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Research Article

IMMUNOPEROXIDASE TEST USING CONVALESCENT SERA TO DEMONSTRATE FOWL ADENOVIRUS TYPE-4 ANTIGEN FOR DIAGNOSIS OF HYDROPERICARDIUM SYNDROME IN BROILER CHICKENS

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ABSTRACT

The present study was conducted to standardize the indirect immunoperoxidase test for demonstration of fowl adenovirus antigen in liver tissue section so that the test can be used for routine diagnosis of hydropericardium syndrome. Use of specific standard convalescent sera raised against fowl adenovirus type-4 (FAV-4) in indirect immunoperoxidase test confirmed the presence of FAV-4 in the liver homogenate.

Key words: Hydropericardium syndrome, indirect immunoperoxidase test

Hydropericardium syndrome (HPS) is primarily a disease of broiler chickens and was reported for the first time in Angara Goath area near Karachi in Pakistan during 1987 (Jaffery 1988; Khwaja *et al.,* 1988). The disease spread to most of the regions of India within very short time (Gowda and Satyanarayana, 1994). The disease is caused by fowl adenovirus type-4 (FAV-4) (Jadhao *et al.,* 1997; Dahiya *et al.,* 2002; Ganesh *et al.,* 2002). Various methods are being used for the diagnosis of HPS like isolation of virus, immunodiffusion, ELISA and PCR (Anil Kumar *et al.,* 2011). In this study, we standardized indirect immunoperoxidase test (IIPT) for the demonstration of FAV-4 antigen in histopathological sections of liver tissue.

MATERIALS AND METHODS

Day-old commercial broiler chicks were procured from the Department of Animal Breeding, Hisar. The chicks were reared under strict hygienic conditions. The HPS seed virus was procured from Indovax Pvt. Ltd., Hisar. Infective dose was calculated as per the method of Reed and Muench (1938). A dilution of 10^{-3} of 20% HPS inoculum was found to be optimum for producing the protracted disease. Each bird was injected with 0.5 ml of the diluted (10^{-3}) HPS inoculum (ID50) subcutaneously.

Immunohistochemical Studies: Immunohistochemical detection of viral antigen was carried out by IIPT in formalin fixed paraffin embedded liver tissue sections taken on lysine coated slides (Gupta, 1999). The liver samples showing typical inclusion bodies collected at 3 and 5 days post infection were selected for standardizing the IIPT.

Convalescent Sera: Convalescent serum from chicken infected with chicken liver cell cultured FAV-4 was used for conducting the IIPT in tissue sections.

Rabbit anti Chicken Unconjugated Whole Serum: It was obtained from Bangalore-Genei and was used at the concentration of 1:10 for IIPT. It was used for blocking the chicken immunoglobulin suspected to be present in the tissue sections.

Conjugate: Rabbit anti-chicken, horseradish peroxidase (HRPO) conjugate (Bangalore-Genei) was used for detecting bound immunoglobulins in tissue sections by immunoperoxidase test.

Substrate: 20 mg of 3, 3-diaminobenzidine (DAB; Sigma St Louis, USA), dissolved in 50 ml of Tris buffer saline (TBS) and 10 µl of hydrogen peroxide was used as a substrate.

Procedure: Immunoperoxidase staining was performed as per protocol of Gupta (1999) with necessary

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modifications. The procedure followed was: Deparaffinization of tissue sections in two changes of xylene followed by rehydration through descending grades of ethanol. The sections were rinsed in water and treated with 1.5% H_2O_2 in methanol for 30 minutes at 37^{0}C. This was followed by three times washings with TBS. The tissue sections were then incubated with 1.0% trypsin solution containing 0.5% calcium chloride ($CaCl_2$) for 45 minutes at 37^{0}C and immediately the slides were dipped in prechilled (4^{0}C) TBS and rehydrated for 5 minutes. Tissue sections were then treated with rabbit anti-chicken unconjugated whole serum (1:10 dilution) for 30 minutes at 37^{0}C in moist chamber to block the chicken immunoglobulins present in tissue sections. The sections were then rinsed with TBS. Non specific blocking was done by immersing the sections in 4% BSA in TBS for 45 minutes at 37^{0}C in moist chamber. Tissue sections were flooded with FAV-4 specific convalescent sera (1:5 dilution in BSA) for 1 hour at 37^{0}C in moist chamber. Then washing was done with TBS for 3-4 times. Sections were then flooded with rabbit anti-chicken HRPO conjugate at a dilution of 1:250 (standardised) for 1 hour at 37^{0}C followed by washing with TBS. The sections were dipped in the substrate (DAB) for 4-5 minutes. Final washing was done in running tap water to stop the reaction and counterstained with 10% Harris haematoxylin for 1 minute. Slides were dehydrated in ascending grades of alcohol, cleared in xylene and mounted with DPX and observed under light microscope. Brownish coloured round bodies representing intranuclear inclusions were considered as positive for FAV-4 antigen.

