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Experimental studies on hydropericardium syndrome in two different synthetic lines of broiler chickens

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ABSTRACT

Hydropericardium syndrome (HPS) was produced after experimental infection of two different synthetic lines of chickens, white (B1) and coloured (B2) plumage broilers in their 3rd and 4th weeks of age. B1 broilers were found more susceptible and mortality ranged from 92.3% to 97%. B2 broilers were less susceptible and mortality ranged from 15.7% to 23.3%. Sixty percent mortality was observed in 3-week-old sentinel B1 broilers. Sixty percent of the B1 broilers chickens infected at eight weeks old died due to HPS, whereas B2 broilers showed no clinical signs, nor died, even at six weeks of age. High mortality was observed in B1 broilers following infection with chloroform-extracted homogenate of infective livers. HPS was a consistent feature in dead birds. Gross pathological lesions were observed in liver, kidney and heart. Microscopic lesions were observed in liver, kidney, Harderian gland, brain, heart, lung, pancreas, spleen and bursa. Some of the hepatocytes contained large basophilic intranuclear inclusion bodies. A difference in susceptibility of two synthetic lines of broiler chickens to HPS disease virus is recorded for the first time.

Key words: hydropericardium syndrome, susceptibility, mortality, gross pathology, histopathology

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Introduction

Hydropericardium syndrome (HPS), a disease primarily of broiler chickens, was first reported in Angora Goth, Karachi, Pakistan, during 1987 (KHAWAJA et al., 1988). Subsequently, the disease has also been recorded in India (GOWDA and SATHYANARAYANA, 1994). The sudden onset of the disease and a mortality rate as high as 75% in well-grown, clinically healthy 3-5-week-old broilers are some of the salient features of the outbreaks (AHMED et al., 1989; ABDUL AZIZ and AL-ATTAR, 1991). Necropsy findings are very characteristic, and include, hydropericardium, hepatic necrosis and pale kidneys (CHEEMA et al., 1989; ABDUL AZIZ and HANSON, 1995). The disease is caused by fowl adenovirus (FAV) serotype 4 (JADHAO et al., 1997). An inactivated vaccine was found to be efficacious in controlling the disease (ROY et al., 1999). This paper describes the susceptibility of two different synthetic lines of broiler chickens following experimental infection with HPS disease virus.

Materials and methods

Samples. Liver tissues collected from broilers during an outbreak of HPS were used for infecting chickens in this experiment. Liver tissues contained FAV serotype 4 (Personal Comm. Dr. A. Koteeswaran). The virus produced cytopathic effects in Vero cell line (ROY et al., 2001).

Chickens. Two synthetic lines of broiler chickens, white (B1) and coloured (B2), were obtained from the Poultry Research Station (PRS), Nandanam, Chennai-35, India. One pure breed, Indian Cornish - 3 (IC-3) was bred in criss-cross fashion with three synthetic lines derived from native and exotic breeds, namely Samrat, Starbrow and Vencob. In the first (F1) generation white and coloured plumage broilers were developed. Coloured plumage broilers were crossed with coloured plumage broilers continuously for about 20 generations to select coloured broilers (B2). Similarly, white plumage broilers were crossed with white plumage broilers to select white coloured broilers (B1). All the chickens were maintained without any vaccine at PRS and brought to the Centre for Animal Health Studies for the purposes of the experiment. Five birds chosen at random were bled before the start of the experiment and were tested for the presence

of FAV serotype 4 antibodies by agar gel immunodiffusion (AGID) test as described earlier (ROY et al., 2001). All chickens were found to be free of the specific antibody.

Experiment design

Trial 1. Supernatant of 30% liver homogenate (samples) in normal saline solution (NSS) was treated with antibiotics (Penicillin – 10,0000 I.U./ml., Streptomycin – 10 mg/ml and Gentamicin – 250 µg/ml). Presence of virus in liver homogenate was tested by counter immuno electrophoresis (CIE) using specific antiserum. Thirty percent liver homogenate showed CIE titre of 3 $\log_2$. One hundred and thirty-two three-week-old B1 broiler chickens were infected as described by CHEEMA et al. (1989). Each chicken was injected with 0.5 ml of liver homogenate subcutaneously. Five age-matched non-infected chickens were reared with the infected group and were used as sentinels.

Trial 2. Twenty-six four-week-old B1 broiler chickens were infected as described in Trial 1. In addition, five chickens were injected subcutaneously (0.5 ml. per chicken) with chloroform extracted liver homogenate (samples) treated with antibiotics as described in Trial 1. Chloroform extraction was done by mixing chloroform with liver homogenate (1:4) thoroughly and after 15 min. the mixture was centrifuged and clear supernatant was used for infection.

Trial 3. Five eight-week-old B1 broiler chickens were infected as described in Trial 1.

Trial 4. Twenty three-week-old B2 broiler chickens were infected as described in Trial 1.

Trial 5. Nineteen four-week-old B2 broiler chickens were infected as described in Trial 1.

Trial 6. Twenty six-week-old B2 broiler chickens were infected as described in Trial 1.

