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Cytokine profile of ducks during duck plague in Assam

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Abstract
Serum cytokine level in three different varieties of duck namely Pati, Khaki Campbell and Nageswari from different parts of the state were evaluated in healthy state and during disease outbreak. The Cytokine profile namely IL-6, IL-4 and IL-10 were estimated using Enzyme Linked immune-sorbent assay and expressed in pg/ml. The IL 6 level of Pati duck during health and disease conditions were recorded as 7.71 ±0.35 pg/ml and 7.40±0.45 pg/ ml respectively. Both The Pati and Nageswari breed of ducks showed a non significant decrease in the IL 6 level during disease outbreak. The present study showed a significant increase (P<0.05) in IL 4 level of diseased Khaki Campbell breed (15.25 ±0.43 pg/ml) than that of the healthy ones (12.32 ±1.39 pg/ml). The IL 4 level in the healthy Nageswari breed of ducks were recorded to be 11.29 ±0.52 pg/ml) and during disease outbreak it was observed to be significantly (P<0.05) higher than the healthy state (15.34 ±0.51 pg/ml). Statistical analysis showed that there is a significant rise (P<0.05) in the level of serum IL 10 during disease outbreak in all the three varieties of duck as compared to that of the healthy ducks. From the present study, it can be concluded that there was a rise in the level of cytokines in the duck during duck plague outbreak which indicates a relationship between various diseases of duck and the host immune responses.

Keywords: Pati, khaki Campbell, Nageswari, cytokine, duck

Introduction
Duck is famous for table egg production across the world and as per 19th livestock census of Govt. of India it constitute about 3% (23.539 millions) of total poultry (729.2 million) in India (GOI, 2012) [4]. The North East India is famous for the number indigenous duck breeds reared under traditional system, where duck farming is a subsidiary income source for all farmers irrespective of caste or religion. Cytokines are a broad category of small proteins involved in autocrine, paracrine and endocrine signalling as immunomodulating agents. Cytokines play an important role in health and disease, specifically in host immune responses to infection, inflammation, trauma, sepsis, cancer, and reproduction. It includes chemokines, interferons, interleukins, lymphokines and tumor necrosis factor etc. Pathological disorders occur in any species, when there is imbalance in cytokine production or dysregulation in a cytokine process (Tayal and Kalra, 2007) [17]. The study of cytokines and chemokines in avian species is still in its infancy as compared to that of the mammals and the availability of literature is also scanty. Keeping this in view, the present investigation was carried out to study certain cytokine profile of duck of Assam under healthy and during duck plague outbreak.

Material and Methods
In the present study, 350 blood samples (5ml) from different varieties of duck namely Pati (150), Khaki Campbell (100) and Nageswari (100) were collected from different parts of the state from healthy as well during disease outbreak. Blood samples were collected from the wing veins of each bird under aseptic conditions and serum was separated. The cytokine profile namely IL-6, IL-4 and IL-10 were estimated using Enzyme Linked immune-sorbent assay procured from Life Technologies (India), Pvt Limited, Delhi-34 and expressed in pg/ml. Data generated from the experiment were analyzed statistically using SAS Enterprise Guide (Version 4.2). Serum samples containing high cytokine levels were repeatedly measured after dilution to ensure that the assay results remained within the standard curve.

Results and Discussion

In the present investigation, serum cytokine *viz*. IL-6, IL-4 and IL-10 level of healthy Pati, Khaki Campbell and Nageswari ducks of Assam were estimated and compared with the same variety of ducks with the blood samples of duck plague outbreak. The cytokine profile estimated in the present investigation is presented in Table 1.The IL 6 level of Pati duck during health and disease conditions were recorded as 7.71 ±0.35 pg/ml and 7.40±0.45 pg/ml respectively. Both The Pati and Nageswari breed of ducks showed a non significant decrease in the IL 6 level during disease outbreak. However, the Khaki Campbell duck showed a non significant rise in the IL 6 level (8.19±0.81 pg/ml) during duck plague outbreak from that of healthy condition (7.25±0.32 pg/ml). Interleukin-6 is one of the most pleiotropic interleukins released at the site of infection or injury (Verdrengh and Tarkowski, 1997) [18]. IL-6 is a multifunctional cytokine produced by a number of cell types and is involved in acute-phase responses, immune regulation and haematopoesis (Hirano, 1998) [6]. The IL-6 cytokine is produced in response to various infections in chickens (Zhou *et al.*, 2007) [20] and mammals (Jia and Sheng, 2006) [7]. The serum IL-6 levels of infected ducklings increased, which might be both an indication of the induction of inflammation and an immune response against the virus. A study conducted by Liang *et al.* (2011) [10] suggested that IL-6 is more robustly induced in chickens than in ducks, which may be responsible for the different symptoms observed in the two species after influenza virus infection. In a study conducted by Lynagh *et al.*, (2000) [12] presence of substantial quantities of IL-6-like factor activity was detected in serum taken from some chickens infected with *E. tenella* during the course of primary infection. Leina *et al*., (2018) [9] observed that proinflammatory cytokines, like IL-6, were potently induced in the chickens, but modestly in ducks at later times in the infection period. The authors concluded that the controlled response in ducks contributed to apparent resistance and decreased mortality. Gu *et al*., (2012) [5] reported no changes in serum IL-6 level in ducklings infected with (Duck Hepatitis Virus) DHV-1 JX within the first 5 days after infection. Only a slight insignificant increase was found at 1 day post infection, in comparison with the control group.

