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EXPERIMENTAL INDUCTION OF IMMUNOSUPPRESSION. AND ITS EFFECTS ON HYDROPERICARDIUM SYNDROME IN BROILER CHICKS

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The prevalence of hydropericardium syndrome (HPS) was studied in broilers of 3-5 weeks age from 10 poultry farms around Faisa labad. The source of feed and managemental conditions did not influence the course of severity of the disease. The liver suspension prepared from field cases of HPS, when inoculated in 10 days old, 20 embryonating eggs caused 100% stunted growth and mortality. In day-old broiler chicks, immunosuppression was achieved by application of colchicine for four consecutive days to the bursa of Fabricius on the eloacal lips. The colchicine treated chicks when challenged with liver suspension of HPS cases showed 90% mortality as against 10% in untreated chicks and zero per cent in untreated unchallenged chicks. Increased mortality was related to immunosuppression caused by colchicine. making the chicks more vulnerable to the causal agent of HPS. The pathological lesions in dead chicks showed distension of pericardial sacs with fluid. The hearts were flabby and showed haemorrhages on the epicardium. Microscopically. there were degenerative changes in the myocardial fibres along with cellular infiltration. Grossly, the livers were enlarged. swollen and mottled in appearance. Histologically there was massive necrosis. dilatation of sinusoid and presence of. basophilic intranuclear inclusion bodies in the hepatocytes. The kidneys were pale in colour and swollen which under microscope showed degenerative changes in 'the epithelial cells of tubules. Deposits of urates were encountered in the ureters.

Key words: broiler chicks, colchicine, hydropericardium, immunosuppression

INTRODUCIlON

Hydropericardium syndrome (HPS) was first recognized in Angara Goth near Karachi, Pakistan, in late 1987. Within a year. diagnoses of HPS were made in most of the intensive broiler growing areas located near the main urban centers of Pakistan. The first cases of HPS occurred in broilers aged 3-5 weeks with flock mortality exceeding 50%. There were no characteristic clinical signs, but on postmortem examination. the accumulation of up to 10 ml straw-coloured fluid in the pericardial sac differentiated this condition from other endemic infections recognized by field diagnosticians. In addition. focal hepatic necrosis was observed in affected broilers.

Originally, the syndrome was attributed to either a toxicity or nutritional deficiency. Possible causes included mycotoxins, toxic fat agent., polychlorinated biphenyl. sodium, chloride. chlordane and phytotoxins, all of which are associated with hydropericardium. Concurrently a virus was suggested as the possible cause based on the observation that the disease could be reproduced by injecting young broilers with a bacteria-free liver homogenate from affected birds. It was also noted that crude vaccines comprising supematant from liver homogenates treated with formalin, provided protection against challenge under experimental conditions.

Detailed histological examination conducted on specimens from a number of flocks revealed the presence of basophilic intranuclear inclusion bodies in hepatocytes. Attempts were made to isolate a viral agent by late 1988. It was found that an adenovirus was in all probability responsible for HPS. The condition was considered to represent a more virulent form of conventional inclusion body hepatitis. an infection of relatively low pathogenicity compared to Angara-HPS. Infectious bursal disease virus and Marek's disease virus which are considered immunosuppressive pathogens are known to exacerbate adenoviruses. These are regarded by many pathologists and virologists as opportunistic agents. The present investigations were initiated to see the effects of immunosuppression on the pathogenesis of HPS in broiler chicks.

MATERIALS AND METHODS

Morbid livers of 50 broiler chicks. aged 1-5 weeks. affected with naturally occurring hydropericardium syndrome (HPS) were collected from 10 poultry farms aronnd Faisalabad, These livers were triturated in normal saline (1:2) to prepare a homogeneous suspension in which penicillin (lOO lU./ml) and streptomycin (Iumg/ml) were added to check bacterial contamination. Of this suspension. 0.1 ml was inoculated in 10 day-old. 20 chicken embryos to determine the pathogenicity of the suspected virus in the liver suspension. Along with this, 20 uninoculated to day-old embryonating eggs were kept as controls. The pathogenicity of the suspected virus was observed' b~ recording the mortality. gross lesions and growth of the embryos.

In the second trial. day-old 100 broiler chicks obtained from a commercial hatchery were randomly divided into three groups comprising 40 chicks in group A. 30 each in group Band C. The chicks of group A were treated with colchicine to deplete the bursa of Fabricius (Romppanen et al 1983). Chicks of groups A and B were slaughtered and bursa of Fabricius and thymus were removed. size of these organs was measured. gross lesions were recorded and histopathological studies were carried out.

The chicks at 30 days age were challenged with liver suspension. For this purpose. 30 colchicine treated chicks of group A and 20 of group B were inoculated intra-peritoneally at a dose rate of I ml per chick. The chicks of group C remained as unchallenged negative controls. Clinical signs and mortality, if any, were recorded. Post-mortem examination of all the inoculated and control chicks was performed and gross and histopathological lesion.swere studied.

