of positive control serum PC (ODPC) as well as from the optical density of tested serum samples ($OD_{TS}$). The results were obtained as S:P (Serum:: Positive) ratio, from the following formula:-

$$S/P = \frac{\text{Mean OD of test sample } (OD_{TS}) - \text{Mean OD of negative control } (OD_{NC})}{\text{Mean OD of positive control } (OD_{PC}) - \text{Mean OD of negative control}}$$

Sample with an S/P of 0.5 or greater were considered as positive.

### Calculation of antibody titre

The following equation relates the S/P of a sample at a 1:100 dilution to an end point titre.

$Log_{10}$ Titre: $1.1 * Log_{10}$ (S/P) + 3.361

Antilog= Titre

| S/P value | Titre range | Antibody status |
|---|---|---|
| 0.499 or less | ≤1070 | No antibody detected |
| 0.500 or greater | ≥1071 | Positive |

### Data analysis

The results obtained were analysed by the Statistical Package for Social Sciences (SPSS) version 26.0. The alpha level was set at 0.05 and 95% confidence interval (CI 95%) was calculated. Pearson's Chi-square test was used to detect significant differences in the seropositivity between the districts, age season and health status. If the probability value (P value) is less than or equal to set alpha level (0.05) then the result was considered as statistically significant.

## RESULTS AND DISCUSSION

Even though IBH-HPS has been reported in the survey zone as well as in the region, the sero-prevalence study of FAdV in this North Eastern Region of India was conducted for the first time.

During the period of study, out of a total of 460 sera samples were tested, out of which 213 (46.38%) were found positive. The spatial distribution of FAdV in different agro-climatic zones of Assam has been shown in Table 1 and Fig 2 (Dot Density Method). Highest (60%) and lowest (36.66%) sero-positivity was recorded in Kamrup (R) and Jorhat district respectively. The seroprevalence of FAdV antibodies have an insignificant association ($\chi^2$= 8.4647, P=0.6711) between the districts. The increased in the prevalence of FAdV antibodies from Kamrup (R) district of Assam might be due to increased movements of poultry from in this area that could result in high rate of contact between birds.

The study demonstrated that FAdV infection is endemic in the state on the basis of serological evidence of the virus activity in the broiler population. Since all the districts from where the samples were collected were well connected with the National Highways, so mechanical of transmission of the disease cannot be ruled out. The sub-clinically infected birds may act as a source of infection through their secretions and excretions and could pose a potential risk of virus dissemination. The preliminary findings of the present study suggested the need for strengthening the surveillance activities in large population at different districts of Assam.

Single dilution i-ELISA was found useful to screen the flock for FAdV antibodies. The test can be easily conducted, broad spectrum *i.e* not serotype specific and is considerably more sensitive than AGPT in early stages of infection (Philippe *et al.*, 2007).

**Table 1:** Sero-prevalence of fowl adenovirus antibodies in different districts of Assam.

| Name of the District | Total sample | Positive sample | Percent positivity | $\chi^2$ |
|---|---|---|---|---|
| Kamrup (M) | 45 | 22 | 48.88 | **8.4647, df=11** |
| Kamrup (R) | 40 | 24 | 60.00 | **P=**0.671171 |
| Nalbari | 40 | 19 | 47.50 | |
| Goalpara | 40 | 21 | 52.50 | |
| Morigaon | 45 | 19 | 42.22 | |
| Nagaon | 40 | 18 | 45.00 | |
| Sonitpur | 40 | 16 | 40.00 | |
| Lakhimpur | 35 | 17 | 48.57 | |
| Dhemaji | 35 | 18 | 51.42 | |
| Karbi-Anglong | 35 | 15 | 42.85 | |
| Golaghat | 35 | 13 | 37.14 | |
| Jorhat | 30 | 11 | 36.66 | |
| **Total** | **460** | **213** | **46.38** | |

**Table 2:** Seroprevalence of fowl adenovirus antibodies at different age groups.

| Group | Age (week) | Total sample | No. of positive sample | Percent positivity | $\chi^2$ |
|---|---|---|---|---|---|
| Group I | 1st | 10 | 1 | 10 | **89.1361, df=4** |
| Group II | 2nd | 95 | 26 | 27.36 | **P**<.00001 |
| Group III | 3rd | 112 | 41 | 32.03 | |
| Group IV | 4th | 115 | 72 | 64.28 | |
| Group V | ≥4th | | 73 | 63.47 | |
| **Total** | | **460** | **213** | **46.30** | |

