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Sero-epidemiology of duck plague in duck population of Assam

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Abstract
Background: Duck Plague Virus (DPV) or Duck enteritis virus (DEV) affect wild, domestic ducks, geese and swans as early as at one week age up to adult age. The first documented report of Duck virus enteritis in Assam dates back to 1980. There are also reports of outbreaks of the disease sporadically time to time from different duck rearing states of India. The disease is also most frequently reported from different districts of Assam due to considerable density of duck population in the state. However, there are not much data available on the sero-prevalence of Duck plague in Assam. The present study was undertaken to obtain the baseline epidemiological information on the prevalence of Duck plague and its association with age, breed, health status and seasons.
Methods: Blood samples were collected from 11 different districts of Assam from different agro-climatic zones at various time periods during the period from February, 2019 to March, 2020. Association of various factors like age, breed, season and health status with the prevalence of the affected ducks were studied. Indirect ELISA was performed by using manually made Duck plague ELISA technique. The results obtained were analyzed by the Optical density (O.D.) of the wells measured at 492nm in ELISA reader (Biorad, USA).
Results: In the present study, a total of 465 serum samples were tested, of which 207 were found positive for DPV antibodies with a sero-positivity of 44.51 percent. Among the different age groups, the highest sero-positivity was recorded in adults (59.90%), followed by growers (29.46%) and ducklings (10.62%). Breed wise, highest sero-positivity was recorded in Pati ducks (29.95%), followed by Indian Runner (22.22%), White Pekin (17.87%), Nageswari (16.42%) and Khaki Campbel (13.52%). Health status-wise, 40.70 percent sero-positivity was recorded in adult ducks followed by growers (35.39%) and ducklings (23.89%) from ailing flock. In apparently healthy flock, adult ducks had sero-positivity of 42.55 percent followed by growers (37.23%) and ducklings (20.21%). Season-wise, highest prevalence was recorded in pre-monsoon season (38.64%) followed by post-monsoon (30.43%), winter (21.25%) and monsoon (9.66%) season.

Keywords: Ducks, duck plague, duck virus enteritis, ELISA, seroprevalence

1. Introduction
Duck rearing is a profitable livestock industry because of its egg, meat and feather. Duck keeping is an integral part of rural farming system in India that provides supplementary income to the small, marginal and landless farmers. The leading states in duck population are Assam, West Bengal, Tripura, Kerala, Andhra Pradesh, Tamil Nadu, Uttar Pradesh, Bihar and Orissa (Narahari, 2009) [2]. Assam is one of the major duck rearing states in country. In Assam, ducks fulfils a great proportion of animal protein like any other developing countries of the world in the form of meat and eggs (Neher, 2016) [27]. Moreover, ducks are more prolific than chicken and more adaptable to free-range system of rearing. They also grow faster than chicken (Rajput *et al*., 2014) [32].
Ducks are suitable for integrated farming systems such as duck-cum-fish farming and duck farming with rice cultivation. It plays an important role in the socio-economic development of poor and landless farmers. Duck farming provides a continuous flow of income, enabling the poor to meet their daily cash requirements. Traditionally, the role of women in duck farming is well recognized (Sahariah, 2016) [33]. The most important constrain in duck rearing is infectious diseases, of which Duck plague (DP) is the most important one (Khan *et al*., 2018).
DP or DVE is an acute, highly contagious and lethal disease of duck, geese and swans, which was first reported from Netherland (Baudet, 1923) [4]. Thereafter the disease has been reported in many countries like North America (Levine and Fabricant, 1950) [21];

China (Jansen and Kunset, 1964) [15]; Belgium (Devos *et al*., 1964) [9]; Canada (Hanson and Willis, 1976) [12]; Bangladesh (Sarkar, 1980) [34]; Denmark (Prip *et al*., 1983) [34]; Austria (Pechan *et al*., 1985) [29]; France (Vuilluame, 1989) [35]; Thailand (Leibovitz, 1991); and Vietnam (Morrissy *et al*., 2004) [22]. In India, the disease was first reported from West Bengal (Mukherjee, *et al*., 1963) [23] and subsequently transmitted to Tamil Nadu (Duraiswami *et al*., 1979) [11]; Assam (Chakraborty *et al*., 1980) [6]; Kerala (Rajan *et al*., 1980) [31] etc.

