VETERINARSKI ARHIV 71 (2), 97-103, 2001

Serological, cytopathological and cytochemical studies on hydropericardium syndrome virus

Parimal Roy*, Agatheeswaran Koteeswaran, and Ramagounder Manickam

Centre for Animal Health Studies Tamil Nadu, Veterinary and Animal Sciences University, Madhavaram Milk Colony, India.

ROY, P., A. KOTEESWARAN, R. MANICKAM: Serological, cytopathological and cytochemical studies on hydropericardium syndrome virus. Vet. arhiv 71, 97-103, 2001.

ABSTRACT

Hydropericardium syndrome (HPS) is an emerging disease of broiler chickens. For diagnosis of the disease agar gel immunodiffusion (AGID) and counter immuno-electrophoresis (CIE) were found useful. Fifty percent liver homogenate from affected chickens showed AGID titre of 3.2±0.2 $\log_2$ and CIE titre of 4.0±0.0 $\log_2$ with HPS antiserum. The causative virus became adapted in VERO cell line after four blind passages and after 96 hours produced appreciable cytopathogenic effects (CPE) characteristic of an adenovirus. Immunoperoxidase test (IPT) was carried out on coverslip cultures. Infected coverslips treated with HPS antiserum showed IPT positive reaction but the reaction was negative when coverslips were treated with Newcastle disease virus antiserum or Infectious bursal virus antiserum. Aetiology of HPS is discussed.

Key words: hydropericardium, broilers, immuno peroxidase test, agar gel immunodiffusion, counter immuno electrophoresis

Introduction

Hydropericardium syndrome (HPS) is a new disease of broiler chickens initially reported from Pakistan (KHAWAJA et al., 1988). Subsequently it was found to be prevalent in India (GOWDA, 1994). HPS was typically observed in three- to six-week-old growing broilers and mortality ranged up to 60 percent (AHMED et al., 1989). The syndrome is characterized by an accumulation of clear, straw-coloured fluid in the pericardial sac,

* Contact address:
Dr. Parimal Roy, Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai 600051, India. Phone: +253 4454 530; Fax: +253 4454 544; E-mail: parimalroy@hotmail.com

ISSN 0372-5480
Printed in Croatia

discoloured liver and enlarged kidneys (ANJUM et al., 1989; CHEEMA et al., 1989). Histopathologically, basophilic intranuclear inclusion bodies were seen in the hepatocytes of liver (ABDUL AZIZ and HANSON, 1995). The exact aetiology of the syndrome remains uncertain but an adeno virus has been isolated from the liver of infected chickens (CHEEMA et al., 1989). An inactivated vaccine prepared from affected livers was found highly effective in controlling the disease (ROY et al., 1999). This paper deals with serological, cytopathological and cytochemical studies on HPS.

Materials and methods

Samples. Liver materials were collected from broiler chickens showing typical lesions of HPS during a natural outbreak.The disease was reproduced experimentally as described by CHEEMA et al. (1989). A thirty percent suspension of liver homogenate in normal saline solution (NSS) treated with antibiotics (Penicillin – 10,000 unit/ml, Streptomycin – 10mg/ml and Gentamicin – 250 µg/ml) was inoculated subcutaneously into three-week-old HPS susceptible broiler chicken. About 80 per cent of chickens died with typical lesions of HPS. Liver tissues collected from the dead chickens were used for this study.

Antiserum. About 20 per cent-infected chickens did not succumb to the disease. Sera samples collected from the survivors 21 days after infection were used as antisera for this experiment.

Antigen for Agar gel immunodiffusion (AGID) and Counter immuno electrophoresis (CIE) tests. Liver tissue was collected from five different chickens showing lesions of HPS. Fifty per cent and 25 per cent suspensions of liver tissues in NSS were prepared individually and thoroughly mixed with chloroform (4:1). After 15 minutes the mixtures were centrifuged and clear supernatants were used as test antigen in AGID and CIE. Antigen samples were titrated against the HPS antiserum.

AGID. The test was done in slides in 1% agarose (low EEO) containing 8% sodium chloride. Antiserum was charged in central well and different dilutions of antigen (liver homogenate) in peripheral wells at a volume of 25 µl. Diameter of wells and distance between wells were 4 mm. The slides were left at room temperature in a humidity box and results were

read at 24 hours, 48 hours and 72 hours.

CIE. The test was done as described earlier (HARIBABU, 1986; ROY and VENUGOPALAN, 1997). Different dilutions of antigen (liver homogenate) were changed to wells connected to the cathode, and antiserum to the wells connected to the anode. The test was run for 45 minutes and results were read under diffused light.

Passage in VERO cell line. VERO cells were grown to confluence in milk dilution bottles with a growth medium MEM Glasgow modification (BHK21) amino acids (Hi-Media, India) supplemented with 10 percent heat inactivated fatal bovine serum, 500 IU Penicillin/ml and 0.25 mg Streptomycin/ml. Growth medium with a reduced level of serum (2%) served as a maintenance medium.

