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Alteration of Liver and Kidney Specific Biomarkers in Indigenous Ducks Infected with Duck Plague Virus

Prasanta Chabukdhara[1], Dhruba Jyoti Kalita[2], Shantanu Tamuly[2], Naba Jyoti Deka[2], Gautam Bordoloi[3], Sanjib Khargharia[4], Ankita Gogoi[5]

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ABSTRACT

Duck plague is an economically devastating viral disease prevalent in ducks which causes massive loss of duck populations annually. High morbidity and mortality are characteristic of the infection and outbreaks. Duck plague is reported from time to time from different parts of our country including Assam. The infected ducks manifest clinical signs like photophobia, partially or completely closing of eyelids, excessive thirst, nasal discharge, anorexia, drooped plumage, ataxia, diarrhea with soiled vents along with tremors in the head, neck and body. Present study was aimed to evaluate the deleterious effects of duck plague virus on liver and kidney condition by estimating liver and kidney specific biomarkers from indigenous ducks, namely Pati, Nageswari and Cinahanh of Assam from different agroclimatic zones. Study revealed significant increase of AST, ALT, ALP, uric acid and creatinine which is indicative of disruption of healthy function of liver and Kidney due to duck plague virus infection.

Key words: Duck plague virus, Indigenous duck, Kidney function, Liver function.

Duck husbandry constitutes a valuable livestock enterprise for marginal and landless farmers which serve to provide an extra source of income. In India, duck population is mainly concentrated in North Eastern, Eastern and Southern states (Veeramani *et al*., 2014). From North eastern region, Assam is considered to be rich in duck genetic resource repository owing to the abundant watershed, marshy areas which provide congenial environment for rearing and breeding of duck (Kalita *et al*., 2009).

One of the most dreaded diseases known to be prevalent among duck populations across the globe is duck plague also called as duck viral enteritis. It is an acute, highly contagious and fatal viral disease with high morbidity and mortality. Duck plague is known to cause heavy economic losses to the duck farming community with concomitant loss of duck populations annually (Dhama *et al*., 2017). The causative organism of duck plague is Anatid herpesvirus type 1, belonging to the family Herpesviridae, subfamily Alpha herpesvirinae and Genus Mardivirus (Fadly *et al*., 2008; King *et al*., 2011). Affected ducks manifest clinical signs of photophobia, partially or completely closing of eyelids, increased thirst, dehydration, inappetence, nasal discharge, excessive lacrimation, greenish-watery diarrhea and soiled vents (Davison *et al*., 1993). Duck plague outbreaks are reported from time to time in different places of Assam. Hence, the present study was conducted to evaluate the liver and kidney function in indigenous ducks of Assam, namely Pati, Nageswari and Cinahanh infected with duck plague virus by estimating liver and kidney specific biomarkers from different agro-climatic zones of Assam.

Ducks under study were divided into three groups as healthy, duck plague infected and recovered. Duck plague outbreaks reported from different agro-climatic zones *viz*. North Bank, Upper Brahmaputra, Lower Brahmaputra and Barrak Valley zones of Assam during the period April, 2020 to August, 2022 were attended to collect blood samples from clinically affected and recovered indigenous ducks. Ducks which came in contact with infected birds were separated and observed for disease progression or recovery from

[1]Department of Veterinary Physiology and Biochemistry, Lakhimpur College of Veterinary Science, Assam Agricultural University, North Lakhimpur-787 051, Assam, India.
[2]Department of Veterinary Biochemistry, College of Veterinary Science, Assam Agricultural University, Khanapara-781 022, Guwahati, Assam, india.
[3]Department of Veterinary Parasitology, Lakhimpur College of Veterinary Science, Assam Agricultural University, North Lakhimpur-787 051, Assam, india.
[4]Department of Veterinary Pharmacology and Toxicology, Lakhimpur College of Veterinary Science, Assam Agricultural University, North Lakhimpur-787 051, Assam, india.
[5]Department of Animal Genetics and Breeding, Lakhimpur College of Veterinary Science, Assam Agricultural University, North Lakhimpur-787 051, Assam, india.