Trial 7. Twenty un-inoculated B1 and B2 broilers were maintained separately up to eight weeks of age and served as controls.

In all the trials infected chickens were observed for three weeks for any clinical signs of disease, and live chickens were sacrificed humanely.

Pathology and histopathology. All chickens that died during the course of the experiment, and sacrificed live chickens at the end of the experiment, were subjected to necropsy examinations. For histopathological examination, Harderian gland, cerebellum, heart, lung, kidney, spleen, pancreas and cloacal bursa were collected from selected chickens exhibiting typical HPS lesions.

Results and discussion

An inactivated HPS vaccine was produced which has a high impact in controlling the disease (ROY et al., 1999). During the course of vaccine production, broiler chickens were obtained from the PRS on different occasions. It should be mentioned that the birds were from either B1 or B2 broiler lines and of different age groups. Based on the interesting mortality pattern observed, batches of chickens obtained are shown under the heading of different trials.

Chickens in the control group (Trial 7) were healthy for an observation period of 8 weeks. In HPS-infected chickens, mortality rate was higher in B1 broilers compared to B2 broilers (Tables 1 and 2). In B1 broilers mortality rates were 97%, 92.3% and 60% in 3- week-, 4-week- and 8-week-old chickens, respectively (Table 1). Three- and four-week-old chickens died within 2 to 5 days, and eight-week-old chickens died within 3 to 7 days after infection. In Trial 1, three-week-old (60%) sentinel chickens died within 3 to 5 days. In Trial 2, 100% 4 week-old chickens died within 2 to 4 days following infection with chloroform extracted liver homogenate.

Table 1. HPS in B1 broilers of different age groups

	Infection		
Age in weeks	Dose	Route	% Mortality
3	0.5 ml	Subcutaneously (s/c)	97% (128/132)
3	Sentinel	Sentinel	60% (3/5)
4	0.5 ml	s/c	92.3% (24/26)
4	0.5 ml (Chloroform extracted)	s/c	100% (5/5)
8	0.5 ml	s/c	60% (3/5)

Figures in parenthesis indicate number of chickens died/number of chickens infected

In B2 broilers, mortality rate was low, ranging from 15.7% to 23.3% in 4-week- and 3-week-old chickens, respectively. Deaths were observed between 2 and 5 days following infection. In six-week-old chicken no deaths were observed (Table 2).

Table 2. HPS in B2 broilers of different age groups

	Infection		
Age in weeks	Dose	Route	% Mortality
3	0.5 ml	s/c	23.3% (7/30)
4	0.5 ml	s/c	15.7% (3/19)
6	0.5 ml	s/c	0% (0/20)

Figures in parenthesis indicate number of chickens died/number of chickens infected

Occurrence of the disease in sentinel birds clearly indicates that HPS is a contagious disease. Similar findings were reported by ABDUL-AZIZ and HANSON (1995). HPS was reproduced earlier following infection with liver homogenate from affected chickens (AHMED et al., 1989; ANJUM et al., 1989; AFZAL et al., 1991) but in the present experiment the interesting finding was the high susceptibility in B1 broilers compared to B2 broilers. The virus usually affects broilers of 3 to 5 weeks old, and mortality ranges from 10% to 60% (AHMED et al., 1989; ABDUL-AZIZ and AL-ATTAR 1991). However, ANJUM et al. (1989) reported high a mortality rate in 3-week-old birds and a lesser rate in those aged 5 or 6 weeks, where the mortality ranged between 20 and 75%. In the present experiment mortality was highest in 3-week-old chicks, followed by those 4 weeks old (Tables 1 and 2). B1 broiler chickens were found susceptible even at 8 weeks of age. Mortality ranged between 60 and 97%. This high percentage could be due to individual infection compared to those reported in field outbreaks. In the present study incubation period of the disease was found to be 2 to 7 days. In Trial 1 (Table 1) 100% mortality was observed after infection with chloroform extracted liver homogenate. This could be due to the high infecting dose, as chloroform extraction removed lipids and other tissue debris. Thus, 0.5-ml inoculum contained more virus. In B2 broilers a 23.3% mortality was observed in 3-weekold birds, whereas the 6-week-old group was found to be resistant, thus indicating reduced susceptibility in B2 broilers.

In all dead chickens the pericardial sac was distended with a clear, straw-coloured fluid, which varied in volume from 2 to 12 ml. In most cases, pericardial fluid transformed into a gelatinous mass on exposure to air. pH of the fluid varied from 7.5 to 8.0. Heart showed areas of congestion and haemorrhages in the myocardium; liver was enlarged, mottled and showed areas of necrosis. Kidneys were haemorrhagic, swollen and pale. Of all the dead chickens, one 8-week-old and one 3-week-old B1 broiler showed haemorrhages in the mucosal surface of the proventriculus-gizzard junction. Both these chickens died 4 days post-infection. Gross pathological lesions were similar to earlier reports (KHAWAJA et al., 1988; ABDUL-AZIZ and AL-ATTAR, 1991; AFZAL et al., 1991). However, haemorrhages in the proventriculus-gizzard junction was observed in some birds, although it was not a consistent feature in all dead birds. High pH of pericardial fluid indicated that it is an exudate due to infection.

