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Inclusion body hepatitis-hydropericardium syndrome in commercial broiler chickens

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ABSTRACT

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Five cases of Inclusion body hepatitis (IBH) - hydropericardium syndrome (HPS) were investigated in commercial broiler chickens of 15-37 days age in Maharashtra state, India. Grossly, livers were enlarged and mottled with a reticular pattern on its surface. Hydropericardium was also observed in few cases. Mortality ranged from negligible to as high as 10% in the studied flocks. In all the cases, intranuclear basophilic inclusion bodies were observed in the hepatocytes along with fatty changes and mononuclear cell infiltration around portal triads. The disease was diagnosed on the basis of histopathological examination, agar gel precipitation test, isolation of virus and demonstration of inclusion bodies in liver cell culture using Macchiavello's technique.

Keywords: Broiler chicks, hydropericardium, inclusion body hepatitis

Inclusion body hepatitis - hydropericardium syndrome (IBH-HPS) is an emerging disease of broiler chickens caused by Adenovirus[1]. It is an economically important disease in commercial broiler chickens, characterized by poor weight gain and mortality[2]. The present manuscript reports the inclusion body hepatitis-hydropericardium syndrome (IBH-HPS) in commercial broiler chickens from Maharashtra state, India.

In the present study, liver samples from dead birds were collected from broiler chickens showing typical lesions of IBH-HPS during a natural disease outbreak. The detailed history regarding vaccination status against IBH-HPS, mortality and morbidity and duration of illness were recorded. Agar gel precipitation test (AGPT) was done with liver homogenate (50%) to confirm the disease[3]. The liver samples from affected birds of different flock were collected in 10% formalin, routinely. These were processed for histopathological examination and stained with haematoxylin and eosin[4]. Isolation of virus was done in liver cell culture prepared from SPF embryos. In short, twenty percent suspension of liver samples from affected flock was prepared (virus inoculums) in normal saline with antibiotic solution (1000IU of penicillin/ml and 1000µg/ml of streptomycin), incubated for one hour and filtered with 0.25µm syringe filter[3]. Afterward, the sample was processed for sterility test on Nutrient agar to rule out any bacterial growth. Chicken embryo liver (CEL) cell culture was prepared from 14-16 days old embryonated SPF chicken eggs. After 80% of liver cell growth on petri plate (Approximately, 24 hr after growth of cell culture), 0.1 ml of liver suspension (inoculums) was inoculated on liver cell culture[3]. The CEL cell culture plates were incubated at 37°C and examined periodically for any cytopathological effects (CPE). Cell culture after confluent growth was stained with Macchiavello's technique (modified) for inclusion body[4].

Clinically, birds showed ruffled feather, reduced feed intake, depression with uneven body weight (up to 10% of birds were underweight) and haemorrhages in thigh muscles. Mortality in affected flock ranged from non significant to maximum of 10% (Table 1). Similarly, Memon *et al.*[5] recorded 2.58 to 13.08 % mortality from 1999 to 2001 in various Taluka of Hyderabad district of Pakistan. However, some authors have reported mortality 10 to 40% in a flock which is in contrast to present observation[6]. In the present study, mortality percent was less and this could be attributed to ubiquitous nature of adenoviruses in environment. Moreover, this could have possibly also been due to the presence of low level circulation of antibodies in birds. Studies have demonstrated the presence of antibodies in healthy poultry, and viruses have been isolated from normal birds. Despite their widespread distribution, the majority of adenoviruses cause no or only mild disease; however, some are associated with specific clinical conditions[7]. History collected from owners revealed that all the flocks were unvaccinated for IBH. On post mortem examination, liver was found to be enlarged with mottled appearance (reticular pattern) and hydropericardium containing 5-15 ml of clear straw coloured fluid (Fig. 1). Kidneys were pale and severely

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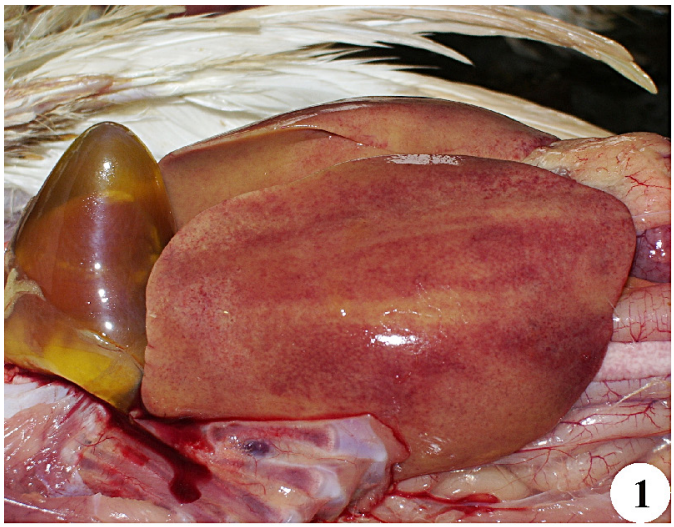