Corresponding Author: Prasanta Chabukdhara, Department of Veterinary Physiology and Biochemistry, Lakhimpur College of Veterinary Science, Assam Agricultural University, North Lakhimpur-787 051, Assam, India. Email: pchabukdhara87@aau.ac.in

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infection. Around 7- 10 ml of blood were collected from wing vein in sterile vials from each bird of the respective groups, healthy (Pati-55, Nageswari-33 and Cinahanh-37), duck plague infected (Pati-71, Nageswari-52 and Cinahanh-59) and recovered ducks (Pati-39, Nageswari-27 and Cinahanh-24) for separation of serum and were used for estimation of Aspartate Aminotransferase (AST), Alanine Aminotrans ferase (ALT), Gamma Glutamyl Transferase (GGT), Alkaline Phosphatase (ALP), Total Bilirubin, Direct Bilirubin and Indirect Bilirubin, Uric Acid (UA), Creatinine and Blood Urea Nitrogen (BUN). Serum indirect bilirubin was determined by subtracting direct bilirubin values from the total bilirubin. The parameters were analyzed in Analytica 705 Semi-Auto Biochemistry Analyzer using commercially available diagnostic kit (Meril Diagnostics Pvt. Ltd., Gujarat, India). All the analyses were carried out in the facilities available at College of Veterinary Science, AAU, Khanapara, Guwahati and Lakhimpur College of Veterinary Science, AAU, North Lakhimpur, Assam. The data obtained were subjected to statistical analyses by statistical software R (version 4.1.1). Two factor ANOVA was used to differentiate the mean values followed by multiple comparison using pairwise t-test with Bonferroni correction. The $p<0.05$ was considered as statistically significant.

Liver and kidney specific serum biomarkers (Mean±SE) of indigenous ducks namely Pati, Nageswari and Cinahanh of healthy, duck plague infected and recovered ducks are presented in Table 1. The infected ducks manifesting different clinical signs have been depicted in Fig 1 to Fig 4.

Results from the current study revealed that liver and kidney specific biomarkers were significantly altered in ducks infected with duck plague virus. Indigenous ducks infected with duck plague virus were observed to have significantly increased ($P<0.05$) AST activity in Pati (60.39±1.61 IU/L),

Table 1: Liver and kidney specific biomarkers (Mean±SE) in healthy, duck plague infected and recovered ducks.