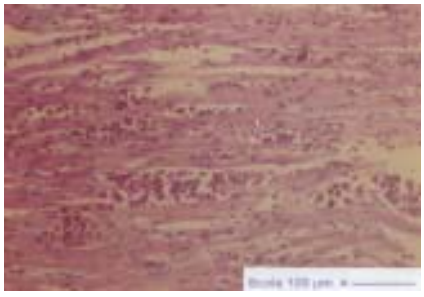

Fig. 1. Heart showing congestion, haemorrhages and degenerative changes in the myocardial fibre, H&E; ×320

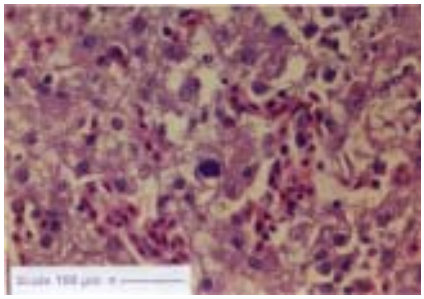

Fig. 2. Hepatocytes showing swelling and vacuolar degeneration. Some hepatocytes contain large intranuclear basophilic inclusion bodies. H&E; ×800

Histopathologically, Harderian glands showed multifocal haemorrhages and depletion of plasma cells. Heart revealed congestion, haemorrhages and hyaline changes in the myocardial fibres (Fig.1). Spleen showed lymphoid depletion in germinal centres. Lungs showed marked hyperaemia in alveoli. Liver showed diffused vacuolar degenerative changes in the hepatocytes, with marked swelling. Some hepatocytes were pyknotic. Sinusoidal spaces were widened, containing erythrocytes and a small number of heterophils. In focal areas the hepatocytes were atrophied and

appeared rounded. Isolated hepatocytes showed enlarged nuclei containing large basophilic intranuclear inclusion bodies (Fig. 2). Focal areas showed hepatocytes with intensely stained eosinophilic cytoplasm and pyknotic nuclei (Fig. 2). Pancreas showed focal infiltration of perivascular mononuclear cells. Kidneys showed multifocal haemorrhages. Tubular epithelium showed marked swelling and granular degenerative changes. Bursa showed marked depletion and necrosis of lymphoid cells in medulla. Microscopic lesions in heart, lung, bursa, spleen, and kidney were in accord with the earlier reports (CHEEMA et al., 1989; ABDUL AZIZ and HANSON, 1995). Microscopic lesions in the Harderian gland, pancreas, cerebellum and liver also have been described here. Pathological changes observed in chickens following infection were similar in both the B1 and B2 broiler groups. However, B1 groups were more susceptible than B2 groups. It is possible that a disease-resistant character was selected in the B2 broilers, which explains their lesser susceptibility to hydropericardium syndrome disease. A difference in susceptibility of broilers chickens of different genetic group to HPS virus infection has not been reported earlier. However, further work is needed to explore the factors responsible for the difference in susceptibility.

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ROY, P., B. MURALIMANOHAR, A. KOTEESWARAN, A.V. OMPRAKASH: Pokusna istraživanja hidroperikardijskog sindroma na dvjema linijama hibrida tovnih pilića. Vet. arhiv 74, 157-164, 2004.

SAŽETAK

Hidroperikardijski sindrom izazvan je u pokusno zaraženih dviju različitih linija hibrida tovnih pilića, bijelog (B1) i pigmentiranog perja (B2), u dobi od tri i četiri tjedna. Pilići linije B1 bili su osjetljiviji na infekciju s pomorom od 92,3% do 97%. Pilići linije B2 bili su manje osjetljivi s pomorom od 15,7% do 23,3%. Pomor od 60% bio je ustanovljen u pridodanih prijemljivih pilića linije B1 u dobi 3 tjedna. Šezdeset posto tovnih pilića linije B1 inficiranih u dobi od 8 tjedana uginulo je od hidroperikardijskog sindroma, dok pilići linije B2 nisu pokazivali nikakve znakove bolesti niti su ugibali u dobi od šest tjedana. Velik pomor ustanovljen je u pilića linije B1 nakon infekcije homogenatom tkiva zaražene jetre obrađenim kloroformom. Hidroperikardijski sindrom redovito je ustanovljen u uginulih pilića. Patoanatomske promjene utvrdene su u tkivu jetre, bubrega i srca. Patohistološki poremećaji dokazani su u tkivu jetre, bubrega, Harderove žlijezde, mozga, srca, plućiju, gušterače, slezene i burze. Neki hepatociti sadržavali su velike bazofilne intranuklearne uklopine. Po prvi put je dokazana razlika u prijemljivosti dviju linija hibrida tovnih pilića na virus hidroperikardijskog sindroma.

Ključne riječi: hidroperikardijski sindrom, osjetljivost pilića, pomor, patoanatomski i patohistološki nalazi