

Biochemical Analysis of Pericardial Fluid in Chicks of *Gallus domesticus* Suffering from Hydropericardium Syndrome

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ABSTRACT : The liver extract of chicks, *Gallus domesticus*, suffering from hydropericardium syndrome (HPS) was inoculated intraperitoneally into healthy chicks to produce HPS. After inoculation the blood serum and the pericardial fluid of the newly infected chicks were analyzed, at regular intervals for a total period of 72 hours for concentration of proteins, K⁺ ions, Na⁺ ions and the LDH activity. The protein content was significantly decreased both in the blood serum (30%) and the pericardial fluid (39%) within 24 hours of inoculation, which was then maintained during the subsequent period. The K⁺ ions and the LDH activity, on the other hand,

were significantly increased in the blood serum (26% and 169%, respectively) as well as the pericardial fluid (131% and 217%, respectively) within 24 hours of inoculation. After 72 hours this increase was, respectively, 43% and 191% in blood serum, and 153% and 200% in the pericardial fluid. Accumulation of K⁺ ions, and decrease of protein and Na⁺ ions in the pericardial fluid indicate homeostatic imbalance, which may prove fatal. The increased LDH activity is indicative of hepatocytic damage. (**Key Words:** *Gallus domesticus*, Hydropericardium Syndrome, LDH Activity, Na⁺ and K⁺ Content, Pericardial Fluid)

INTRODUCTION

Since October, 1987, the poultry industry is facing a serious killer disease, Hydropericardium Syndrome (HPS), which was first noticed in August/September, 1987 in Angara Goth, a Village near Karachi and hence was also called Angara Disease. About 25% of broiler farms were closed in Pakistan because of its speedy spread throughout the country revealing heavy losses to the farmers.

The disease was first reported by Schmittle et al. (1958), which was later diagnosed to be due to toxic fat in broiler feed. The disease was seen almost exclusively in 3-6 weeks old broiler chicks (Tariq, 1988). Rare cases were reported in layers and breeder pullets (Anjum, 1988). The course is usually 10-14 days during which 30-60% mortality was reported (Cheema et al., 1988). Usually in healthy flocks mortality suddenly shoots up in the start of third week, peaks at fourth week and subsides by the end of 5th or 6th week. Well grown and healthy birds are the victims of this disease. Morbidity rate gradually increases with the advance of the disease. The affected chickens show grey white mucoid droppings for a few days, appear depressed, inclined to sit continuously, may gasp and finally die (Anjum et al., 1988).

Many biological and chemical agents such as fat toxicity (Peckham, 1984), aflatoxins (Rao et al., 1985), *Aspergillus* infection (Tesprateep, 1981), biotin deficiency (Bains, 1979), reo virus (Bains et al., 1974; Hussain and Spradbrow, 1981), adenovirus (Bains, 1979; Cheema et al., 1988) are thought to be responsible for this type of syndrome. Adenovirus has been isolated in high concentrations from liver of affected birds where it caused intranuclear inclusion bodies in the hepatocytes (Cheema et al., 1989; Ahmed et al., 1990).

Since the HPS is characterized by accumulation of fluid in the pericardial sac the present study was designed to analyze the biochemical nature of pericardial fluid with reference to total proteins, Na⁺ and K⁺ concentrations and lactate dehydrogenase (LDH) activity. The object was to determine whether ionic imbalance and cellular damage caused the death of animal. The various biochemical changes in the pericardial fluid have been correlated with the onset of disease. These changes have been followed in conjunction with similar changes in the blood serum.

MATERIALS AND METHODS

Chicks

One hundred, one day old broiler chicks of *Gallus domesticus* were obtained from Big Bird, Gulberg,

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Lahore. They were kept in the Animal House of the Department of Zoology, Punjab University, Lahore, where temperature was maintained at 30°C. Chicks were fed on mash feed No. 4 (protein $16.03 \pm 0.29\%$, fat $4.93 \pm 0.25\%$, moisture $9.96 \pm 1.34\%$, ash $7.83 \pm 0.63\%$, fiber $5.8 \pm 0.16\%$, and nitrogen-free extract $55.53 \pm 1.0\%$) of Punjab Feeds, Lahore, Pakistan. They were given antibiotic Neotera 50 for the first five days. After 7 days of age they were vaccinated against HPS (local name of disease, Ranikhate). They were also provided with vitamins as a commercial preparation known as Vitasloe super for six days in a specified amount.