Parameter	Breed	Healthy	Infected	Recovered
Liver specific biomarkers				
Aspartate aminotransferase (IU/L)	Pati	48.95 ^{A,b}±1.40	60.39 ^{A,a}±1.61	47.58 ^{A,b}±1.92
	Nageswari	50.68 ^{A,b}±1.55	62.97 ^{A,a}±1.40	47.23 ^{A,b}±1.65
	Cinahanh	48.51 ^{A,b}±1.00	56.97 ^{A,a}±1.48	45.90 ^{A,b}±0.77
Alanine aminotransferase (IU/L)	Pati	38.66 ^{A,a}±1.90	43.97 ^{A,a}±1.29	39.61 ^{A,a}±2.16
	Nageswari	37.65 ^{A,b}±1.31	46.64 ^{A,a}±1.67	40.16 ^{A,b}±1.23
	Cinahanh	40.10 ^{A,b}±1.34	46.54 ^{A,a}±1.03	42.09 ^{A,b}±1.39
Gamma glutamyl transferase(IU/L)	Pati	1.00 ^{A,a}±0.06	1.06 ^{A,a}±0.09	0.86 ^{B,a}±0.04
	Nageswari	1.09 ^{A,a}±0.08	1.09 ^{A,a}±0.08	1.16 ^{A,a}±0.09
	Cinahanh	0.94 ^{A,a}±0.04	0.99 ^{A,a}±0.05	0.97 ^{AB,a}±0.06
Alkaline phosphatase (IU/L)	Pati	57.89^{A,b}±2.46	67.29 ^{A,a}±2.57	57.53 ^{A,b}±2.35
	Nageswari	60.53^{A,b}±2.18	68.97^{A,a} ±1.17	55.48 ^{A,b} ±2.24
	Cinahanh	58.29^{A,b}±1.45	65.49^{A,a}±1.51	57.70 ^{A,b} ±1.81
Total bilirubin (mg/dl)	Pati	0.43^{B,a}±0.02	0.51 ^{B,a}±0.03	0.43 ^{B,a}±0.02
	Nageswari	0.44 ^{B,a}±0.03	0.47 ^{B,a}±0.05	0.44 ^{B,a}±0.03
	Cinahanh	0.58 ^{A,a}±0.03	0.69 ^{A,a}±0.03	0.63 ^{A,a}±0.04
Direct bilirubin (mg/dl)	Pati	0.17 ^{B,b}±0.01	0.22 ^{B,a}±0.01	0.18 ^{B,ab}±0.01
	Nageswari	0.18 ^{B,a}±0.02	0.21 ^{B,a}±0.02	0.18 ^{B,a}±0.02
	Cinahanh	0.26 ^{A,a}±0.02	0.28 ^{A,a}±0.02	0.26 ^{A,a}±0.02
Indirect bilirubin (mg/dl)	Pati	0.25 ^{A,a}±0.02	0.30 ^{B,a}±0.03	0.25 ^{B,a}±0.01
	Nageswari	0.27^{A,a}±0.02	0.31^{B,a}±0.03	0.28 ^{B,a}±0.02
	Cinahanh	0.32 ^{A,b}±0.02	0.41 ^{A,a}±0.02	0.37 ^{A,ab}±0.03
Kidney specific biomarkers				
Uric acid (mg/dl)	Pati	4.35 ^{A,a}± 0.14	4.41 ^{A,a}±0.15	4.33 ^{A,a}±0.13
	Nageswari	4.53 ^{A,ab}±0.17	5.02 ^{A,a}±0.15	4.45 ^{A,b}±0.14
	Cinahanh	4.35 ^{A,a}±0.13	4.74 ^{A,a}±0.16	4.26 ^{A,a}±0.14
Creatinine (mg/dl)	Pati	0.71 ^{A,b}±0.04	0.80 ^{A,a}±0.05	0.69 ^{A,b}±0.04
	Nageswari	0.83 ^{A,a}±0.05	0.84 ^{A,a}±0.05	0.79 ^{A,a}±0.06
	Cinahanh	0.68 ^{A,b}±0.04	0.80 ^{A,a}±0.03	0.65 ^{A,b}±0.05
Bun (mg/dl)	Pati	1.95 ^{A,a} ±0.14	2.11 ^{A,a}±0.07	1.98 ^{A,a} ±0.15
	Nageswari	1.76 ^{A,a}±0.17	1.95 ^{A,a}±0.16	1.90 ^{A,a}±0.18
	Cinahanh	1.75 ^{A,a}±0.08	1.87 ^{A,a}±0.09	1.87 ^{A,a}±0.08

Means bearing different superscripts (Capital letters) differ significantly ($P<0.05$) in columns and means bearing different superscripts (Small letters) in a row differ significantly ($P<0.05$).

Nageswari (62.97±1.40 IU/L) and Cinahanh (56.97±1.48 IU/L) as compared to healthy and recovered ducks. Nageswari and Cinahanh infected with duck enteritis virus were recorded to have significantly ($P<0.05$) increased activities of ALT. GGT did not reveal any significant alteration in different groups of ducks. However, recovered Nageswari ducks (1.16±0.09 IU/L) had significantly ($P<0.05$) higher activities as compared to recovered Pati ducks (0.86±0.04 IU/L). Alkaline phosphatase activity was significantly increased ($P<0.05$) in all the indigenous ducks infected with duck plague virus (Pati 67.29±2.57, Nageswari 68.97±1.17 and Cinahanh 65.49±1.51 IU/L) compared to healthy and recovered counterparts. The study documented significantly higher ($P<0.05$) mean levels of total bilirubin in all three groups of Cinahanh ducks as against corresponding total bilirubin levels in all three group of Pati and Nageswari ducks. Cinahanh birds belonging to all three groups were seen to have significantly higher ($P<0.05$) mean values of direct bilirubin also in contrast to Pati and Nageswari ducks. Significantly elevated levels ($P<0.05$) of direct bilirubin were observed in diseased Pati ducks (0.22±0.01 mg/dl) compared to healthy birds (0.17±0.01 mg/dl). The significantly ($P<0.05$) increased activities of AST, ALT, GGT and ALP in duck plague virus infected ducks indicated that the hepatic tissue was markedly injured as a consequence of infection. Moreover, liver specific enzyme activities are commonly seen to be increased when the hepatocytes undergo necrosis and suffer canalicular membrane or biliary epithelial cells damages due to varied etiologies including viral infections (Giannini *et al*., 2005; Padda *et al*., 2011).