The etiological agent of DP or DVE is known as Duck Enteritis Virus (DEV) or Anatid herpesvirus-1. According to 10th International Committee on Taxonomy of Virus (ICTV, 2019) [1], DEV was grouped under the genus Mardivirus, sub-family Alpha-herpesvirinae of the family Herpesviridae (King *et al*., 2011) [18]. Various serological tests like AGPT, CIE, serum neutralization, immune-peroxidase, immune-fluorescence immune-diffusion and Indirect-ELISA were used for studying the seroprevalence of duck enteritis virus infection in ducks (John *et al*., 1989) [16].

In this study, we have reported the prevalence of duck plague viral antibodies in duck population of different places of Assam in order to take applicable measures to control the disease which causes economic loss to duck industry.

2. Materials and Method

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

2.1 Ethical approval: The present research work was approved by the Institutional Animal Ethics Committee (IAEC) vide letter No: 770/GO/Re/S/03/CPCSEA/FVSc/AAU/IAEC/19-20/775, dated. 23.12.2019.

2.2 Study Area: The study was conducted in 5 agro-climatic zones of Assam *viz*. Lower Brahamaputra Valley, North Bank plain zone, Central Barak Valley zone, Barak Valley zones and Hill Zones.

Blood samples were collected from unvaccinated flock of duck 11 different districts of Assam *viz*. Kamrup (M), Kamrup (R), Nalbari, Dhuburi, Nagaon, Sonitpur, Lakhimpur, Dhemaji, Cachar, Karbi-Anglong and Kokrajhar during the period from February, 2019 to March, 2020 (Fig. 1).