Preparation of inoculum

Morbid liver samples were obtained from Veterinary Research Institute, Lahore. About 50 g of liver tissue was homogenized in 80 ml of saline solution. To the mixture antibiotic Polybiotic (100 mg/25 ml) was added and filtered. Filtrate was stored at 4°C and was used as inoculum.

Procedure adopted

Twenty one days old chicks (both sexes) were divided into two groups, 20 control and 50 experimental chicks. They were kept in isolated cages. All the chicks were weighed, the experimental chicks were administered with 0.2 ml of inoculum. Ten chicks from the experimental group and four chicks from control group were slaughtered 24, 36, 48, 60 and 72 hours after inoculation. Blood samples were collected in sterilized glass tubes after slaughtering. Pericardial fluid was taken with the help of 5 ml syringe. Blood and pericardial fluid were obtained from the same animal. The blood and pericardial

fluid were centrifuged at 3,000 rpm for 15 min. Serum and supernatant pericardial fluid was collected in another set of tubes and analyzed biochemically and by SDS-polyacrylamide gel electrophoresis. The blood serum and pericardial fluid were used for estimation of proteins according to Lowry et al. (1951), K^+ concentration according to Teitz (1976), Na^+ concentration according to Henry (1974), and LDH activity according to Cabaud and Wroblewski (1958). SDS-PAGE was used for the evaluation of variety of proteins of blood serum and pericardial fluid according to Laemmli (1970).

Statistical analysis

Duncan's Multiple Range Test was performed to determine significant differences between and within different experimental and control groups.

RESULTS

No mortality was recorded during 72 hours of experimental period. The treated chickens appeared depressed and inclined to sit. The most prominent gross lesion observed was the accumulation of crystal clear, colourless and sometimes yellowish fluid in the pericardial sac. The heart appear hypertrophied. Liver appeared enlarged, swollen and was darkened. Ascites was not seen during the present experimental period.

Biochemical analysis

The biochemical changes in blood serum of chicks after inoculation of pericardial fluid are shown in table 1, whereas table 2 shows similar type of changes in the pericardial fluid of infected chicks. In blood serum the

Table 1. Biochemical analysis of blood serum of broiler of *Gallus domesticus*, administered with 0.2 ml of liver extract of chicks, suffering from hydropericardium syndrome

Parameters	Control	24 Hours (n = 20)	36 Hours (n = 10)	48 Hours (n = 10)	60 Hours (n = 10)	72 Hours (n = 10)
Proteins (g/100 mL)	2.26 ^a ±0.33	1.58 ^b ±0.13	1.69 ^b ±0.16	1.63 ^b ±0.15	1.51 ^b ±0.08	1.60 ^b ±0.09
K^+ ions (mmol/L)	9.94 ^a ±0.86	12.56 ^b ±0.50	12.96 ^{ab} ±0.22	13.13 ^{ab} ±0.23	13.48 ^{ab} ±0.29	14.24 ^a ±0.20
Na^+ ions (mmol/L)	168.6 ^a ±9.76	160.9 ^a ±11.12	159.12 ^a ±12.96	163.5 ^a ±12.48	158.62 ^a ±10.37	164.5 ^a ±5.45
LDH activity (IU/L)	185.31 ^a ±2.21	498.76 ^d ±1.48	515.25 ^c ±1.50	529.41 ^b ±2.00	532.86 ^b ±0.94	538.36 ^a ±0.25

Means sharing same letters are not significantly different at 5% level of Duncan's Multiple Range Test.