Mahmoud (2015) too reported the AST, ALT, ALP and GGT activities to be significantly increased in the Egyptian ducks infected naturally with avian influenza. Kudair and Al-Hussary (2010) too reported significant elevation of aspartate aminotransferase (AST) activity in broiler chickens in response to vaccines administered against infectious bursal disease, infectious bronchitis and Newcastle disease viruses. Significantly increased activities of AST, ALT and ALP were also reported by Mandrawy and Ismail (2017) in Egyptians broilers birds infected with Newcastle disease virus. Duck circovirus infection was also seen to prominently elevate the serum activities of AST, ALT, GGT, ALP and serum levels of total bilirubin in ducks (Zhu *et al*., 2019). Significantly elevated activities of ALP in Amyloidotic ducks as compared to healthy ducks were also documented by Malkinson *et al.* (1980).

Duck plague virus infection was also responsible to elevate the kidney specific biomarkers. Duck plague virus infected Nageswari ducks had significant higher ($P<0.05$) mean uric acid (5.02±0.15 mg/dl) as compared to recovered ducks (4.45±0.14 mg/dl). Significantly ($P<0.05$) elevated levels of mean serum creatinine were recorded in duck plague virus infected Pati (0.80±0.05) and Cinahanh (0.80±0.03) as compared to healthy and recovered groups. Serum blood urea nitrogen levels in the current study did not reveal any significant alteration among different groups.

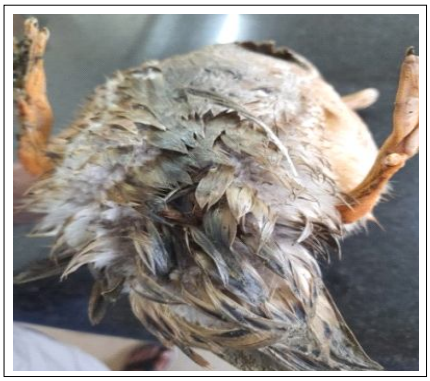

Fig 1: Soiled vents due to diarrhea.

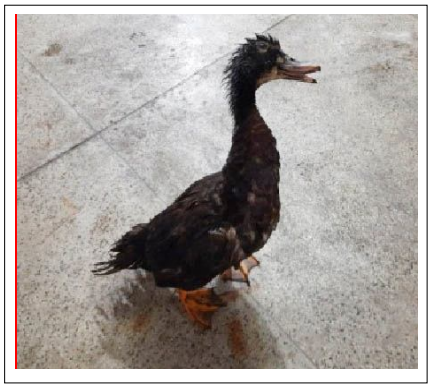

Fig 2: Ducks showing nervine signs.

Fig 3: White discoloration of eye mucous membrane.

Fig 4: Excessive lacrimation.

Observations on similar lines were reported by Mahmoud (2015) in avian influenza infected Egyptian domestic ducks which had prominent higher mean values of serum uric acid and creatinine. Malkinson *et al*., (1980) also revealed similar findings in an investigation performed in white Pekin ducks suffering from amyloidosis where they documented significantly elevated levels of serum uric acid in the affected ducks. Mandrawy and Ismail (2017) found that uric acid and creatinine levels were significantly increased in the Egyptian chickens infected with Newcastle disease virus as compared to the vaccinated broiler chickens. Significantly elevated levels of serum uric acid and creatinine were also reported by Okorie-Kanu *et al*., (2016) in Pekin ducks experimentally infected with Newcastle disease virus. Kalita *et al*., (2020) too reported significantly elevated levels of BUN in Pati ducks infected with duck plague virus in contrast to healthy ducks. Observed higher serum levels of uric acid, creatinine and BUN in the duck plague virus infected ducks in the present study might be due to possible kidney injury caused by infection (Okorie-Kanu *et al*., 2016). Dehydration usually seen in duck plague virus infection might have also played a role in elevating the serum uric acid and creatinine levels (Okorie-Kanu *et al*., 2016). The internal hemorrhages caused in different internal organs commonly seen in duck plague virus infection and consequent blood loss might also have caused elevated synthesis of different serum constituents (Echols, 2009).

CONCLUSION

In the present study, liver and kidney specific biomarkers in indigenous ducks of Assam infected with duck plague virus were evaluated. Findings from the study revealed significant derangement of hepatic and renal functions in the infected ducks which was evident from the significantly increased serum levels of liver and kidney specific biochemical markers.

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Conflict of interest: None.

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