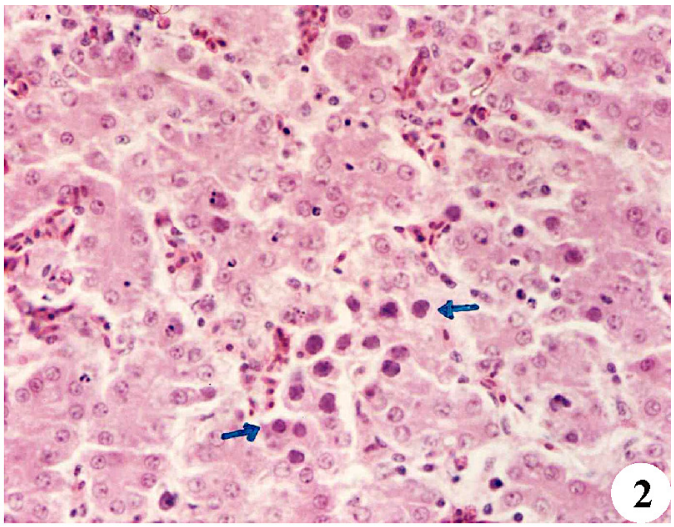

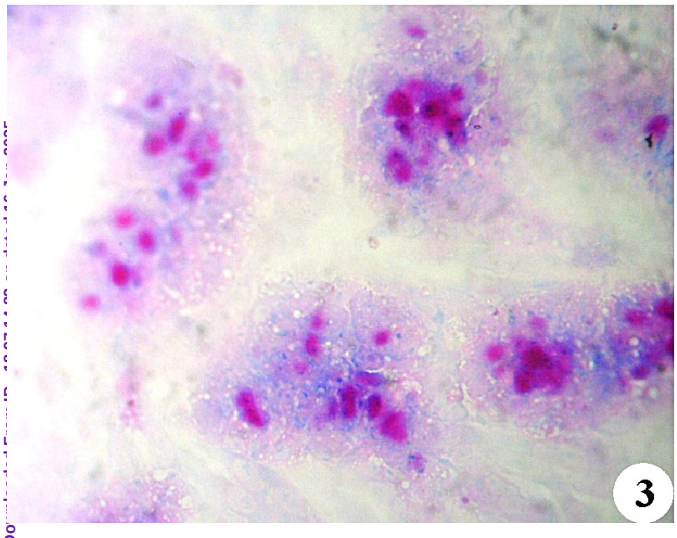

Fig. 1. Enlarged and mottled liver with hydropericardium; **Fig. 2.** Section of liver showing numerous intranuclear, basophilic inclusion bodies (Arrow) H&E x400; **Fig. 3.** Liver cell culture inoculated with IBH virus showing inclusion bodies, Pink; Macchiavello's stain x400.

enlarged (almost double than the normal size). Gizzard showed mucosal erosion with detachment of koilin layer. Histopathological examination of liver sample revealed diffused hepatitis with intranuclear, basophilic inclusion body in hepatocytes and mononuclear cell infiltration around portal triads in all cases (Fig. 2). In few cases, there were also fatty changes/ vacuolar degeneration in hepatocytes. Kidney lesions were inconsistent and showed tubular degeneration, interstitial nephritis to proliferative glomerulonephritis with mononuclear cell infiltration. Lungs showed pulmonary edema and congestion. Lesions in liver, kidneys, lungs and gizzard were in agreement with those of previous workers[8,9,10,11]. On rare occasion, eosinophilic inclusion has also been reported by author[11], which is in contrast to the present investigation. Gizzard showed severe mucosal congestion, haemorrhages and necrosis with mononuclear cell infiltration. Koilin layer was more eosinophilic and was infiltrated by mononuclear cells. In one case, basophilic intranuclear inclusion body was observed in gizzard mucosa along with inclusion body in hepatocytes. Similar microscopic lesions in gizzard were also observed by previous authors[12].

Growth of virus in CEL liver cell culture showed enlargement and rounding of cells after 3-4 days of inoculation. The virus was confirmed by agar gel precipitation test (AGPT). Macchiavello's staining of liver

Table 1. Detailed history of poultry flock affected with IBH-HPS.

Farm no.	Flock size	Type	Age	Duration of Outbreak	Mortality %
Farm 1A	8235	CB	22 days	12 days	6
Farm 1B	8589	CB	15 days	12 days	6.1
Farm 1C	7835	CB	18 days	10 days	3.5
Farm 2	1530	CB	31 days	8 days	9.3
Farm 3	3420	CB	37 days	NA	Non-significant
Farm 4	3000	CB	22 days	14 days	2.6
Farm 5	2000	CB	35 days	10 days	10 (10% underweight)

cell culture revealed pink coloured inclusion body (Fig. 3), which is a special technique to demonstrate inclusion bodies[4]. Similarly, isolation of IBH virus has been carried out by various authors from liver samples using CEL cell culture[13,14]. Although diagnosis of the disease is mainly done by gross pathological and histopathological observations but with the availability of known antiserum, the disease can be diagnosed by AGPT using 50% liver homogenate since it showed higher titre values[14]. Histopathology is a time-consuming process and hydropericardium occurs in several diseases conditions. CPE in liver cell culture were mainly aggregation, clumping of cells resembling clusters of grapes and detachment of cells. Buxton and Fraser[15] described that the CPE of adenovirus may be very characteristic, with rounding and clumping of affected cells into regular clusters resembling "bunches of grapes". Moreover, staining of virus inoculated culture with Macchiavello's technique can be used as confirmatory test to diagnose IBH-HPS.

In conclusion, IBH- HPS outbreak was recorded in broilers chickens of 15-37 days of age. Disease was confirmed by isolation of virus, Macchiavello's staining technique on chicken liver cell culture and AGPT test as well as by histopathological examination of liver sample. Present report indicates that Macchiavello's technique can be used to confirm the virus in cell culture within 24 hrs.

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