Table 2. Biochemical components of pericardial fluid of broiler of *Gallus domesticus*, administered with 0.2 ml of liver extract of chicks, suffering from hydropericardium syndrome

Parameters	Control (n = 20)	24 Hours (n = 10)	36 Hours (n = 10)	48 Hours (n = 10)	60 Hours (n = 10)	72 Hours (n = 10)
Proteins (g/100 mL)	2.26 ^a ±0.33	1.37 ^b ±0.11	1.29 ^b ±0.31	1.38 ^b ±0.22	1.24 ^b ±0.14	1.30 ^b ±0.37
K ⁺ ions (mmol/L)	9.94 ^c ±0.86	22.94 ^b ±0.42	23.53 ^{ab} ±0.42	23.70 ^{ab} ±0.30	24.06 ^{ab} ±0.75	25.12 ^a ±0.49
Na ⁺ ions (mmol/L)	168.6 ^a ±9.76	142.03 ^b ±2.36	147.53 ^b ±3.66	144.8 ^b ±3.18	150.00 ^b ±4.54	145.72 ^b ±5.89
LDH activity (IU/L)	185.31 ^a ±2.21	587.54 ^a ±1.44	578.00 ^b ±1.89	573.92 ^b ±1.15	568.51 ^c ±0.76	556.25 ^d ±0.72

Means sharing same letters are not significantly different at 5% level of Duncan's Multiple Range Test.

protein content decreased, whereas K⁺ concentration and LDH activity increased, as the infection progressed (table 1). Within 24 hours of infection the proteins content decreased 30%, whereas K⁺ and LDH activity increased 26% and 169%, respectively. The proteins content are maintained at this reduced level until the end of observation period i.e. 72 hours, whereas the increasing trend is maintained for K⁺ concentration and the LDH activity. After 72 hours of inoculation the K⁺ concentration and LDH activity showed an increase of 43% and 191%. Na⁺ concentrations remained unchanged throughout this period. In pericardial fluid the protein and Na⁺ content decreased 39% and 16%, respectively within 24 hours after inoculation, but these changes were maintained as such during the subsequent post-inoculation period. K⁺ content and LDH activity increased 131% and 217%, respectively, within 24 hours post-inoculation. After 72 hours of inoculation this increase was respectively, 153% and 200% (table 2).

The pericardial fluid of infected chicks had 13, 24, 15, 18 and 19% lesser protein contents than in blood serum of infected chicks at 24, 36, 48, 60 and 72 hours after inoculation. The blood serum of chicks infected with HPS had 30, 25, 28, 33 and 29% lesser protein content than blood serum of healthy chicks. K⁺ contents of pericardial fluid were 83, 81, 81, 79 and 76% were greater than that of blood serum of infected chicks 24, 36, 48, 60 and 72 hours after inoculation, respectively. The Na⁺ contents of pericardial fluid were 12, 18, 11, 17 and 16% lesser than that of blood serum of infected chicks at 24, 36, 48, 60 and 72 hours after inoculation, respectively. Pericardial fluid showed 18, 12, 8, 7 and 3% increase in LDH activity as compared to that of blood serum of infected chicks at 24, 36, 48, 60 and 72 hours after inoculation.

SDS-PAGE pattern of proteins

Figures 1 and 2 show the SDS-PAGE pattern of blood serum and pericardial fluid of control and infected chicks. Generally the banding pattern is almost the same in all the groups, except for protein bands number 2, 5, 7, 10 and 14 which are absent in the control serum but are present in the experimental samples. Protein bands number 2 and 14 are present in both the blood serum and the pericardial fluid of infected chicks, whereas band no. 5 and 10 are exclusively present in the pericardial fluid (figure 2).

DISCUSSION

The angara disease is a highly contagious and fatal disease of broiler chickens, which has been identified in all regions of Pakistan. Almost all varieties of commercial broilers, fed on different brands of commercial rations, are affected with a rapid and persistent spread in all parts of the country within few months of its emergence (Ahmad et al., 1989; Jaffery, 1989). Interestingly the disease has not so far been reported across the border and this has drawn the attention of a number of investigators. Many are of the belief that etiological factors of this disease are endemic and are related with contaminated feed or contaminated vaccines manufactured locally on embryonating eggs from non-specific pathogen free source (Ahmad et al., 1992).

