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# Pathological studies on experimentally induced hydropericardium syndrome in immunosuppressed broiler chickens

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## ABSTRACT

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The effects of cyclosporine A (T-cell suppressor) was studied in chickens experimentally infected with hydropericardium syndrome (HPS) virus. The clinical signs of HPS were more severe and lasted longer due to administration of cyclosporine A (CsA) in HPS infected birds. The mortality due to HPS was more in CsA plus HPS group as compared with HPS group at different intervals. The characteristic gross and microscopic lesions in different organs observed due to HPS virus infection were of severe magnitude and longer lasting in CsA plus HPS group as compared to the HPS group at different intervals. HPS infected birds had significantly decreased delayed type hypersensitivity (DTH) response in comparison with the control birds against dinitrochlorobenzene antigen and this reduction in DTH response was significantly more in CsA plus HPS group as compared with the HPS group. It may be concluded that CsA enhanced the severity of HPS infection and thus suggesting the role of T-cells in limiting the pathology and pathogenesis of HPS.

**Keywords:** Chickens, cyclosporine A, delayed hypersensitivity, hydropericardium syndrome, pathology



## INTRODUCTION

Hydropericardium syndrome (HPS) is an emerging viral disease of three to six weeks old broiler chicks and has an important impact on economy of poultry industry due to high mortality and morbidity.[1] HPS was first reported from Pakistan and within a short span of time; the disease got introduced in India and other countries.[2] The etiological agent of HPS is a fowl adenovirus serotype 4.[3,4,5] However, field observations suggest that the high mortality in HPS affected broiler chicken flocks depends upon concurrent presence of infectious immunosuppressive agents such as infectious bursal disease and non infectious factors such as stress and aflatoxins.[6,7,8] Furthermore, chicken anemia virus, which causes T-cell depletion has been reported to enhance the severity of HPS.[9] Little experimental information is available in the literature on the effects of T cell suppressive agents on pathogenesis of HPS in chickens. The present work was undertaken to study the effect of cyclosporine A, a known T-cell suppressor, on pathogenesis and pathology of HPS in broiler chickens.

## MATERIALS AND METHODS

### Experimental chicks and feed

One-day old commercial broiler chicks were procured from a local hatchery having no history of HPS in the parent flock. Birds' management protocol and experimental procedures were undertaken as per guidelines of the Animal Welfare Board of India, Prevention of Cruelty to Animals Act, 1960. All the birds were given *ad libitum* mycotoxin free standard chick feed and provided clean drinking water throughout the experiment.

### Hydropericardium syndrome virus inoculum and cyclosporine A

HPS seed virus (freeze dried liver extract from HPS infected birds) was procured from Indovax Pvt. Ltd., Hisar. $10^{-3}$ diluted HPS virus infected liver inoculum ($ID_{50}$) was used as the infective dose, which was calculated using the standard method[10]. Cyclosporine A was dissolved in a solution containing 5.5 parts absolute ethanol and 13 parts sterilized castor oil to get the dose rate of 100 mg/kg body weight.

### Experimental design

Ninety broiler chicks at the age of 18 days were divided randomly into two groups viz. group A and group B of 45 chicks each. Birds of the group A were injected with cyclosporine A @100 mg/kg body weight by intramuscular route on 18$^{th}$ day of age and subsequently at 3 days intervals up to the age of 30 days, whereas the birds of group B were injected with placebo of ethanol and castor oil. At the age of 21 days, three birds from each group were sacrificed and then each group was further divided into two subgroups i.e. group A into groups A1 and A2 of 27 and 15 chicks and group B into group B1 and B2 of 27 and 15 chicks, respectively. Birds of groups A1 and B1 were injected 0.5 ml of 10-3

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diluted HPS virus inoculum (ID50), subcutaneously. Birds were closely observed daily for clinical signs and mortality, if any.

On 0, 2, 4, 6, 9 and 13th day post infection, three birds were sacrificed and subjected to thorough post-mortem examination from each group. Sections of liver, heart, lungs, spleen, kidneys, thymus and bursa of Fabricius were collected in 10 per cent buffered formalin for histopathological studies. The formalin-fixed tissues were processed for paraffin embedding technique and sections were stained with haematoxylin and eosin H&E)[11].

