IN VIVO PATHOGENECITY STUDY OF INCLUSION BODY HEPATITIS (IBH) VIRUS IN EXPERIMENTALLY INFECTED BROILER CHICKS AND CHICKENS

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ABSTRACT

Inclusion body hepatitis (IBH) virus primarily affects the broiler birds between the ages of 3 and 5 weeks under field conditions. This investigation was planned to study the pathogenicity of IBH virus by infecting broilers through intramuscular route using liver homogenate of IBH affected birds. Total 20 birds of two age groups were selected for the in vivo study, which included day-old broiler chicks (n=10, Group A) and twenty days old chickens (n=10, Group B). In both Group A and B, five birds each were inoculated with 0.2 ml infected liver homogenate suspension by i/m route, i.e. treatment groups (T) and remaining five birds injected i/m with 0.2 ml normal saline served as control groups (C). All birds were kept under observation for five days to record morbidity and mortality. In infected/treated chicks of Group A, 100% mortality was observed on third day, while in infected/ treated chickens of Group B only 40% mortality was recorded within five days of observation period. Clinical signs like reduced feed intake and depressed appearance were observed in both the *in vivo* infected groups. However, hydropericardium syndrome was observed in infected birds of only Group B. Eosinophilic intranuclear inclusion bodies were observed in the hepatic cells of both the infected groups, which confirmed the disease as inclusion body hepatitis. The results indicated that the 0.2 ml liver homogenate of IBH affected birds was more pathogenic in day-old chicks than the older ones due to age factor.

KEYWORDS: Broiler Chick, Chicken, Hydropericardium, IBH, *In vivo* Pathogenicity.

INTRODUCTION

Inclusion body hepatitis (IBH) of poultry has emerged as devastating disease in many parts of the world. It is causing heavy economic losses more than Rs. 80 million/annum (Khalid, 2003) to the poultry farmers. IBH has been observed in broiler birds, layers and breeder pullets and is responsible for low morbidity but heavy mortality (Khawaja et al., 1988). The causative agent is characterized as fowl adenovirus FAV-4 (Jadhao et al., 1997). In India, the disease was observed in early 1993 in Jammu and Kashmir and Punjab states and after that the disease spread in other parts of the country within a very short period. Typical postmortem lesions of IBH such as pulmonary edema, hepatitis and accumulation of clear straw-colored fluid in the pericardium are characteristics of this disease. Demonstration of basophilic and eosinophilic intranuclear inclusion bodies in hepatocytes is considered as pathognomonic lesions for diagnosis. The IBH disease has been reproduced in broiler chickens by the inoculation of liver homogenates from affected birds (Muneer et al., 1989; Afzal et al., 1991). The objective of the present study was to investigate the pathogenicity of IBH virus causing IBH-HPS in different age groups of broilers.

MATERIALS AND METHODS

Liver samples were collected in 20% glycerol saline during necropsy examinations of naturally affected bird with IBH showing the typical lesions of IBH disease, and a homogenate suspension (w/v) was prepared by triturating the same.

In all 20 broiler birds were selected to know the pathogenecity of IBH virus from the liver homogenate of IBH affected birds. These birds included day-old broiler chicks (n=10, Group A) and 20-days

old broiler chicken (n=10, Group B). Birds of both the groups A & B were subdivided in treatment (T, n=5) and control (C, n=5) groups. The chicks and chickens of treatment (T) groups were inoculated intramuscularly with 0.2 ml of above liver homogenate, whereas their control (C) groups received 0.2 ml normal saline as inoculum intramuscularly. Feed and water were provided *ad libitum* to all the birds in both the groups. The clinical signs and mortality were noted until 5 days in all groups. The necropsy was carried out for the dead birds from both groups and samples of organs were collected in 10% formal saline for histopathological examinations. These included liver, heart, spleen, kidneys, thymus and bursa of fabricius.

RESULTS AND DISCUSSION

The present study was conducted to evaluate potential pathogenicity of IBH virus. The birds were monitored for 5 days after inoculation of 0.2 ml infected liver homogenate i/m for behavioral changes / clinical signs and mortality, if any.

Clinical Signs and Mortality Rate

The clinical signs were observed within 2 days in infected/treated (T) day-old chicks of Group A, which included reduced feed intake, ruffled feathers, depressed appearance, and inability to get up and walk (Fig. 1). All the five birds in this group died on 3rd day giving 100 % mortality. Nakamura et al. (1999) and Wang and Chang (2000) found similar clinical signs and mortality rate in day-old SPF pigeon chicks. Wang and Chang (2000), however, inoculated SPF chicks of day-old age with higher dose than the present one, while with lower dose mortality reached 90% at 14th day post-inoculation. In the infected/treated (T) 20-days old chickens of Group B, clinical signs were observed after 3rd day and included reduced feed intake, depressed appearance with mortality up to 40% by the end of 5 days observation period. Nakamura et al. (1999) also found 40% mortality in five-weeks old chicken inoculated with cell culture of IBH adenovirus that compared favourably with the findings in Group B. Anjum (1990) studied pathogenicity of virus by inoculating 0.25 ml infected-liver homogenate subcutaneously in 12 to 15 days old broiler chickens and all birds died suddenly after 2 to 5 days challenge without any premonitory signs. Pathogenicity studies in commercial chickens with fowl adenovirus have shown variable clinical signs and mortality suggesting that fowl adenovirus pathogenesis is not necessarily serotype dependent (Saifuddin and Wilks, 1990). Route of inoculation and dose are an important factors contributing to the pathogenicity of IBH virus. There was lower mortality rate in 20 days old broiler chickens as compared to day-old broiler chicks. This may be due to the presence of maternal antibodies in older chicks.