RESULTS AND DISCUSSION

On 3 and 4 DPI, the birds of infected groups revealed severe hydropericardium and enlarged mottled liver having necrotic and haemorrhagic spots. Histological observations revealed mild to severe lesions in liver of infected chicks at different intervals. On 2 DPI, the liver of the HPS infected chicks showed hepatitis characterized by the presence of a few focal areas of necrosis of hepatocytes, congestion of sinusoids, and mild infiltration of lymphocytes and heterophils. At later stages, severe hepatitis with hydropic changes, congestion and haemorrhages, fatty changes along with intranuclear inclusion bodies in the hepatocytes was observed. The nuclei of some hepatocytes showed pyknosis and karyorrhexis with dark and hyperchromatic nuclei. Inclusion bodies seen in many hepatocytes were surrounded by a clear halo in some of the hepatocytes, while in many hepatocytes; the inclusions were round, oval or irregular in shape completely filling the nucleus. The liver sections stained with IIPT revealed brown coloured intranuclear inclusion bodies (Figs. 1,2) in hepatic cells indicating the presence of virus antigen in the cells.

Immunohistological techniques are frequently used to demonstrate a variety of infectious agents as well as other tissue antigens in routinely processed material

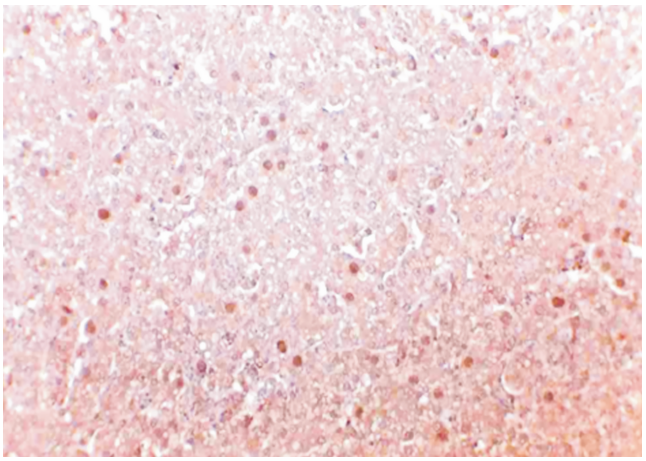

Fig 1. Section of liver of an HPS infected chick at 3 DPI showing brown coloured intranuclear inclusion bodies with IIPT staining. (IIPT x 66)

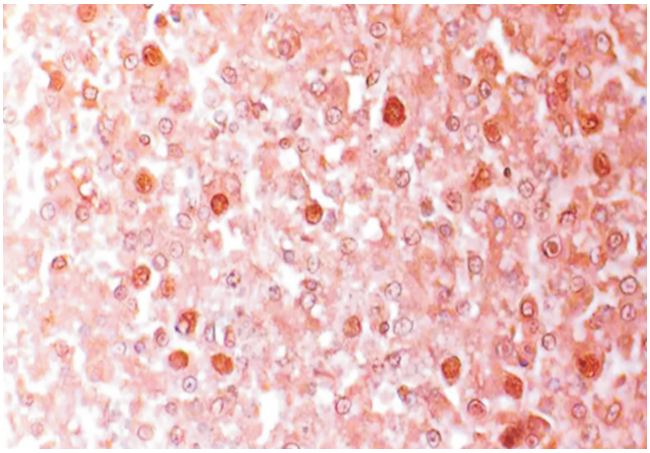

Fig 2. Section of liver of an HPS infected chick at 3 DPI showing brown coloured intranuclear inclusion bodies with IIPT staining. (IIPT x 132)

(Turfrey, 1985; Cruz-Coy *et al.,* 1993; Gupta, 1999). Immunohistology can be a major aid for the diagnosis of those viral diseases whose causal agents do not grow or do so poorly in usual isolation procedures or grow very slowly. The combined use of immunohistology and cultural techniques may be more useful and accurate for evaluation of spread of virus. The results of the present study using IIPT for the demonstration of viral particles in tissue sections using convalescent sera of chicken infected with FAV-4 as source of primary antibodies were encouraging, and the histopathological lesions could be correlated with the presence of dark brown deposits of viral antigen intranuclearly adding confirmation of their pathogenic role. The use of highly sensitive immunoperoxidase method for demonstration of viral antigen aids to diagnosis. Furthermore, immunoperoxidase technique can be used for routine field diagnosis (Domingo *et al.,* 1986). Moreover, the use of specific standard convalescent sera raised against FAV-4 as a source of primary antibody in immunohistological technique in the present study confirmed the presence of FAV-4 in the liver homogenate inoculum used for reproduction of hydropericardium syndrome.

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