Cell-mediated immune response (CMI) was assessed by delayed type hypersensitivity (DTH) skin test using di-nitrochlorobenzene (DNCB) as an eliciting antigen[12]. The chicks were sensitized with DNCB on 11$^{th}$ day post infection. The increase in skin thickness due to DTH was measured at 24 hours post challenge of the eliciting antigen. In order to study the cellular reaction of DTH, a portion of the skin was collected in 10 per cent buffered formalin and processed for H & E staining. Statistical significance was assessed by using analysis of variance[13].

## RESULTS

### Clinical signs and mortality

On days 3 and 4 PI, birds in both the infected groups showed anorexia, restlessness, ruffled feathers and abnormal posture i.e. they appeared to have resting their chest on floor while sitting with closed eyes and neck tucked between the legs. These clinical signs were more severe in CsA plus HPS group. On day 5 PI, birds of HPS group appeared to be normal but the birds of CsA plus HPS group exhibited the clinical signs up to day 8 PI.

On day 2 PI, two birds from HPS group and three birds from CsA plus HPS group died. On day 3 PI, three birds from HPS and six birds from CsA plus HPS group were found to be dead. On day 4 PI, three birds in HPS group and five birds in CsA plus HPS group died. On day 5 PI, two birds were died in CsA plus HPS group only.

### Gross lesions

On day 2 PI, birds of both HPS infected groups revealed hydropericardium, congested and slightly swollen liver with a few petaechial haemorrhagic spots on the surface but the amount of fluid in pericardial sac and enlargement of liver in CsA plus HPS group were comparatively more. On days 3, 4 and 6 PI, birds of both the infected groups either died or sacrificed, showed severe hydropericardium, enlarged liver having necrotic and petechial haemorrhagic spots, congested and oedematous lungs but these changes were of higher magnitude in CsA plus HPS group as compared with HPS group. On day 9 PI, no gross lesions could be observed in the birds from HPS group, whereas hydropericardium and enlargement of liver with mottled appearance were noticed in the birds of CsA plus HPS group.

### Histological lesions

On day 2 PI, the liver of HPS infected chicks' revealed hepatitis characterized by the presence of a few focal areas of fatty changes and necrosis of hepatocytes and mild infiltration of lymphocytes and heterophils alongwith intranuclear basophilic inclusion bodies in a few hepatocytes. On days 3 and 4 PI, hepatitis became more severe and intranuclear basophilic inclusion bodies were observed in a number of hepatocytes. On day 6, liver of HPS infected birds showed mild hepatitis characterized by the presence of mild lymphocytic infiltration and only a few focal necrotic areas alongwith lymphoid follicles near central veins. These microscopic lesions in CsA plus HPS infected birds were more severe and intranuclear basophilic inclusion bodies were noticed in large number of hepatocytes (Fig. 1). Even on day 13 PI, liver of CsA plus HPS infected birds exhibited mild hepatitis. Hearts of both HPS infected groups on days 3, 4 and 6 PI, revealed focal areas of myocarditis characterized by infiltration of mononuclear cells and heterophils, haemorrhages, oedema and necrotic areas of cardiac muscles (Fig. 2). On day 13 PI, the heart of HPS infected birds did not reveal any significant change but CsA plus HPS infected chicks showed mild haemorrhages alongwith mild oedema and mild mononuclear cells infiltration in myocardium.

The lungs of HPS infected chicks on days 3 and 4 PI, revealed congestion, perivascular oedema (Fig. 3) and mononuclear cells infiltration in the interstitial tissue.