At present the only source rich in infectious agent is liver of naturally or experimentally infected commercial broiler chickens. The disease can be reproduced by the inoculation of a bacteria free liver homogenate from an affected bird (Khawaja et al., 1989; Ahmad et al., 1989; Cheema et al., 1989; Muneer et al., 1989).

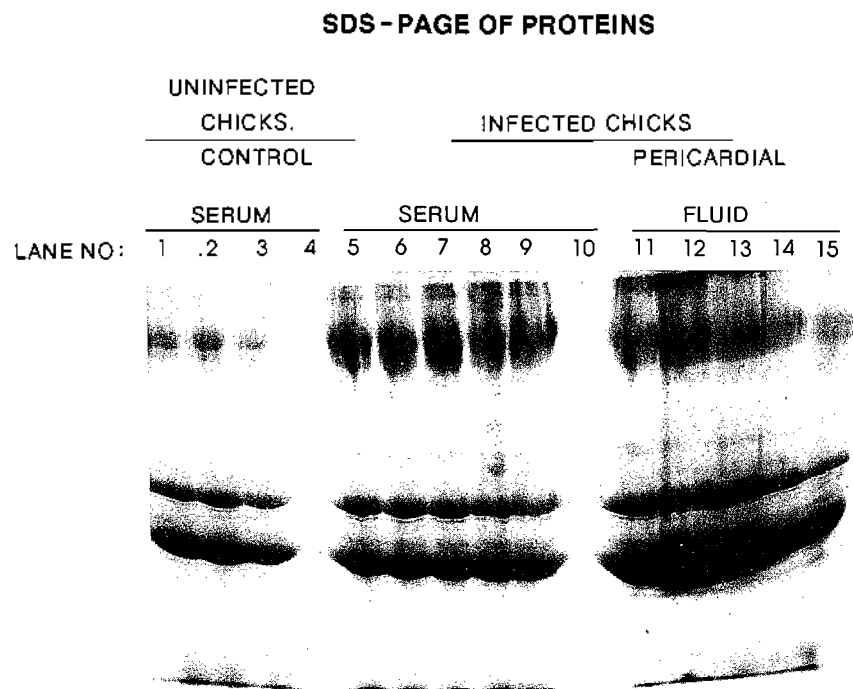


Figure 1. PAGE pattern of proteins of blood serum and pericardial fluid of chicks suffering from hydropericardium syndrome.

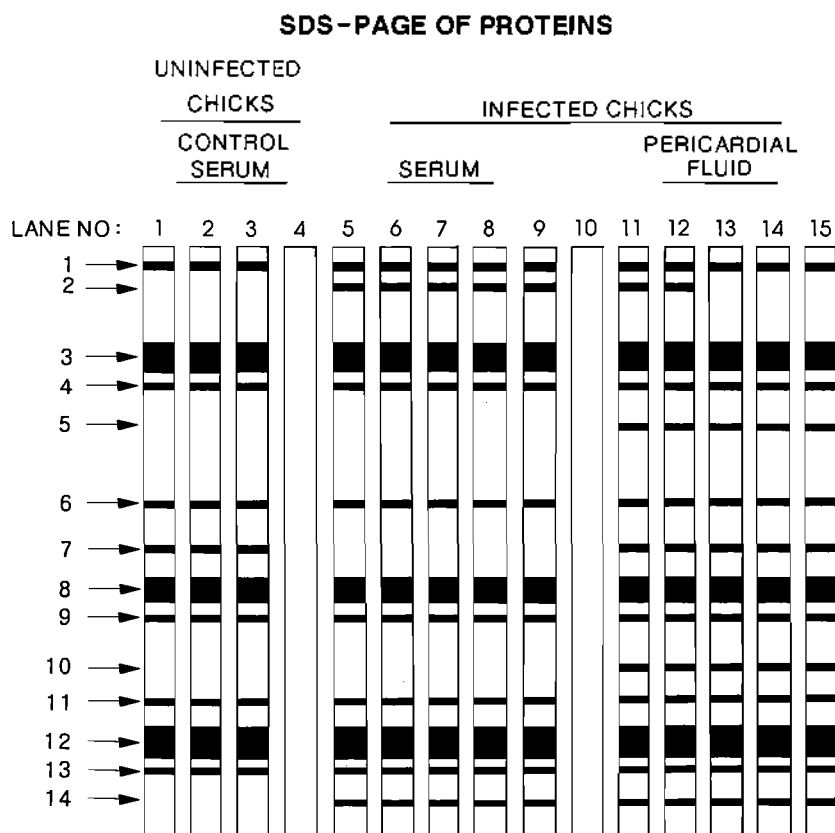


Figure 2. Diagrammatic representation of the PAGE pattern of proteins of blood serum and pericardial fluid of chicks suffering from hydropericardium syndrome.