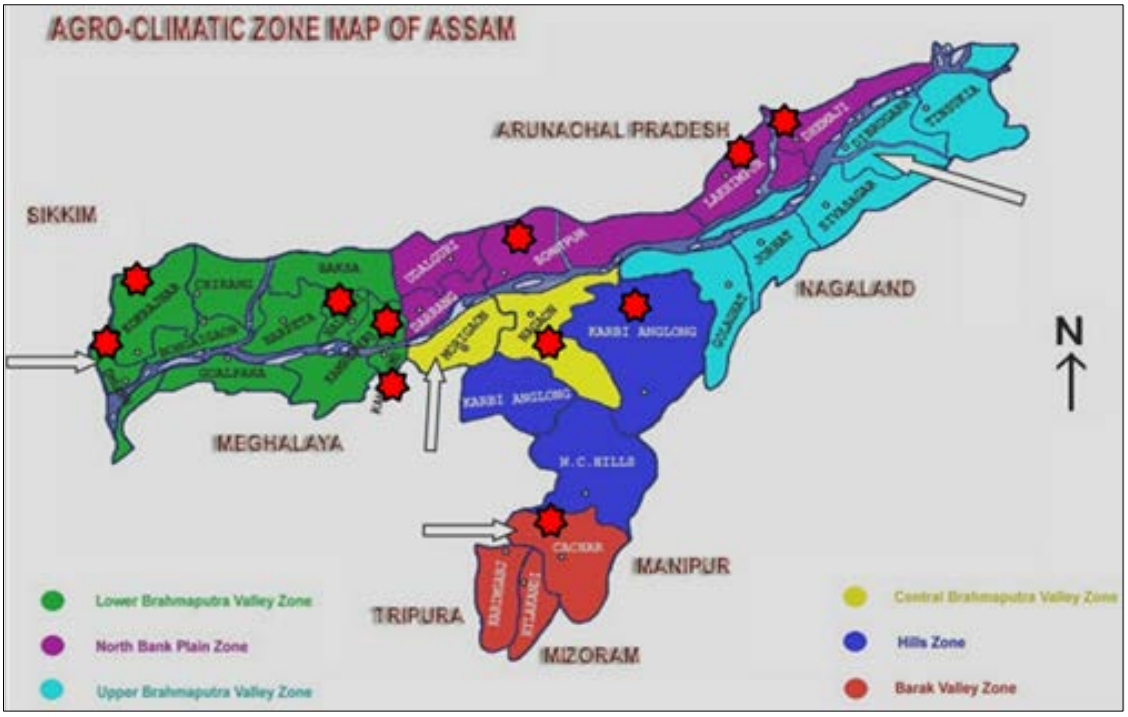

Fig 1: Map of Assam showing different districts of blood collection.

2.3 Design of Study and sampling

A structured questionnaire was prepared to collect epidemiological data such as breed, age, health status, previous occurrence of any other diseases etc.

The sample size was calculated as per Thrust field (1995) considering the 50% prevalence as expected prevalence, 95% confidence interval and 5% absolute precision. The calculated value with the following formula comes out as 384, however in order to improve the precision, the sample size was increased upto 465.

$n = 1.962 \times P^{exp} (1-P^{exp}) /d2$

n =1.962 X 0.5%(1 – 0.5)/0.052 1.96 – Z value of 95% CI

n = 384

n=required sample size

P^{exp}= expected prevalence

d2 =desired absolute precision

2.4 Association of different factors

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) as per Regional Meterological Centre, Barjhar, Guwahati, Assam.

Age: The duck population were divided into three (3) categories *viz*.

Ducklings	:	0-8 weeks
Growers	:	8-20 weeks
Adults	:	>20 weeks

Breed: The different breeds of duck used in the present study were-

- Indian Runner
- Khaki-Campbel
- Pati duck
- White Pekins
- Nageswari

Health status: Blood samples were collected from ailing as well as apparently healthy flock.

2.5 Sample Collection

About 2ml of blood samples were collected from the medial metatarsal vein in clot activator vial (Peerless Biotech Pvt. Ltd) from unvaccinated ailing as well as apparently healthy ducks. The serum was separated from blood by centrifuging at 3000 rpm for 10 min. Then the separated serum was collected in a screw capped plastic vial and stored at -20 ºC until they were tested.

2.6 ELISA Test

Indirect ELISA was performed as per the method described by Neher *et al*. (2019) [28] and the purified attenuated DPV was obtained from the Department of Veterinary Microbiology, C.V.Sc., A.A.U, Khanapara, Guwahati, Assam. Briefly, 96 well ELISA plates (Polysorp, Nunc) were coated with 10 µg of purified virus in carbonate-bicarbonate buffer (pH 9.6). Antigen was added to all the wells (Row 1 to Row 7) except antigen negative (Ag -ve) control wells (Row 8) and incubated overnight at 4 ^{0}C. After incubation, plates were washed thrice with washing buffer, PBS+-T (0.0002mol/L diluted PBS containing 0.05% Tween 20). After thorough washing, pre-blocking was done by using blocking buffer containing 5% BSA and 2% LAH @ 50 µl to each well and the plate was kept for 1 hour at 37 ^{0}C. Two fold serial dilution of serum samples with initial dilution at 1:40 were made in blocking buffer and added in 50 µl volume and incubated. Controls included positive and negative sera: a) 50 µl of diluted strong positive serum to each well of row 6; b) 50 µl of negative serum to each well of row 7. After incubation, unbound antibodies were washed thrice with washing buffer and a volume of 50 µl diluted anti duck HRPO conjugate (KPL, USA) diluted in wash buffer @1:200 was added to each well and incubated at 37 ^{0}C for 1 hour. After washing 50 µl of freshly prepared OPD substrate + H_2O_2 solutions was added to each well. After 15-20 minutes of incubation, colour reaction was stopped by adding equal volume of 1M H_2SO_4 to all the wells. Optical density (O.D.) of the wells measured at 492nm in ELISA reader (Biorad, USA).