**Table.** Delayed type hypersensitivity response in different experimental groups.

| Groups | Specific increases in skin thickness (mm) | Histological cellular reaction |
|---|---|---|
| (Control) | 0.63±0.14$^{a}$ | +++ |
| (CSA) | 0.20±0.05$^{bc}$ | ++ |
| (HPS) | 0.26±0.02$^{b}$ | ++ |
| (CSA+HPS) | 0.13±0.03$^{c}$ | + |

+ mild; ++ moderate; +++ intense

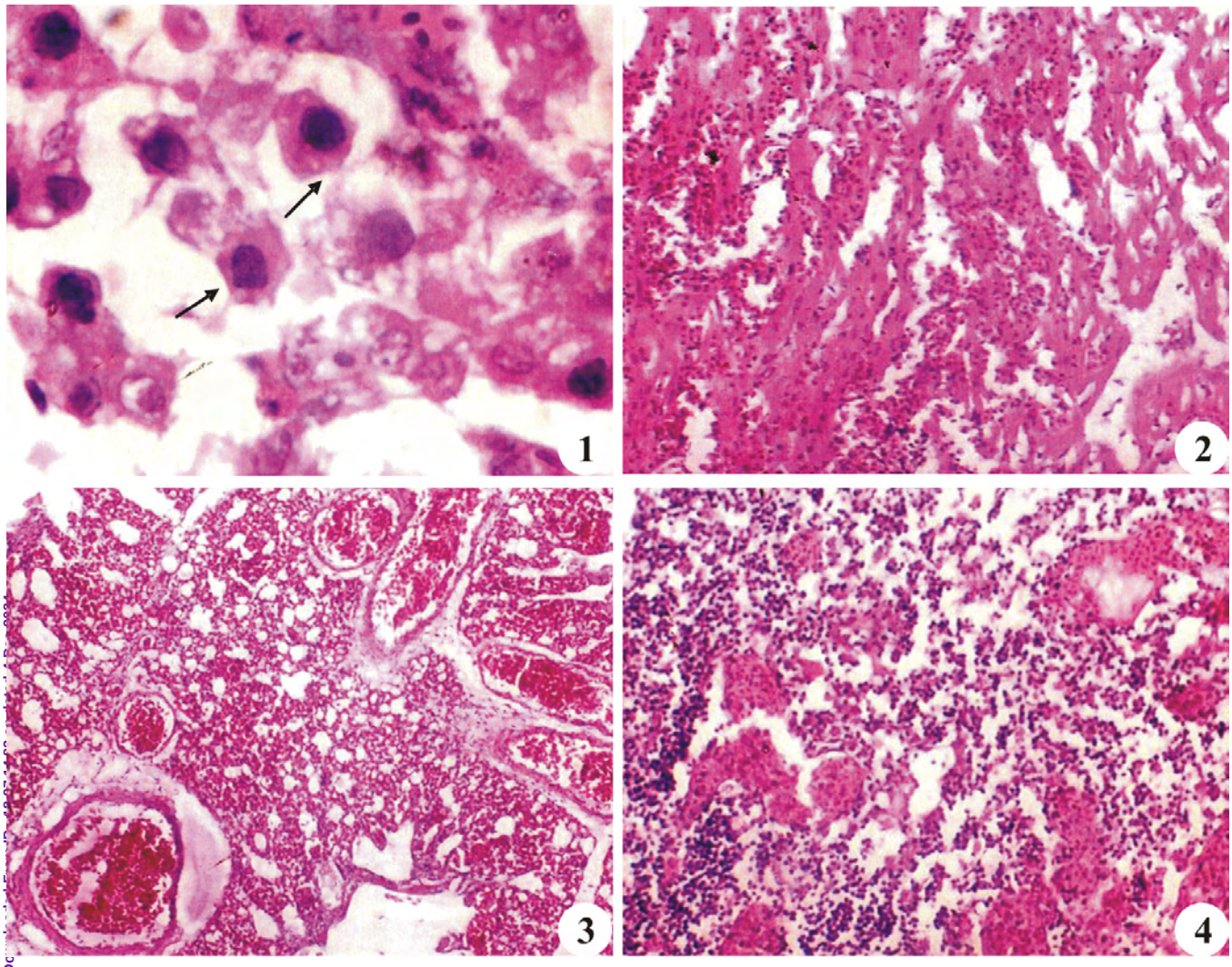




**Fig. 1.** Liver of CsA HPS infected chick showing intranuclear basophilic inclusion bodies (arrows) in hepatocytes on day 3 PI. H&E x200; **Fig. 2.** Heart of CsA HPS infected chick showing myocarditis alongwith severe haemorrhages and oedema in cardiac muscles on day 4 PI. H&E x200; **Fig. 3.** Lung of CsA HPS infected chick showing severe congestion and perivascular oedema on day 3 PI. H&E x200; **Fig. 4.** Thymus of CsA HPS infected chick showing necrosis and depletion of lymphocytes alongwith haemorrhages on day 4 PI. H&E x200.