Fig. 1: *Experimentally IBH infected broiler chick (A group) showing depressed appearance and inability to get up.*

Gross and Microscopic Lesions

Post-mortem lesions in infected birds of Group A included congestion and hemorrhages on epicardium of heart and pale enlarged liver (Fig. 2) with multiple pinpoint necrotic foci. There was no any lesion in lungs, bursa of fabricius, kidneys, thymus, spleen etc. Post-mortem lesions in infected birds of Group B included jaundiced carcass, hydropericardium with moderate to enlarged friable hemorrhagic liver with fatty changes (Fig. 3), pale friable swollen kidneys and edematous lungs. McCracken et al. (1976) inoculated adenovirus from typical cases of inclusion body hepatitis (IBH) into SPF birds by a combined oral and intranasal route, but failed to see any pathological

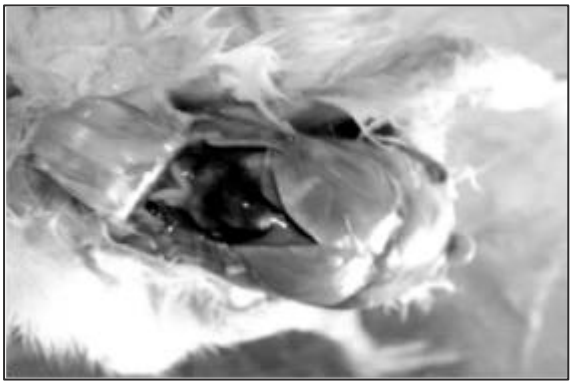

Fig. 2: *Experimentally IBH infected broiler chick (A group) showing hemorrhages on epicardium with pale enlarged liver.*

Fig. 3: *Experimentally IBH infected broiler chicken (B group) showing hydropericardium with moderate to enlarged, friable hemorrhagic liver with fatty changes.*

changes. A typical hydropericardium syndrome was observed in experimental chickens 72 hrs post-inoculation in commercial broilers birds during 1990 to 2003 in Pakistan by Shamim et al. (2009), but no such syndrome was observed in the present study.

Microscopically infected chicks of group A showed diffuse parenchymatous degeneration with focal areas of necrosis, fatty changes and lymphocytic infiltration in liver tissue. Many hepatic cells showed presence of eosinophilic intranuclear inclusion bodies which confirmed the disease as inclusion body hepatitis (Fig. 4). In the heart, congestion, hemorrhages and also infiltration of mononuclear leukocytes were seen in myocardial fibers. In infected chickens of group B, diffuse fatty degeneration and formation of eosiophilic intranuclear inclusion bodies were consistent lesions in hepatic cells. Also mononuclear cell infiltration, severe vascular changes, edema and hemorrhages were found in heart, mild to severe congestion, and focal to diffuse intertubular hemorrhages in kidneys. There were no any changes during experiment in both the control groups (A & B). Like present findings, common characteristic lesions of IBH, viz., fatty degeneration, intranuclear inclusion bodies, swelling of hepatocytes and multiple necroses etc have also been observed by earlier researchers (Itakura et al., 1974; Abdul-Aziz and Hasan, 1995; Philippe et al., 2005). Intranuclear inclusion bodies were also seen in gizzard, proventriculus, duodenum, caecum, kidneys, and lungs of chicks inoculated at day-old age (Nakamura et al., 2000). Romanova et al. (2009) observed the highest virus replication in the liver, ceacal tonsil and bursa of fabricius following intramuscular inoculation of fowl adeno virus. In present study no lesion was found in thymus, but Asrani et al. (1997) reported lymphocytolytic activity leading to poor cellularity in cases of HPS in thymus.

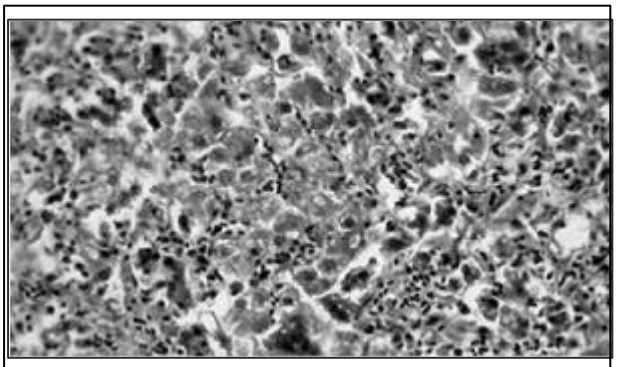

Fig. 4: *Liver from experimentally IBH infected broiler chicken (B group) showing hemorrhages, necrosis, fatty changes, lymphocytic infiltration and eosinophilic intranuclear inclusion bodies.*

Pathogenicity of IBH virus in different age group of broiler birds via same routes using infected liver homogenate suspension showed intranuclear inclusion bodies which confirmed the disease as inclusion body hepatitis, but different mortality rate was observed in different age groups probably due to the presence of maternal antibodies in older chicks. Comparison of present findings with

other studies suggested that, apart from age, route of inoculation and dose are also important factors contributing to the pathogenicity of IBH virus.

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