Confluent monolayers were infected with 1 ml of sample (50% homogenate in NSS of livers collected from chickens that had died due to HPS was sterilized through membrane filter of 0.45µm porosity and used as samples). After incubation at 37 ºC for one hour to allow virus adsorption, the inoculum was decanted and fresh maintenance medium was added. The cells were incubated at 37 ºC and examined periodically for any cytopathological effects (CPE). For blind passages, cells were harvested 4 days after infection and clear supernatant was inoculated to the confluent monolayer as described above and examined periodically for CPE. Similarly, corresponding healthy cells were also maintained without any infection as controls. Coverslip cultures were also prepared for infection and controls. Coverslips were infected with 0.2 ml of inoculum. Infected and corresponding control coverslips were fixed in acetone and stained with haematoxylene and eosin for microscopic examination and photography.

Immunoperoxidase test (IPT). IPT was carried out as described earlier (ROY et al., 1997). Infected and control coverslip cultures were fixed with acetone. After washing with phosphate buffered saline (PBS) coverslips were divided into three sets – the first set treated with Newcastle disease virus (NDV) antiserum, the second set with Infectious bursal disease virus (IBDV) antiserum and the third set with HPS antiserum for one hour at 37 ºC. Following washing with PBS, all the coverslips were treated with methanol-hydrogen peroxide (97ml:3ml) for 30 minutes at room

temperature. All coverslips were washed in PBS and treated with 1:500 dilution of conjugate (anti-chicken IgG conjugated with HRPO, Sigma, USA) for 40 minutes at 37 ºC. Coverslips were washed thoroughly and treated with 0.05% DAB (diamino benzidine tetrahydrochloride) containing 0.05% fresh hydrogen peroxide. The reaction was halted after 10 minutes by washing in tap water. Coverslips were counter-stained with haematoxylin and observed under a light microscope.

Results and discussion

Geometric mean (GM) AGID titres in five different 50% and 25% liver homogenate samples were 3.2±0.2 $\log_2$ and 2.2±0.2 $\log_2$, respectively, corresponding GM-CIE titre values were 4.0±0.0 $\log_2$ and 2.8±0.2 $\log_2$, respectively. Although diagnosis of the disease is mainly by gross pathological and histopathological observations but with the availability of known antiserum, the disease can be diagnosed by AGID and CIE using 50% liver homogenate since it showed higher titre values. Histopathology is a time-consuming process and hydropericardium may be a condition in several diseases. But with the help of CIE the disease can be diagnosed within one hour. In cell culture, no characteristic CPE could be observed during four blind passages, but on the fifth passage (by about 96 hours) CPE could be observed in infected cultures. CPE were mainly aggregation

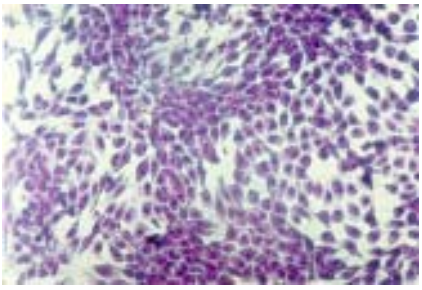

Fig. 1. VERO cells infected with HPS virus. Aggregation and clumping of cells into irregular clusters were seen. H&E; ×400.

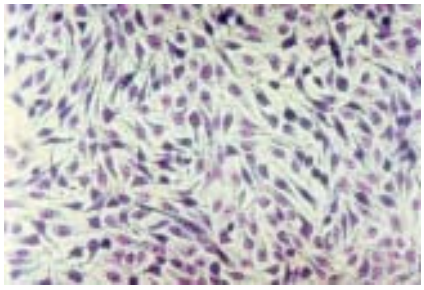

Fig. 2. Uninfected control VERO cells. No abnormal changes. H&E; ×400.

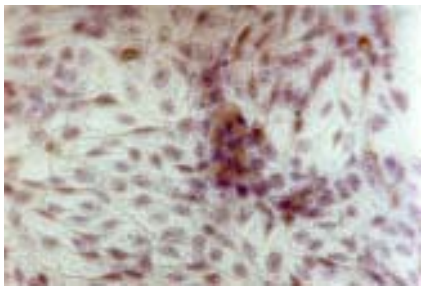

Fig. 3. HPS virus infected VERO cells. IPT positive brown complexes are seen around the nucleus. H&E; ×400.

