Pak. j. life soc. sci. (2003), 1(2): 141-143

Pakistan Journal of
Life and Social Sciences

Reverse Passive Haemagglutination (RPHA) Test for the Detection and Quantification of Hydropericardium Syndrome Virus (HPSV)

Manzoor, S. and I.Hussain
Department of Veterinary Microbiology, University of Agriculture, Faisalabad-Pakistan

Abstract

The reverse passive hamagglutination test was performed to detect hydropericardium syndrome virus (HPSV) using 1% suspension of sheep erythrocytes, treated with 1:400 dilution of 0.1M chromium chloride and labelled with a known positive anti-HPS serum. Liver samples of 116 poultry birds were collected including 16 broilers (6 livers were from clinically positive), 50 Desi and 50 commercial layers (adult). A liver homogenate was produced. The presence of antigen in the test liver homogenate was shown by agglutination of sheep erythrocytes sensitized with anti-HPS antiserum. The titres were expressed as the reciprocal of the highest dilution showing agglutination. All of the six clinically positive liver samples gave positive results with titres ranging from 1:32 to 1:128. Of these 6 positive, 3 samples, had 1:32, 2 samples had 1:64 and 1 sample had 1:128 tire. While none of Desi and commercial layer was found to be positive. The results indicate that RPHA is simple and inexpensive test to detect and titrate HPS virus in the infected liver samples. All the HPS virus isolates from HPS infected broiler livers and adenovirus group-I obtained from NARC, Islamabad and VRI, Lahore showed RPHA titre (1: 64) with specific HPS antibody labeled sheep erythrocytes.

Key words: RPHA Test , Hydropericardium syndrome virus, Quantification.

Introduction

Hydropericardium syndrome is an acute infectious disease of chickens characterized by high morbidity and mortality, distension of pericardial sac along with accumulation of transparent jelly-like fluid of white, amber or occasionally green color. Multifocal hepatic necrosis and severe involvement of kidneys is also seen. HPS was most exclusively seen in broilers at the age of 3-6 weeks of age.

Corresponding author: I. Hussain
Department of Veterinary Microbiology, University of Agriculture, Faisalabad -Pakistan
E.Mail: driftikharuaf@hotmail.com

The duration of disease is 10-14 days. Although the specific etiology has yet to be defined, available evidence suggests the condition is caused by a pathogenic group-I adenoviruses. Other agents may serve as potentiators and increase the severity of disease under field conditions (Cowen , 1992).

This disease was first seen in Karachi near Angara Goth in 1987. High mortality and morbidity about 30-60% was recorded (Khawaja et. al., 1988). In India HPS infectious agent was identified in liver homogenates by using antisera raised in laboratory animal by using Counter immunoelectrophoresis and demonstrated a precipitin line in positive cases (Kumar et. al ., 1997) .

Haq *et al.*, 1997 characterized the HPS aget at molecular level. They collected liver samples from 30 commercial broilers during an outbreak of HPS in Faisalabad, Pakistan. HPS agent was purified by sucrose gradient centrifugation and polypeptides of HPS agent were separated through sodium dodecylsulphate-polyacrylamide gel electrophoresis. Eight polypeptides ranging in molecular weights from 15.7 to 119 kDa were observed. The nucleic acid of HPS was extracted and a single DNA band of 23 kbp was determined by agarose gel electrophoresis.

Indirect haemagglutination (IHA) test as a simple procedure for quantitatively measuring antibodies against HPS was described. The etiological agent contained in the liver of HPS affected chicks did not directly haemagglutinate chicken, Guinae pig and rat RBCs . The antibody titre measured through IHA test varied from 1:16 to 1:256 in 28 serum samples taken at 3-4 weeks of outbreak. Considering the need for quantitative measurement of HPS virus, a reverse passive haemagglutination (RPHA) test has been developed. It has given useful results for detection and quantification of HPS virus in test samples (liver homogenates) as well as for adenovirus group-I isolates.

Materials and Methods

Raising of hyper immune HPS antiserum

A commercial HPS vaccine (Sanna Laboratory, Fsd) was purchased from local market and hyper immune antiserum against this vaccine was raised in a laboratory rabbit. Briefly, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0,

and 2.0 ml HPS vaccine was inoculated subcutaneously in the rabbit on alternate days. Ten days after the last injection the rabbit was slaughtered and serum was separated.

Processing of poultry livers

Poultry livers were triturated separately in NS along with antibiotics penicillin and streptomycin, each 1000 µgs/ml and the homogenate was centrifuged at 1500 rpm for 5 minutes. After centrifugation, the liver proteins were precipitated by treating with chloroform @1:2 (chloroform: supernatant). The chloroform supernatant was again centrifuged at 1500 rpm for 5 minutes. The supernatant was used for HPS virus (Rahman et. al., 1997).

Sensitization of sheep Erythrocytes

A 0.75 ml of packed sheep erythrocytes were thoroughly mixed in 1.0 ml of 1:400 dilution of chromium chloride solution (pH 6.8) and 2.0 ml of NS and 2ml of hyper immune antiserum. The mixture was placed at room temperature for 10 minutes. The erythrocytes were then separated by centrifugation and washed thrice with vernal buffer and resuspended to give one percent suspension.