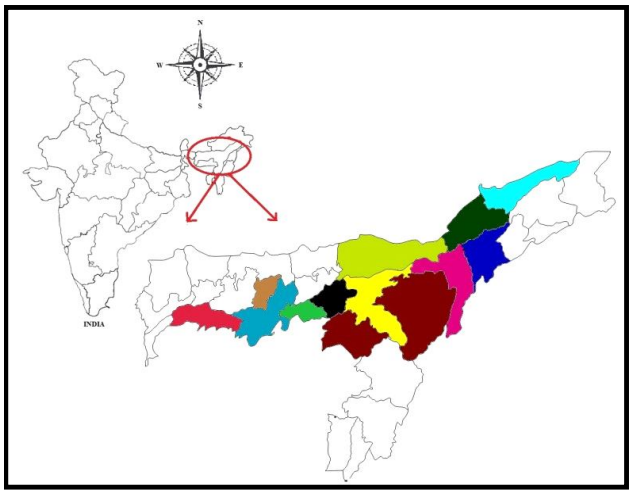


**Fig 2:** Seroprevalence of Fowl adenovirus antibodies in different districts of Assam by Dot Density Method.

FAdV antibodies was observed in all age grouped birds (Table 2). Highest (64.28%) positivity was recorded in the birds of 4th week of age, followed by above 4th week (63.47%), 3rd week (32.03%), 2nd week (27.36%) and 1st week (10.0%). The seroprevalence of FAdV antibodies have significant difference ($\chi^2$= 89.1361, P<0.00001) among the age groups. Seroprevalence of FAdV-1 and FAdV-4 in broiler chick of < 2 weeks of age was recorded as 58.1% and 12.9% respectively by Chang and Tsai (2006), whereas Ito *et al*. (2007) recorded 6.5% seroprevalence in broiler chicken against serotype- 8. Rahman *et al*. (1997) titered the IHA antibodies against FAdV in the sera of chickens above 4th week of age. The present observations indicated that the parent stocks were not vaccinated against IBH-HPS, as no maternal antibodies against FAdV could be detected in the chicks of 1st week of age. Sero-positivity implies the picking up of infection by the birds from the premises or other risk factors associated in such situation. Immunologically, the chicks were naive against FAdV antibodies and highly susceptible to infection. Therefore the vaccination is utmost essential in these endemic areas to protect the birds from IBH-HPS.

Health status-wise 68.97 percent sero-positivity was recorded in the affected flock, where as 32.51 percent in apparently healthy flock (Table 3).There is insignificant association ($\chi^2$= 3.4784, P=0.6217) between the health groups In relation to the age group, 100 percent sero-positivity was recorded the birds of group-V *i.e*. above 4th weeks of age from infected flock. In apparently healthy flock, antibodies could be detected in group IV and Group V. Presence of FAdV antibodies in apparently healthy flock,indicated that the birds might be experienced the infection during their life (Hussain *et al*., 2003).

The presence of the FAdV antibodies in apparently healthy birds is a suggestion that the disease might be endemic in this area but there is absence or lack of proper reporting system. Also, outbreak and clinical disease might occur but confused or mistaken for other diseases.

Season-wise, highest (61.53%) prevalence was recorded in post monsoon season followed by monsoon

**Table 3:** Health status-wise sero-prevalence of fowl adenovirus antibodies.

| Health status | Sample tested(Nos.) | Positive(Nos.) | % Positivity | $\chi^2$ |
|---|---|---|---|---|
| Infected flock | 174 | 120 | 68.97 | **3.4784, df=4** |
| Apparently healthy | 286 | 93 | 32.51 | **P**=0.0621 |
| **Total** | **460** | **213** | | |

**Table 4:** Seroprevalence of fowl adenovirus antibodies in different season.

| Season | Sample tested(Nos.) | Positive(Nos.) | % Positivity | $\chi^2$ |
|---|---|---|---|---|
| Pre-monsoon (March- May) | 71 | 23 | 32.39 | **68.9342, df:3** |
| Monsoon (June- September) | 200 | 98 | 49.00 | P<0.00001 |
| Post-monsoon (October- November) | 104 | 64 | 61.53 | |
| Winter (December- February) | 85 | 28 | 32.49 | |
| **Total** | **460** | **213** | | |

(49.00%), winter (32.49%) and pre-monsoon (32.39%). The seroprevalence of FAdV antibodies have significant difference ($\chi^2$= 68.9342, P<0.00001) among the different seasons (Table 4). Although the positive antibody titer was present in all the seasons, the occurrence of the disease could only seen in monsoon and post-monsoon. The effect of season in disease outbreak pattern was assured earlier (Yunus *et al*., 2009). The heavy rainfall, warm temperature and high humidity during monsoon season increased stress to the birds leading to occurrence of disease. The highest prevalence in the post monsoon season might be due to preceded infection in the monsoon season.

## CONCLUSION

The evaluated commercial ELISA for the detection of FAdV antibodies in broiler chicken of twelve districts of Assam was successfully applied. The overall seropositivity was recorded as 46.38 per cent. The assay was found to be rapid and sensitive so that timely measures can be taken to prevent spread of the infection.

The high prevalence of FAdV antibodies suggests that the disease is endemic in the state. Since this is the first study on the prevalence of Fowl adenovirus infection, there is an urgent need of detail study with a large numbers of population throughout the state, which will be of helpful for formulation of control and eradication programmes.

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### Conflict of interest

The authors declared that they have no any conflict of interest**.**

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