Total body water contents of one day old chick is about 85% and it decreases gradually with age. About 50% of total body water is stored in the cells and the rest outside the cells. Blood plasma and pericardial fluid are parts of extracellular fluid. Fluid separation is done by semi-permeable membranes having individual characteristics. Content of fluid in these storing sites depends upon the body fluid dynamics or haemodynamics. Abnormal accumulation of fluid may be due to infection of regulatory sites, i.e., heart, liver and kidney and accumulation depends upon the severity of infection.

Many plasma proteins including albumin, fibrinogen and most-globulins are formed in liver. The concentration of total proteins is of limited value. It may be altered by changes in plasma volume. Albumin is the main contributor of the plasma colloid osmotic pressure, counteracting the effect of the capillary blood pressure which tends to force water into tissue spaces.

After SDS-PAGE it was noted that quantitatively no significant change was observed in protein content both in blood serum and pericardial fluid, but a slight decrease is observed in serum, while in the pericardial fluid reverse pattern is observed. There are certain proteins which are found only in the serum and some are confined to the pericardial sac. Otherwise most of the proteins are found to be common which shows the relative permeability of physiological membrane.

Sodium and potassium constitute electrolyte and form bulk of fixed base. Further potassium is also responsible for membrane permeability. Quantitatively significant change is observed in K^+ content of pericardial fluid which gradually increase with the infection but the blood serum of infected chicks shows comparatively a slight increase in K^+ content. This indicates the influx of K^+ into the pericardial sac with the onset of the disease.

All the cell membranes of the body have a powerful Na^+ , K^+ pump which continually pump Na^+ outside the cell and K^+ ions inside the cell. Quantitatively no significant change is observed in Na^+ ions content of blood serum, though a slight decrease is observed in pericardial fluid. This deviation from the control in Na^+ and K^+ levels in the blood is presumably responsible for accumulation of fluid in the pericardial sac as has been reported by Bhatti et al. (1989).

Bhatti et al. (1989) observed a marked increase in triglycerides, calcium and uric acid which is indicative of decrease in urinary excretions due to kidney disorder. They also observed decrease in plasma proteins, Na^+ , content and an increase in K^+ content. The observations were from a field poultry farm showing acute symptoms of HPS.

HPS appears suddenly in a flock without showing any premonitory symptoms. According to Moss et al. (1974) estimation of serum enzymes is an important tool in the diagnosis of acute and chronic disease. The evaluation of specific enzymes in the serum of body fluid always reflects some pathological changes in specific organs. The target organs of HPS are the heart, liver and kidneys showing various pathological changes and are supposed to release specific enzymes in the plasma. Frankel et al. (1970) reported that the activity of LDH reflect the myocardial and pulmonary infarcts. The mean level of LDH increased highly significantly in HPS affected chicks as compared to those of normal. LDH content of pericardial fluid show highly significant increase but it gradually decreases during the course of disease, while in blood serum it gradually increases during the course of disease. Zaman and Khan (1991) observed a significant increase in serum enzymes LDH, creatine phosphokinase and alkaline phosphatase in HPS infected broilers as compared to normal chicks. HPS apparently disturbs the normal molecular and ionic balance, which is normally maintained at a specific level in blood. Accumulation of K^+ ions and decrease of protein and Na^+ ions in the pericardial fluid indicate disturbance of homeostatic balance which may prove fatal. This imbalance is corroborated by similar pattern in blood serum, though to a lesser degree. Increase in LDH activity indicates cellular damage. A serious attention is desired to control this disease to save such a valuable and cheap animal food.

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