2.7 Interpretation

The cut-off value was determined on the basis of the negative serum reactivity. When the Mean OD_{492} value of test sample - Mean OD_{492} of negative sample was more than or equal to 0.1 (≥0.1) it was considered as the end point of serum dilution.

2.8 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 s*et al*pha level (0.05) then the result was considered as statistically significant.

3. Results and Discussion

During the present study, the sero-prevalence of Duck plague was carried out in 11 districts of Assam. The spatial distribution of Duck plague in different agro-climatic zones of Assam has been shown in Table 1 and Figure 2.

Table 1: Sero-prevalence of DPV in different districts

Name of the District	Sample tested (Nos.)	Positive (Nos.)	% positivity	χ^2
Kamrup (M)	49	28	13.52	
Dhubri	35	23	11.11	
Nalbari	55	17	8.21	
Kamrup (R)	56	34	16.42	
Nagaon	50	23	11.11	
Sonitpur	38	18	8.69	*P*<0.01
Lakhimpur	40	16	7.72	
Dhemaji	42	14	6.76	
Karbi-Anglong	30	12	5.79	
Kokrajhar	32	9	4.34	
Cachar	38	13	6.28	
Total	465	207	44.51	

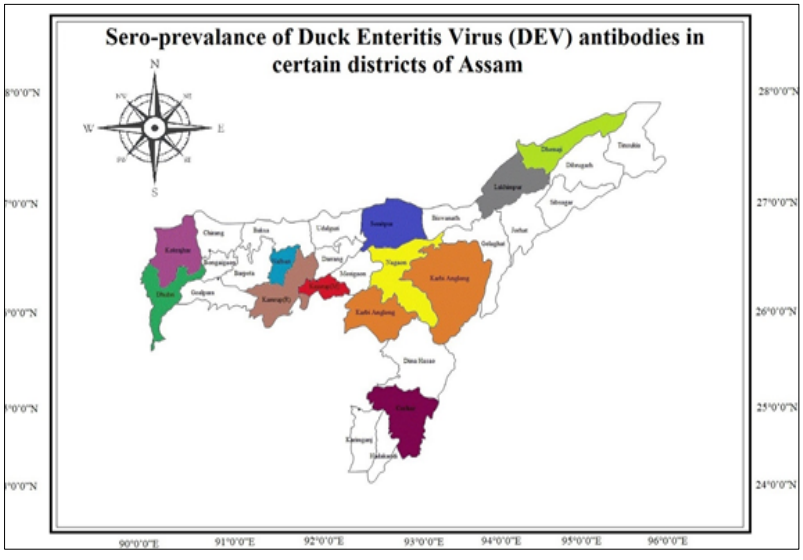

Fig 2: The spatial distribution of DPV antibodies in different districts of Assam

Out of the total 465 sera samples tested, 207 (44.51%) positive by iELISA. Highest (16.42%)) and lowest (4.34%) sero-positivity was recorded in Kamrup (R) and Kokrajhar district respectively. The sero-prevalence of Duck plague antibodies have significant association (**P<0.01) between the different districts. Earlier seroprevalence of Duck plague in Assam was recorded by Sahariah (2016) [33] with a sero-positivity 38.42%. Neher *et al*., (2019) [28] also reported higher sero-positivity (65.39%) from the duck population of Assam. Indirect ELISA was most convenient diagnostic tool for the detection of antibodies against various viral infections in sero-epidemiological surveys (Bhanuprakash *et al*., 2006 and Balamurugan *et al*., 2007) [5, 2]. Indirect ELISA allowed rapid detection of duck plague antibodies in ducks (Morrissey *et al*., 2004) [22]. However some researcher (Dargiri and Butterfield, 1969; Jacobsen *et al*., 1976; Mukit, 1985; Hien *et al*., 2004; Morrissy *et al*., 2004 and Kaleta, 2007) [8, 14, 24, 13, 22] reported that virus neutralization test was more suitable for diagnosis of Duck virus Enteritis (DVE) viral antibody from field samples.

