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PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Adenovirus Induced Hydropericardium-hepatitis Syndrome in Broiler Parent Chickens in Chittagong, Bangladesh

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Abstract: A disease outbreak was investigated in a commercial broiler parent stock farm. The outbreak began at the mid of May 2000 and continued more than one month. During the period mortality was recorded around 4 % in the affected shed, flock strength of which was 5000 birds. During post mortem examination the pericardial sac was found engorged with straw colour pericardial fluid. Numerous subcapsular petechial and ecchymotic haemorrhages were seen in liver. No bacterial growth was found after 48 hours of incubation on Muller-Hinton and MacConkey agar surfaces inoculated from the pathological sample. But poor viral growth was recorded in chick embryo fibroblast cell culture propagated inoculums making from heart and liver. Harvested virus using as antigen source from the growth was found reacted positively in agar gel precipitation test with reference conventional adenovirus polyvalent antiserum. Two of the four randomly collected serum samples of the affected flock at day 20 of the outbreak were also found reacting positively with the known reference antigen produced by the same institute. Not having the type specific antiserum the present study failed to elucidate the stereotype (s) that involved in the said outbreak. Nevertheless, the study seems to be the first report of adenoviral induced hydropericardium-hepatitis syndrome or leechi heart disease affecting chicken in Bangladesh.

Key words: Adenovirus, hydropericardium, hepatitis, angara disease, leechi heart disease, harvest virus, chicken mortality

Introduction

Adenoviruses are DNA viruses, which replicate and frequently produce inclusion bodies in the nuclei of infected cells. There are three major groups or types of adenoviruses; each type possesses different members, which may share group antigen. Type II avian adenoviruses encompass the viruses causing marble spleen disease in pheasants and hemorrhagic enteritis in turkey. The type III group adenovirus is responsible for egg drop syndrome, 1976 (EDS, 76) (VanEck *et al.*, 1976). The members of first group are conventional avian adenoviruses which differentiate from III group by haemagglutination test. Unlike group I adenovirus EDS virus agglutinates avian but not mammalian erythrocytes to high titres. A lot of reports have been published on EDS, 76 and inclusion body hepatitis in chickens (Firth *et al.*, 1981; Cernik, 1985 and Gupta *et al.*, 1985).

Conventional adenoviral infections are very common in poultry and other avian populations. There are 12 distinct serotypes of this type identified. However, the exact role that the members of this type plays in chickens is unclear. They are suspected of playing a primary or secondary role in a variety of diseased conditions including inclusion body hepatitis, splenomegaly, hydropericardium, and miscellaneous respiratory, arthritic, encephalitic and enteric syndromes.

Earlier 1987, in Pakistan, adenovirus induced hydropericardium syndrome in broiler caused heavy mortality in young chicks. Despite the extensive use of vaccine against the disease it is still considered a great problem to poultry sector in Pakistan (Qureshi, 1998).

Adenoviral hydropericardium-hepatitis syndrome is known as Angara disease in Pakistan, Leechi heart disease in India (Karunamoorthy *et al.*, 1998), virulent inclusion body hepatitis in Mexico and other countries in Latin America. This is an endemic and economically important disease in those countries that cause huge economic loss to poultry sector owing to chick mortality and decreased productivity. Since the disease has been reported from Iraq, Kuwait, Japan and many other countries, advances in an understanding of the pathogenesis and control of the disease are important to poultry producers worldwide (Shane, 2000). Being a neighboring country to India and having a porous boundary in terms of animal movement and disease control strategy, there is every possibility that Bangladesh might already have got the leechi

heart disease or Angara disease in its territory. However, due to lack of proper poultry disease surveillance and diagnostic system it might be still unnoticed in the country as one may fail to find any reference in literature for the diseased conditions. The present study seems therefore to be the first report of adenovirus induced hydropericardium-hepatitis syndrome in broiler chickens in Bangladesh.

Materials and Methods

A disease outbreak was investigated in a commercial broiler (Kasila Halb Chick Strain) parent stock farm, flock strength of which was 64000. The outbreak began at mid of May 2000 and continued more than a month. After 7 days of the outbreak 8 (eight) 25 weeks old dead chickens were collected from the farm and subjected to thorough postmortem examination at the Dept. of Microbiology, Chittagong Govt. Veterinary college. The outbreak was recorded only in one of the sheds having flock strength of 5000 birds. Prior to outbreak the flock of the affected farm was reported to have treated with gentamycin followed by tylosine tartrate. All the birds have been vaccinated against Newcastle disease, infectious bronchitis, Egg drop syndrome, 76, infectious coryza and avian mycoplasmosis. After the postmortem examination samples were taken from the heart, pericardial fluid, bone marrow and spleen and inoculated onto Muller-Hinton agar and MacConkey agar surfaces for bacterial cultivation.