Reverse passive haemagglutination test

A two-fold dilution of each liver homogenate was made in normal saline (0.9% NaCl) in micro-titration plate (U-shaped wells). Fifty microlitres of sheep erythrocytes sensitized with anti-HPS antiserum were dispensed in each well of micro-titration plate. Then the plate was placed at room temperature for 30 minutes was examined; a positive reaction consisted of marked haemagglutination, while a negative reaction showed an evidence of button formation at the bottom of the wells.

Results and Discussion

A total of 116 poultry liver homogenates including 16 broilers (6 livers were clinically positive), 50 Desi and 50 commercial layers, were subjected to reverse passive haemagglutination test. Out of these 116 liver homogenates only 6 clinically positive homogenates showed positive results which had RPHA titres from 1:32 to 1:128. Out of which, 3 had 1:32, 2 had 1:64 and 1 samples had 1:128 titre (Table 1) These results show that HPS occur only in broilers and Desi or commercial layers are not affected with this disease.

Haemagglutination (HA) may be of two types, direct and indirect haemagglutination. In direct HA virus directly agglutinates RBCs to titrate specific antibodies. While in indirect HA virus does not directly agglutinate RBCs but virus has to be mounted on the RBCs and then that virus is used to titrate specific serum. This indirect HA is also called as passive HA for the titration of specific antibodies. Now if titration of virus is required then specific antibodies are mounted on the RBCs and these antibodies coated RBCs are used to titrate the specific virus Indirect HA in which quantification of virus is done and RBCs are coated with specific antibodies (with respect to the virus to be titrated) is called as "Reverse passive HA" (RPHA) test. The passive and reverse passive both are indirect HA tests, the difference is only that former test is used for the quantification of antibodies and latter test is used for the quantification of virus and we have used latter one for the quantification of HPS virus.

Table 1: Prevalence of HPS in Domestic Poultry

Poultry Birds	Total Livers	Reverse Passive Haemagglutination Test (RPHA)							
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
Broilers (3-6wks)	10+6* = 16	-	-	-	-	3	2	1	-
Desi	50	-	-	-	-	-	-	-	-
Layers (Adults)	50	-	-	-	-	-	-	-	-

* Clinical positive cases.

Table 2: Control Positive of RPHA Test

HPS VIRUS	1:2	1:4	1:8	1:16	1:32	1:64
Adenovirus group-I (NARC*)	+	+	+	+	+	+
Adenovirus Group-I (VRI**)	+	+	+	+	+	-

* National Agriculture Research Council, Islamabad.

** Veterinary Research Institute, Lahore.

In indirect HA test (whether passive or reverse passive), some coupling agent is to be used. Rajeswar et al; (1999), used glutaraldehyde as coupling agent for detection of infectious bronchitis virus, Jamil et al; (1993) used tannic acid as well as glutraldehyde as coupling agent for detection of Foot & Mouth disease virus, while in our study we have used chromium chloride as coupling agent successfully. Adenoviruses group-I were arranged from NARC, Islamabad and VRI, Lahore (Table 2). These viruses were titrated with RPHA test as control positive and their respective titres were 1:64 and 1:32. The virus in the clinically positive broiler livers was also detected by two standard procedures e.g. Agar gel precipitation test (AGPT) and counter current immunoelectrophoresis (CIE) test. The liver samples were also found positive with these tests. Further, it was also observed that reverse passive haemagglutination test is a rapid and simple method for quantitative measuring Angara disease agent (HPS-adeno virus) in livers of the affected birds.

References

Khawaja, D.A., Shabbir, A., Rauf, A. M., Muhammad, Z., Iqbal, S. M. and Mehmood-ul-Hassan, Isolation of Adenovirus from Hydropericardium syndrom in Broiler chicks. Pak. J. Vet. Res., 1988. 1:2-7.

Cowen, B.S. Inclusion body hepatitis aneamia and hydropericardium syndrom: Aetiology and control. World Poult. Sci. J. 1992. 48: 247-254.

Rahman, S.U., Ashfaque, M., Anjum, A. D. and Sindhu, T. A. Indirect haemagglutination test for detecting Angara disease (Hydropericardium syndrome) agent antibodies. Pak. J. Liv. Stock and Poult., 1997. 3(4): 176-178.

Rajeswar, J.J. and Dorairajan, N. Reverse passive haemagglutination of infectious bronchitis virus. Ind. Vet. J. 1999. 76(3):191-192.

Kumar, R., Chandra, R., Shukla, S. K., Agrawal, D. K. and Kamar, M. Hydropericardium syndrome (HPS) in India: A preliminary study on the causative agent and control of the disease by inactivated vaccine. Trop. Anim. Hlth. Prod; 1997. 29(3): 158-164.

Haq , I., Hussain, I. and Anjum, A.A. Polypeptides and Nucleic acid identification of hydropericardium syndrome agent. Pak. Vet. J. 1997. 19 (1):21-23.

Jamil. H., Ashfaque, M., Afzal, H. and Lodhi, L.A. Indirect haemagglutination test for assaying antibodies against FMD virus types O and Asia – I using glutraldehyde and Tannic acid as coupling agent. Pak. Vet. J. 1993. 13 (3): 128-132.