In CsA plus HPS infected birds, these lesions were more severe and even on day 6 PI. Connective tissue proliferation was also observed in the interstitial tissue and around blood vessels. On day 4 PI, the bursa of Fabricius of HPS infected group revealed depletion of lymphocytes in both cortical and medullary regions. In CsA plus HPS infected group, the bursa of Fabricius revealed proliferation of retriculoendothelial cells in cortex in addition to lymphocytic depletion in the bursal follicles. On day 4 PI, the spleen of HPS group showed lymphyocytic depletion in white pulp alongwith reticuloendothelial cells hyperplasia. A number of secondary lymphoid follicles were observed in the spleen of HPS infected birds from day 6 PI onwards. In CsA plus HPS group, there were haemorrhages in the white pulp on day 2 PI. On days 3 and 4 PI, spleen of CsA plus HPS infected birds showed lymphocytic depletion in white pulp and reticuloendothelial hyperplasia in addition to haemorrhages. Thymus of HPS infected group on day 4 PI, showed mild depletion of lymphocytes in cortical areas alongwith mild congestion but from day 6 PI onwards, thymus appeared to be normal. In CsA plus HPS group, there was severe congestion of blood vessels and lymphocytic depletion in the cortical area on day 3 PI and there were haemorrhages along with necrosis of lymphocytes in cortical area from day 4PI (Fig. 4).

**Delayed type hypersensitivity (DTH) response**

At 24 hrs post application of the eliciting antigen, the swelling at the site of application of antigen became indurated and nodular but no such swelling was noticed at the site where only olive oil was applied. The

measurement of swelling revealed that both the infected groups had significantly lower values of mean increase in skin thickness (MST) due to DTH when compared to control (Table) Furthermore, this increase in skin thickness in CsA plus HPS group was significantly less as compared to HPS group. CsA group also had significantly lower value of increase in skin thickness as compared to control. Histological studies of the indurated areas of skin from the different experimental groups revealed infiltration of mononuclear cells and a few heterophils in the dermis. The cellular infiltration was intense in control group, moderate in CsA group and HPS group whereas it was of very mild degree in CsA plus HPS group. No oedema or cellular reaction was noticed in sections from the skin applied with only olive oil.

## DISCUSSION

HPS could be produced successfully in broiler chickens by subcutaneous inoculation of HPS infected liver homogenate. The clinical signs observed in HPS infected birds of the present study were almost similar to those reported in natural cases of HPS. These clinical signs were more severe and longer lasting due to administration of CsA in HPS infected birds. Furthermore, the mortality occurred in birds injected with CsA and infected with HPS at the different intervals was considerably more than that observed in the birds infected with HPS virus alone. Nevertheless, on day 5 PI, two birds died in CsA plus HPS group but there was no mortality in HPS group. The differences in the severity of clinical signs and mortality between the infected groups might be related to cell-mediated immunity since CsA is a selective immunosuppressor of the T-cell system. [14] Thus, it appears that cell-mediated immunity might be important in surviving the acute stage of HPS infection. These findings further suggest that the mortality in field cases of HPS infection may reflect not only the virulence of the virus but also the immune status of the birds where concurrent infection or ingestion of immunosuppressive substances such as mycotoxins could increase morbidity and mortality due to HPS infection. [15] The results of present study with respect to mortality and severity of clinical signs observed in both the HPS infected groups were almost similar to those observed by other workers[9] who have reported that chicken anaemia virus (CAV) causing T-cell immunosuppression enhanced the mortality in specific pathogen free light chicks due to inclusion body hepatitis/hydropericardium syndrome infection. However, these experimental studies did not explain that interaction between these viruses to induce IBH/HPS in the field could be either of CAV or synergistic effect between CAV and FAV.