The present study showed a significant increase ($P<0.05$) of IL 4 level during duck plague outbreak in all the three varieties. IL 4 level in Khaki Campbell breed was recorded as 15.25 ±0.43 pg/ml during healthy condition and 12.32 ±1.39 pg/ml during duck plague outbreak. Similarly, for Nageswari breed IL 4 during healthy condition was 11.29 ±0.52 pg/ml and during disease outbreak it was observed as 15.34 ±0.51 pg/ml. The Pati duck variety also showed an increase in the serum level of IL 4 (11.82±1.48) during disease outbreak as compared to the healthy state (11.22±0.31). IL-4 is an important in the stimulating process of B lymphocytes and T lymphocyte proliferation, as well as for the formation of Th2 cells by differentiation (Fietta and Delsante 2008) [19]. An investigation carried out by Cherry *et al.*, (2018) [1] demonstrated that IL 4 is an important cytokine which inhibits inflammatory responses of lymphocytes in spleen during different pathogen attack in duck. IL-4 is cytokine with diverse biological functions which not only promotes immune response and homeostasis but is also involved in immune regulation, particularly the balance among Th1, Th2, and Th17cytokines (Min *et al*., 2013) [13]. Recent studies show that dysregulated expression of IL-4 is implicated in allergic inflammation and asthma (Liang *et al*., 2012). [11]

Xie *et al.*, (2018) [19] conducted a study where pro inflammatory cytokines (IL-1β, IL-6, and TNF-α) and anti-inflammatory cytokines (IL-4, IL-10) were assessed in the liver and blood samples of ducklings with virulent CH Duck Hepatitis A Virus -1(DHAV-1) strain and the attenuated CH60 commercial vaccine strain. The expression levels of IL-1β, IL-6 and IL-10 in the liver were significantly up-regulated after CH infection and reached a peak at 36 hours post infection and maintained the same levels until 48 hours post infection. However, IL-10 expression in the blood was downregulated before 48 hours post infection. Both the CH and CH60 strains inhibited IL-4 and TNF-α expression during the early stage of infection, and both cytokines were up-regulated after 60 hours post infection. IL 10 also revealed a significant ($P<0.05$) increase during duck plague outbreak in all the three varieties of duck as compared to that of the healthy birds. The IL 10 level in Pati duck during healthy and during duck plague outbreak were found to be 17.02 ±0.86 pg/ml and 18.25 ±2.14 pg/ml respectively. The serum IL 10 level of healthy Khaki Campbell ducks was recorded to be 16.25±1.48 pg/ml and during disease outbreak it was 19.36±0.79 pg/ml. Similarly in the Nageswari the IL 10 level was 11.79±0.37 and 19.23±0.61 pg/ml in healthy and duck plague outbreak. The IL-10 cytokine has been shown to have immune-modulatory functions in mammals (Murphy and Reiner, 2002) [14]. IL 10 is an anti inflammatory cytokine and is important to balance inflammatory responses during bacterial, fungal, protozoal, helminthic infection and even during chronic viral infections (Couper *et al*., 2008 [2]; Sun *et al.* 2009) [16]. IL-10 is increases significantly ($P<0.05$) in all three varieties of ducks as it might protects the host against inflammatory agents, microbial invasion and injury (Laskin and Pendino, 1995) [8]. The function of IL-10 in avian species is similar to that in mammals (Rothwell *et al*., 2004) [15] however, evidence on the function of IL-10 in birds is not sufficient to elucidate its role in the immune system.

From the present study it can be concluded that there was a rise in the level of cytokines in the duck during duck plague outbreak which indicates a relationship between various diseases of duck and the host immune responses. Further detail study will be necessary to determine the protective role of cytokines in ducks during disease outbreak.