Virological investigation was carried out at Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka taking heart and liver considering the most potential sources for viruses. At 20th day of the outbreak blood serum were taken randomly from 4 apparently healthy chickens. The sera were subjected for Agar gel precipitation test with reference viral antigen produced by the Animal Health Research Institute, Shukuba, Japan. For virus cultivation inoculum was made from the heart containing the pericardial fluid and liver of the affected birds. The inoculum (10 % suspension) was propagated in chick embryo fibroblast cell culture (CEF). Virus was harvested as tissue homogenate from the growth in the cell culture and the crude viral preparations was used as a source of antigen. Agar gel precipitation test was done using this antigen with reference polyvalent sera possessing antibodies of 12 known serotypes of conventional avian adenoviruses, produced by the same institute.

Results and Discussion

During the surveillance period most of the affected birds were found dead without any prodromal signs. In rare cases some respiratory distress was recorded. Birds 5-7 were being found dead every day during the outbreak period and total mortality was recorded around 4 %. Feed consumption and egg production were virtually unaffected.

At postmortem examination the pericardial sac was found engorged with straw colour pericardial fluid. The fluid was aspirated by sterile needle and syringe and measured 10-12 ml per sac.

The liver was found congested. In addition to this, numerous subcapsular petechial and ecchymotic haemorrhages were seen. Besides the heart and liver there was no pathological change recorded in lungs, spleen, kidneys, intestines or other organs. The pathological changes of heart and liver are shown in Fig. 1 and 2, respectively.

The growth of the virus on the chick embryo fibroblast cell culture presumed only having inclusion bodies in some cells. Other cytopathic effects like rounding, shrinking, syncytium formation etc. were not found in the used cell culture. Nevertheless, the crude antigen preparation as tissue homogenate from viral growth in CEF was found with positive precipitation test to a known polyvalent sera in agar gel, which revealed that the pathological condition hydropericardium could have been caused by avian adenoviruses. On the other hand, 2 of 4 serum samples collected at day 20 of the outbreak were found positive agar gel precipitation test with the reference antigen. Although the antibody titre was not assessed, all the same, it could be assumed that 50 % sero-conversion in the flock might have been resulted from early adenovirus invasion.

Hydropericardium and lesion of heart that was observed in our study was found in clear conformity with the classical presentation of Shane (2000) and Karunamoorthy *et al.* (1998). In the present study poor CPE was recorded in chick embryo fibroblast cell culture. Watanabe (1984) showed a round cell CPE produced by adenovirus in the same cell culture. This variation in observation might be due to poor growth of the virus in prepared chick embryo fibroblast cell culture.

Rhee *et al.* (1979) reported, in Korea among the 12 serotypes, type 1, 2 and 4 were more prevalent, where as Hussain *et al.* (1981) claimed sero type 1, the most dominant type in Australia. Serotype 8 is said to be responsible for causing inclusion body-hepatitis-hydropericardium syndrome in chickens in Pakistan (Qureshi, 1998). Not having the type specific antiserum the present study failed to elucidate the serotype (s) that might have caused the disease outbreak.

Vasicek and Kopera (1979) claimed agar gel precipitation test as an effective serological tool for detecting avian adeno and reovirus affecting chickens. Dawson *et al.* (1980) reported that ELISA was a reliable and more sensitive test than agar gel precipitation for detecting antibodies to avian adenovirus and adenovirus-associated virus in chickens. The direct ELISA for detecting avian adenovirus antigen in cell cultures was described and shown (Schorlemer, 1985) to be a more suitable and alternative to the agar gel precipitation test. In present investigation agar gel precipitation test was used for detecting viral antigen but ELISA is the most effective test being practiced widely in sero-typing the conventional avian adenoviruses.

As the viruses produce inclusion bodies, demonstration of typical microscopic lesions in the liver, including inclusions, is often used as a basis for the diagnosis of adenovirus induced inclusion body hepatitis. In present study intranuclear inclusions were found in virus propagated cell culture but no effort had been made to conduct histopathological investigation on heart or liver.