The characteristic gross lesions i.e. hydropericardium, congested and swollen liver with few petaechial haemorrhagic and necrotic spots on the surface, oedematous lungs and congested thymus, observed in HPS infected birds of the present study were almost similar to those reported in natural cases.[16] Histopathological changes observed in the HPS infected birds were mainly in liver, heart, lungs, bursa of Fabricius, spleen, kidneys, and thymus. These lesions in CsA plus HPS group were of severe magnitude from day 2 PI and remained up to 9th day PI indicating that CsA administration caused enhancement in the severity of HPS infection. Furthermore, the pericardial fluid in CsA plus HPS group was comparatively more in amount and thick in consistency. In liver, necrosis of hepatocytes, hepatitis and haemorrhages in CsA plus HPS group were severe as compared to HPS group at different intervals. The intranuclear inclusion bodies noticed in a number of hepatocytes of CsA administered - HPS infected chickens reflect the number of viral particles in liver of CsA plus HPS affected birds. This finding further suggests that the HPS adenovirus infected the hepatocytes more severely in immunosuppressed birds. Cardiac lesions might be the indirect affect of HPS adenovirus infection since adenoviral antigens were not detected in the heart and inoculation of pericardial fluid from HPS could not reproduce the HPS infection in chickens[17,18].

The pulmonary lesions observed in the present study have also been reported by other workers [16, 17, 19] in chickens suffering from HPS. Perivascular oedema as observed in lungs and other organs of HPS infected chickens might be a reflection of marked increase in vascular permeability. The lymphocytic depletion in the lymphoid organs i.e. spleen, bursa of Fabricius and thymus due to HPS was markedly increased in CsA plus HPS infected group presumably due to immunosuppressive effect of CsA. Furthermore, some workers[20] reported that HPS adenovirus antigens were detected in bursa of Fabricius and thymus of chickens inoculated with HPS adenovirus and these affected chickens showed immunosuppression. In previous studies, comparable gross and microscopic lesions have been described in experimentally HPS infected chickens[20,21,22,25].

HPS infected birds had significantly decreased DTH response in comparison to the control birds against DNCB antigen indicating depressed cell-mediated immune (CMI) response due to HPS infection. Furthermore, the reduction in DTH response was significantly more in CsA plus HPS group as compared to HPS group indicating CsA administration further suppressed CMI response in HPS infected chicks. Nevertheless, CsA alone also caused decrease in DTH response. These results are in consistent with our

histopathological observations of thymus in which there was necrosis and depletion of lymphocytes due to HPS infection as well as CsA administration. CsA administration has been reported to cause dose related impairment of DTH response to tuberculin, sheep RBC and DNCB antigen in mice and guinea pigs[24]. HPS virus has been reported to cause reduction in both antibody-mediated and cell mediated immune responses of broiler chickens against Newcastle disease virus[8,20].