Table 1: Cytokine Concentration (pg/ml) of indigenous Ducks (Mean ± SE)

Parameter	Pati variety		Khaki Campbell		Nageswari		Level of significance
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	
IL-6	7.71 ±0.35	7.40±0.45	7.25±0.32	8.19±0.81	7.35±0.61	7.21±0.5	NS
IL-4	11.22±0.31	11.82±1.48	12.32 ^{a} ±1.39	15.25 ^{b} ±0.43	11.29 ^{a} ±0.52	15.34 ^{b} ±0.51	0.05
IL-10	17.02 ^{a} ±0.86	18.25^{b}±2.14	16.25^{a}±1.48	19.36^{b}±0.79	11.79^{a}±0.37	19.23^{b}±0.61	0.05

NS- Non significant, Significant at 5 % (P≤ 0.05)

* Superscripts bearing the different letter in columns for same cytokine are significant

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References

1. Cherry PF, Fahmid A, Rochelle AF, Woo HK, Jipseol J, Suk K *et al*. Identification of duck IL-4 and its inhibitory effect on IL-17A expression in R. anatipestifer-stimulated splenic lymphocytes. Molecular Immunology 2018;95:20-29.
2. Couper KN, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection. Journal of Immunology 2008;180:5771-5777.
3. Fietta P, Delsante G. The effector T helper cell triade. Rivista di Biologia 2008;102:61-74.
4. GOI. 19th Livestock census. All India report, New Delhi, Ministry of Agriculture, Department of Animal Husbandry, Dairying and Fisheries 2012.
5. Gu CQ, Xie CQ, Hu XY, Zhang WP, Bi DR, Cheng GF. Cytokine gene expression in the livers of ducklings infected with duck hepatitis virus-1 JX strain. Poultry Science 2012;91(3):583-591.
6. Hirano T. Interleukin 6. In: AE Thomson (editor). The Cytokine Handbook 3rd edition, Academic Press, San Diego 1998, 197-227.
7. Jia HY, Sheng JF. Study on the changes of serum cytokines in patients with chronic hepatitis C before and after IFN treatment. Modern Medical Health 2006;22:1919-1921.
8. Laskin DL, Pendino KJ. Macrophages and inflammatory mediators in tissue injury. Annual Review of Pharmacogy and Toxicology 1995;35:655-677.
9. Leina BS, Laura DS, Daniel E, Ximena FC, Sai M, Robert GW. IFN and cytokine responses in ducks to genetically similar H5N1 influenza A viruses of varying pathogenicity. Journal of General Virology 2018;99(4):464-474.
10. Liang QL, Luo J, Zhou K, Dong JX, He HX. Immune-related gene expression in response to H5N1 avian influenza virus infection in chicken and duck embryonic fibroblasts. Molecular Immunology 2011;48:924-930.
11. Liang HE, Reinhardt RL, Bando JK, Sullivan BM, Ho IC, Locksley RM. Divergent expression patterns of IL-4 and IL-13 define unique functions in allergic immunity. Nature Immunology 2012;13:58-66.
12. Lynagh GR, Bailey M, Kaiser P. Interleukin-6 is produced during both murine and avian *Eimeria* infections. Veterinary Immunology and Immunopathology 2000;76:89-102.
13. Min W, Kim WH, Lillehoj EP, Lillehoj HS. Recent progress in host immunity to avian coccidiosis: IL-17 family cytokines as sentinels of the intestinal mucosa. Developmental and Competitive Immunology 2013;41:418-428.
14. Murphy KM, Reiner SL. The lineage decisions of helper T cells. Nature Reviews Immunology 2002;2:933-944.
15. Rothwell L, Young JR, Zoorob R, Whittaker CA, Hesketh P, Archer A *et al*. Cloning and characterization of chicken IL-10 and its role in the immune response to *Eimeria maxima*. Journal of Immunology 2004;173:2675-82.
16. Sun J, Madan R, Karp CL, Braciale TJ. Effector T cells control lung in-flammation during acute influenza virus infection by producing IL-10. Nature Medicine 2009;15:277-284.
17. Tayal Vand Kalra BS. Cytokines and anti-cytokines as therapeutics- an update. European Journal of Pharmacology 2007;579:1-12.
18. Verdrengh M, Tarkowski A. Role of neutrophils in experimental septicemia and septic arthritis induced by *Staphylococcus aureus* arthritis. Infection and Immunity 1995;65:2517-2521.
19. Xie Wang M, Cheng A, Zhao X, Liu M, Zhu D, Chen S *et al*. Cytokine storms are primarily responsible for the rapid death of ducklings infected with duck hepatitis A virus type 1. Scientific reports 2018;8:6596.
20. Zhou Q, Gu CQ, Hu XY, Wang DH, Zhou SQ, Cheng GF. Role of interleukin-6 in the pathogenesis of an avian model of an avian model of *Staphylococcus aureus* arthritis. Poultry Science 2007;86:1245-1250.