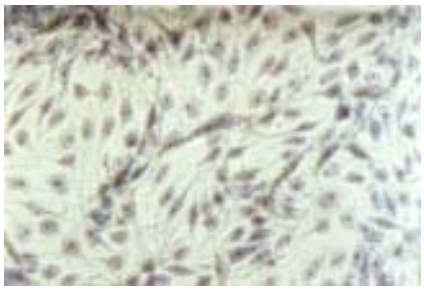

Fig. 4. Uninfected control VERO cells. IPT negative. H&E; ×400.

and clumping of cells resembling clusters of grapes (Fig. 1) but corresponding uninfected controls did not show such changes (Fig. 2). BUXTON and FRASER (1977) described that the CPE of adenovirus may be very characteristic, with rounding and clumping of affected cells into regular clusters resembling "bunches of grapes". Human strains grow best in HeLa, Hep-2 cell lines or rabbit and swine kidney cells. But in the present study the VERO cell line was used because of its easy availability, and the suspected liver material produced CPE which was characteristic of adeno viruses as described above. The present findings are in accordance with reports by CHEEMA et al. (1989) which state that an adeno virus is involved in the production of HPS in broilers.

In IPT, brown complexes were seen around the nucleus (Fig. 3) in infected coverslip cultures treated with HPS antiserum, but such effects was not seen in control coverslip cultures (Fig. 4) or infected coverslip cultures treated with NDV or IBDV antiserum. This clearly indicates that neither NDV nor IBDV were involved in HPS, and that the agent is a separate entity. The availability of reference antiserum is unclear. Although the CPE produced in vero cells resembles adeno virus induced CPE, confirmation of aetiology is in progress.

References

ADBULAZIZ, T. A., S. Y. HANSON (1995): Hydropericardium syndrome in broiler chickens: its contagious nature and pathology. Res. Vet. Sci. 59, 219-221.

AHMED, I. M., AFZAL, M. I. MALIK, Z. HUSSAIN, W. HANIF (1989): Disease patterns and etiology of hydropericardium syndrome (Angora disease) in broiler chickens in Pakistan. Pakistan J. Agril. Res. 10, 195-199.

ANJUM, A. D., M. A. SABRI, Z. IQBAL (1989): Hydropericarditits syndrome in broiler chickens in Pakistan. Vet. Rec. 124, 247-248.

BUXTON, A., G. FRASER (1977): Animal Microbiology, Vol.-II. Blackwell Scientific Publications, Oxford, pp. 725-735.

CHEEMA, A. H., J. AHMED, M. AFZAL (1989): An adenovirus infection of poultry in Pakistan. Epiz. Res. Sci. Tech. (OIE) 8, 789-795.

GOWDA, R. N. S. (1994): Leechi disease – A mysterious and an emerging threat to poultry industry in India. Poult. Guide (Nov/Dec), 33-37.

HARIBABU, Y. (1986): Counter immuno electrophoresis technique in diagnosis of Marek's Disease. Indian J. Poult. Sci. 21, 357-358.

KHAWAJA, D. A., S. AHMED, M. A. RAUF, M. S. ZULFIQUAR, S. M. I. MAHMOOD, M. HUSSAN (1988): Isolation of adenovirus from hydropericardium syndrome in broiler chicks. Pakistan J. Vet. Res. 1, 2-17.

ROY, P., A. T. VENUGOPALAN, B. M. MANOHAR (1997): Demonstration of Newcastle disease virus specific antigen by fluorescent antibody technique and immunoperoxidase test. Indian J. Anim. Sci. 67, 964-966.

ROY, P., A. T. VENUGOPALAN (1997): Agar gel immunodiffusion and counter immuno-electrophoresis for diagnosis of Newcastle Disease. Trop. Ani. Hlth. Prod. 4, 231-234.

ROY, P., A. KOTEESWARAN, R. MANICKAM (1999): Efficacy of an inactivated oil emulsion vaccine against HPS in broilers. Vet. Rec. 145, 458-459.

Received: 31 August 1998
Accepted: 20 April 2001

ROY, P., A. KOTEESWARAN, R. MANICKAM: Serološka, citopatološka i citokemijska istraživanja virusnog hidroperikardijalnog sindroma pilića. Vet. arhiv 71, 97-103, 2001.

SAŽETAK

Hidroperikardijalni sindrom bolest je tovnih pilića kojoj se u posljednje doba pridaje veliko značenje. Kao najprikladnije metode za dijagnostiku te bolesti ističu se geldifuzijski precipitacijski (GDP) test i protusmjerna imunoelektroforeza. Pretragom homogenata jetre inficiranih pilića GDP

testom utvrđen je 3.2±0.2 $\log_2$, a protusmjernom imunoelektroforezom titar 4.0±0.0 $\log_2$. Izdvojeni virus adaptiran je na staničnu liniju VERO nakon četiri slijepe pasaže. Citopatski učinak na stanicama zabilježen je 96 sati nakon infekcije. Proveden je i imunoperoksidazni test na inficiranim stanicama primjenom specifičnog antiseruma čime je dobiven pozitivan nalaz. Negativna je reakcija dobivena upotrebom antiseruma za virus newcastleske i gumborske bolesti. U radu je razmotrena i etiologija bolesti.

Ključne riječi: hidroperikardijalni sindrom, tovni pilići, imunoperoksidazni test, geldifuzijski precipitacijski test, protusmjerna imunoelektroforeza