Qureshi (1998) noticed in Pakistan, a disease condition termed hepato-nephrosis where enlargement of the liver with different degrees of discolouration and even haemorrhages were seen at postmortem examination. The said disease condition in liver might be very similar with liver lesion attributed to adenoviral invasion,



Fig. 1: Heart of affected bird showing characteristic hydropericardium

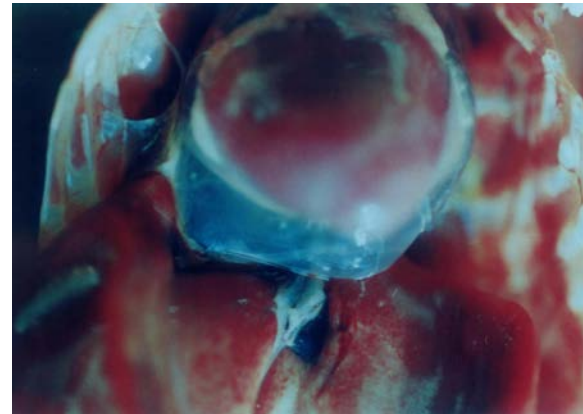


Fig. 2: Characteristic accumulation of fluid around the heart and liver showing sub-scaphular hemorrhages

but in case of the former disease condition kidneys were also reported showing extreme swelling and distension with urates. Concerning the probable etiology of the said hepato-nephrosis in chicken in Pakistan, feed quality problems due to contamination with mould and mycotoxin were indicated. Inclusion body hepatitis and hydropericardium syndrome were reported to be caused by different serotypes of group 1 adenovirus but in the literature one might fail to find any reference for the lesions of nephrosis induced by adeno viruses. In the present study no pathological lesion was recorded in kidneys and the liver was congested and haemorrhagic which is in conformity with Qureshi (1998).

Watanabe (1984) claimed that hydropericardium syndrome in chickens might be of bacterial origin particularly caused by *Escherichia coli* under the influence of stress factors. In present study no bacterial growth was observed on Muller-Hinton agar and MacConkey agar surfaces inoculating with pathological samples. This finding revealed that no bacterial involvement could be considered in inducing the said hydropericardium lesion.

A large number of articles reported that birds predisposed to infectious bursal disease and subsequently rendering immune-compromised were more vulnerable to adenovirus infection. Fadly *et al.* (1980) showed adenovirus induced high mortality with inclusion body hepatitis and hydropericardium syndrome from chemically bursectomized progeny of breeder flock maintained under SPF conditions. The chemical immune-suppression appeared

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to trigger clinical disease in the progeny and to the recognition of virus infection in parent flock. Cernik (1985) in Czechoslovakia recorded 2.9 % flocks positive for adenoviruses and 38.4 % infectious bursal disease virus in 1980 whereas in 1981 the prevalence rate for the viruses were 34.8 and 79.9 %, respectively, that means increased incidence of infectious bursal disease triggered the rate of adenoviral infections. No such correlation was established in our study as antibody level of infectious bursal disease virus was neither assessed after vaccination nor evaluated during the outbreak period.

Erny *et al.* (1991) confirmed that the hydropericardium inducing strains of adenoviruses demonstrated age specificity. The younger the age of infection is more severe the pathology. He demonstrated mortality in meat producing chickens aged 4-9 weeks between 2-10 % and up to 30 %, however Shane (2000) reported a case of hydropericardium syndrome in Japan in a 5000 hen flock of mature broiler breeders demonstrating 6 % mortality. In our study mortality rate was obtained 4 % which is nearly corroborates with the observation.

Erny (1991) suggested that stress factors might predispose adenoviral outbreaks. The affected flock was treated with gentamycin followed by tylosine tartrate with 10 times higher than the normal doses. This overdosing of antimicrobials might cause stress to birds that could have initiated the outbreak or it might be caused other reasons that were not known. But the initial source of the virus could be considered to be vertically transmitted from the grand parent chickens and was imported along with day old parent chicks. During the outbreak the virus was supposed to be disseminated within the flock laterally and by fomites.

Based on the evidence generated the present study might be considered the first ever report of conventional adenoviral induced hydropericardium syndrome in broiler parent chickens in Bangladesh. The disease seems to be a new emerging one in poultry sector. Nevertheless, before claiming its prevalence and endemic nature, specific serotype (s) that have induced the outbreak yet to be elucidated.

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