DPV antibodies could be observed in all age grouped ducks (Table 2). Highest positivity was recorded in adults (59.90%), followed by growers (29.46%) and ducklings (10.62%). The sero-prevalence of DPV antibodies have significant association (*P<0.05) between the different age group. The data pertaining to the sero-prevalence of DPV antibodies in different age groups could not be traced out in available literature. Sero-positivity in the ducks of all group integrated that ducks picked the infection from the premises or other risk factors associated in such situation. Therefore the vaccination is utmost essential in these endemic areas to protect the ducks from Duck plague.

Table 2: Sero-prevalence of DPV antibodies at different age groups.

Age group	Sample tested (Nos.)	Positive (Nos.)	% positivity	χ^2
Ducklings (0-8 wks)	79	22	10.62	
Growers (8-20 wks)	135	61	29.46	P<0.05
Adults (>20 wks)	251	124	59.90	
Total	465	207	44.51	
Age group	Sample tested (Nos.)	Positive(Nos.)	% positivity	χ^2
Ducklings (0-8 wks)	79	22	10.62	
Growers (8-20 wks)	135	61	29.46	P<0.05
Adults (>20 wks)	251	124	59.90	
Total	465	207	44.51	

DPV antibodies could be detected in different breeds of ducks (Table 3). Highest positivity was recorded in Pati ducks (29.95%), followed by Indian Runner (22.22%), White Pekin (17.87%), Nageswari (16.42%) and Khaki Campbel (13.52%). The sero-prevalence of DVE antibodies have significant association (**P<0.01) between the different breeds. Although, DEV is prevalent in India yet literature on breed-wise sero-prevalence could not be traced out. The study indicated that all breeds of duck were susceptible to DPV infection.

Table 3: Sero-prevalence of DPV antibodies in different breeds

Breed	Sample tested (nos.)	Positive (nos.)	% Positivity	χ^2
Indian runner	102	46	22.22	
White pekin	87	37	17.87	
Khaki campbel	65	28	13.52	P<0.01
Pati ducks	117	62	29.95	
Nageswari	94	34	16.42	
Total	465	207	44.51	

Health status-wise highest percent sero-positivity was recorded in adults (40.70%) followed by growers (35.39%) and ducklings (23.89%) from ailing flock. In apparently healthy flock, adult ducks had sero-positivity of 42.55 percent followed by growers (37.23%) and ducklings (20.21%) (Table 4) which indicated that, they might be experienced the infection during their life. Till the date, data related to health status wise presence of DPV antibodies could not be detected in available literature. DPV antibodies have insignificant association between the health groups.

Table 4: Health status-wise Sero-prevalence of DPV antibodies

Health Status	Sample tested (Nos.)	Positive (Nos.)	% positivity	χ^2
Infected flock	214	113	52.80	
Apparently healthy flock	251	94	37.47	P=0.1866
Total	465	207		

Season-wise, highest prevalence was recorded in pre-monsoon season (38.64%) followed by post-monsoon (30.43%), winter (21.25%) and monsoon (9.66%) (Table 5). The sero-prevalence of DPV antibodies have significant association (*P<0.05) between the different seasons. Presents of positive antibodies in all the seasons indicated the persistence of viral infection throughout the year.

Table 5: Sero-prevalence of DPV antibodies in different seasons

Season	Sample tested (Nos.)	Positive (Nos.)	% positivity	χ^2
Pre-monsoon	148	80	38.64	
Monsoon	73	20	9.66	
Post-monsoon	131	63	30.43	P<0.05
Winter	113	44	21.25	
Total	465	207	44.51	

4. Conclusion

The overall sero-positivity was recorded as 44.51 percent. The Indirect ELISA was found to be rapid and sensitive so that timely measures can be taken to prevent spread of the infection.

The presence of duck plague viral antibodies in ducks indicates that the population was exposed to DPV infection

naturally, either directly or indirectly. However, collection of more number of samples from all parts of Assam will elucidate the exact epidemiological picture of duck virus enteritis, which will be of helpful for formulation of control and eradication programmes.

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6. Conflict of interest: The authors declared that they have no any conflict of interest.

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