RESULTS AND DISCUSSION

In the present studies. hydropericardium syndrome (HPS) was recorded in broiler. flocks of different sizes which indicated that the size of the flock had no influence on the occurrence of the disease. Broilers of single or multiple age groups were equally prone to HPS in conformity with the reports of Qureshi (1988) and Khan and Siddique (1989). This disease was observed at various farms. where different commercial feeds were being used. OCcurrence of HPS was slightly more in some breeds than others as has also been pointed out by Khan and Siddique (1989) and mortality was slightly higher in male broilers than in females as described by Shafique and Shakoori (1994). HPS resulted in high mortality varying from 2 to 28%. However, Jafferv et al. (1989) reported a high mortality up to 70% whicl~ might be due to different climatic factors prevailing in Punjab ad Karachi.

Most characteristic and pathognpmonic lesions were distension of pericardial sac with watery fluid, hepatomegaly, . mottling of liver as well as nephrosis, the same as described by Khawaja et al. (1995). The pathogenicity of liver suspension of HPS cases, was determined by inoculation into 10 day-old chicken embryos by recording the stunted growth and mortality of embryos. Stunted growth was observed bv ascertaining the length, width and weight ofinoculat~d embryos and comparing these parameters with those of uninoculated ones. The inoculated embryos showed significantly less measurements ($P<0.05$) which were suggestive of stunted growth. Control and inoculated embryos revealed stunted growth and underdeveloped wings. Mean weight of inoculated embryos was 2.79 ±.0.160 g and those of uninoculated controls was 3.576± 0.36 g. The mortality of the inoculated embryos was 100%. COlchicine.treated chicks when challenged.with liver suspension of HPS cases showed' 90% mortality as against 10% in untreated counterparts (Table I).

Table I.Pattern of mortality in chicks chllllcn~ed with liver suspension

Postinoculation (days)	Colchicine treated chicks (No.)	(%)	Untreated control chicks (No.)	(%)
3	3	10.00	I	5.00
4	2	6.66	–	–
5	6	20.00	I	SW
6	10	33.33	–	–
7	6	20.00	–	–
Total	27	S000	:2	10.00

Fadlyet al. (1980) recorded high mortalitym adenovirus infection and concluded that chemical immunosuppression triggered the clinical disease. Romppancn ct al, (19~D) reported that chemical bursectomy with colchicine ca~lses suppression of B-cell functions. Grossly. the colchicine-treated chicks. showed atrophy of bursa of Fabricius The reduction in length was significant, whereas thc width of bursa showed no marked difference (Table II. These findings were in agreement with those of Romppancn et al. (1983) who reported that bursal weight of colchicine-treated chicks was only 10% of those of non-treated controls. The significant reduction in the Icngth of the bursa was perhaps due to necrosis and severe follicles. Photomicrograph of .colchicine-treated bursa of Fabricius showed that bursal follicles were markedly depleted, with relative prominence of stromal elements and marked papillary folding surface epithelium H & E (x 4(0). In some cases cavities were seen in place of centrally located lymphoid cells. Abramoff and Lavia (1970) stated that colchicine is extremely lymphocytic, inhibits mitosis in metaphase and destroys the dividing and non-dividing cells,

Table 2. Range and mean ± SE values of various measurements of colchicine-treated and untreated bursa of Fabriefus and thymus

Bursa of Fabricius	Untreated	Colchicine-treated
Weight	0.7313 ± .6987 (0.4 - 1,0)	.27122± .2407^{NS} (0.06 - 0.5)
Length	1.3140 ± 1.286 (1,0 - 1,5)	.7576 ± .7424* (06 - 0.9)
Width of bursa of Fabricius	1.0345 ± .9455 (0.9 - 1,2)	.55X5± .5215^{NS} (0.1 - 0.9)
Weight of thymus	2.1804 ± 1.8196 (1.8 - 2.3)	.5X89 ± .5511** (O~ - 0.8)

Figures given in parentheses indicate range: N.S. = Non-significant; * Significant at $P < 0.05$; ** Highly significant at $P < 0.01$.

The mean weight of the thymus of colchicine-treated chicks was also significantly less ($P<0.05$) as compared to those of untreated chicks which appeared to be due to depletion of the cellular elements. Photomicrograph of thymus from colchicine-treated chicks showed atrophy of the lymphoid follicles (x 100). The dead colchicine-treated and untreated chicks were necropsied, gross and histopathological lesions of HPS were recorded. Grossly, the pericardium was distended with a watery fluid having a maximum quantity oP 15 ml as has also been recorded by Tooba and Khan (1989). The epicardium showed haemorrhagic spots while the heart appeared flabby. On incision jhe myocardium appeared congested. Degenerative changes of the myocardial fibres along with cellular infiltration were recorded. Gross lesions of the liver comprised hepatomegaly, mottling and in some cases icteric appearance and friable consistency. The parenchyma showed massive necrosis, congestion, dilatation of sinusoid and cellular infiltration. Intranuclear inclusion bodies were conspicuously present. The kindneys showed enlargement. swelling, congestion and petechial haemorrhages. Deposits of urates were commonly encountered in the ureters. There was coagulative necrosis of the urinary tubules and congestion.

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