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## REFERENCES

1. Kumar R, Chandra R and Shukla SK. 2003. Isolation of etiological agent of hydropericardium syndrome in chicken embryo liver cell culture and its serological characterization. *Indian J. Expt. Biol*., **41**: 821-826
2. Gowda RNS and Satyanaryana ML. 1994. Hydropericardium syndrome in poultry. *Indian J. Vet. Pathol.,* **18:** 159-161.
3. Dahiya S, Srivastava RN, Hess M and Gulati BR. 2002. Fowl adenovirus serotype associated with outbreaks of infectious hydropericardium in Haryana, India. *Avian Dis.,* **46:** 230-233.
4. Nakamura K, Shoyama T, Mase, M, Imada, T and Yamada M. 2003. Reproduction of hydropericardium syndrome in three-week-old cyclophosphamide-treated specific-pathogen-free chickens by adenoviruses from inclusion body hepatitis. *Avian Dis*., **47**:169–174.
5. Schonewille E, Singh A, Göbel T W, Garner W, Saalmüller A and Hess M. 2008. Fowl adenovirus (FAdV) serotype 4 causes depletion of B and T cells in lymphoid organs in specific pathogen-free chickens following experimental infection. *J. Vet. Immunol. Immunopathol.,* **121**:130–139.
6. Toro H, Prusas C, Raue R, Cerda L, Geisse C, Gonzalez C and Hess M. 1999. Characterization of fowl adenovirus from outbreaks of inclusion body hepatitis /hydropericardium syndrome in Chile. *Avian Dis*., **43**:262–270.
7. Shivachandra S B, Sah R L, Singh S D, Kataria J M and Manimaran K. 2003. Immunosuppression in broiler chicks fed aflatoxin and inoculated with fowl adenovirus serotype-4 (FAV-4) associated with hydropericardium syndrome. *Vet. Res. Comm.,* **27:**39–51.
8. Balamurugan V and Kataria J M. 2006. Economically important non-oncogenic immunosuppressive viral diseases of chicken–Current status. *Vet. Res. Comm*., **30**:541–566
9. Toro H, Gonzalez C, Cerda L, Hess M, Ryes E and Geisse C. 2000. Chicken anemia virus and fowl adenovirus: association to induce the inclusion body hepatitis/hydropericardium syndrome. *Avian Dis.,* **44:** 51-58.
10. Reed L V and Muench H. 1938. A simple method of estimating fifty per cent and point. *Am. J. Hygiene,* **27:** 493-497.
11. Luna L. G. 1968. *Manual of histologic staining methods of the Armed Forces Institute of Pathology*. 3rd edn. McGraw Hill Book Co., New York, pp. 230.
12. Tiwari BK and Goel MC. 1985. Contact sensitivity to DNCB in normal and cell-mediated immunity deficient chickens: In vivo detection and correlation with lymphocyte trans-formation and graft-virus-host reaction. *J. Vet. Immunol Immunopathol.,* **8:** 329-339.
13. Snedecor W G and Cochran G W. 1967. *Statistical Methods*. The Iowa State University Press, Ames, Iowa, USA.
14. White S. 1986. Cyclosporine: Prototype of a T-cell selective immunosuppressant. *J. Am. Med. Assoc.,* **189:** 566-569.
15. Survashe BD, Pandit SV, Ghalsasi GR and Mallikarjun S. 1996. Inclusion body heptitis/hydropericardium syndrome (Leechi heart disease) in India – An overview. Proc. 20th World's Poult. Cong. New Delhi. 2-5 Sept.: 375-380.
16. Asrani RK, Gupta VK, Sharma SK, Singh SP and Katoch RC. 1997. Hydropericardium hepatopathy syndrome in Asian poultry. *Vet. Rec.,* **141**: 271-273.
17. Abe T, Nakamura K, Tojo H, Mase M, Shibahara T, Yamaguchi S and Yuasa N. 1998. Histology, immunohistochemistry, and ultrastructure of hydropericardium synodrome in adult broiler breeders and broiler chicks. *Avian Dis.,* **42**: 606-612.
18. Ahmad K, Ahmad I, Akram-Muneer MA and Ajmal M. 1992. Experimental transmission of Angara disease in broiler fowls. *Stud. Res. Vet. Med.,* **1** (1): 53-55.
19. Soni SK. 1999. *Studies on ultra-structural pathology and pathogenesis of hydroperi cardium synodrome in experimentally infected broiler chicken.* Ph.D. Thesis, CCS HAU, Hisar.
20. Naeem K, Niazi T, Malik SA and Cheema AH. 1995. Immunosuppressive potential and pathogenicity of an avian adenovirus isolate involved in hydropericardium syndrome in broilers. *Avian Dis*., **39**:723–728.
21. Trivedi S, Chandra R and Shukla SK. 1996. Hydropericardium syndrome (Leechi disease): An emerging threat to broiler industry of India. *Poultry Adviser,* **XXIX (IV):** 21-24.
22. Jong Nyeo Kim, Seong Hwan Byun, Min Jeong Kim, Jeom ju Kim, Haan Woo Sung, and In Pi Mo. 2008. Outbreaks of Hydropericardium Syndrome and Molecular Characterization of Korean Fowl Adenoviral Isolates. *Avian Dis.,* **52(3):**526-530
23. Munir K, Muneer J M, Tiwari A, Masaoud E and Chaudhry R M. 2009. Dietary arginine stimulates humoral and cell-mediated immunity in chickens vaccinated and challenged against hydropericardium syndrome virus. *Poultry Sci.,* **88**:1629-1638
24. Thomson AW. 1983. Immunobiology of cyclosporine A-Review. *Aust. J. Expt. Biol. Med. Sci.,* **61:**147-172.