POLYPEPTIDES AND NUCLEIC ACID IDENTIFICATION OF HYDROPERICARDIUM SYNDROME AGENT

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ABSTRACT

Hydropericardium syndrome (HPS) agent was purified by sucrose gradient centrifugation. The polypeptides of HPS agent were separated through sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and identified by known protein markers. Eight polypeptides having molecular weights ranging from 119.0 to 15.7 KDa were observed. The nucleic acid of HPS agent was extracted and determined through agarose gel electrophoresis, compared with Hind III λ DNA marker. A single DNA band of 23 KDa was observed.

INTRODUCTION

Hydropericardium syndrome (HPS) or Angara disease has caused huge economic losses to the broiler poultry in Pakistan since September 1987, when it was first reported at Angara Goth, an exclusively broiler growing area in Karachi (Jaffery, 1988). The disease is mainly seen in broiler and broiler breeders (Ahmad *et al.*, 1989). Initially, it was believed that HPS was a nutritional disorder (Qureshi, 1989). Reproduction of the disease by inoculation of liver homogenate proved that disease was infectious in nature (Anjum, 1988). Several studies have been performed on aetiology of HPS agent (Khawaja *et al.*,1988; Afzal *et al.*, 1991). The present study was designated for identification of polypeptides and nucleic acid determination of HPS agent.

MATERIALS AND METHODS

Isolation and identification of HPS agent.

Thirty liver samples were collected from commercial broilers suffering from hydropericardium syndrome in field outbreaks of the disease in and around Faisalabad. Liver samples were homogenized, centrifuged at 1200 g for five minutes and supernatant was collected. Supernatant was sonicated by placing in an ice bucket for 30 seconds by giving two strokes. The supernatant was treated with chloroform at 1:2 ratio to remove the tissue debris and centrifuged at 1200 g for five minutes. A clear supernatant was obtained and the presence of HPS agent was checked through Indirect haemagglutination (IHA) test described by Rehman *et al.* (1989).

Purification and confirmation of HPS agent (Nagy and Lominiczi,1984).

Sucrose gradient centrifugation technique was adopted for purification of HPS agent. A gradient was prepared by pouring 6 ml of 30% sucrose solution and keeping at -20°C for two hours, later 4 ml of 20% sucrose solution was layered, and kept at -20°C for one hour. Then 10 ml of previously cleared fluid was poured and centrifuged at 27,000g for 3 hours at 4°C by using JA-20 rotor (Courtesy NIBGE, Faisalabad). A pellet was obtained which was dissolved in NTE (100mM NaCl, 20M Tris-HCl, 2mM EDTA, pH 6.8) buffer. Above process was again repeated and the pellet was preserved at -70 °C for further use.

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE).

Vertical electrophoretic system with discontinuous buffer described by Laemmli (1970) was adopted for segregation of polypeptides of HPS agent. Stacking gel (3.5%) and separating gel (12.5%) were poured between the vertical glass slabs. Polypeptides from purified HPS agent field isolate were dissociated by heating for two minutes in boiling water in the presence of bromophenol blue and 2X sample buffer. For protein markers of known molecular weights 1X sample buffer was used. Electrophoresis was performed by constant current supply of 100 volts for 8 hours. Gels were stained with Coomassie brilliant blue and photographed. Molecular weights of polypeptides of HPS agent were calculated from a standard curve drawn between Rf

values and log molecular weights of protein markers.

Nucleic acid extraction and identification (Maniatis *et al.*, 1982).

Preserved pelleted samples were dissolved in NTE buffer and mixed gently. An amount of 33 µl of pelleted preparation was dissolved in 3 µl of 5% SDS. Along with the samples Hind III DNA marker was also used for identification of nucleic acid. Pelleted samples were placed in water bath for 10 minutes at 65 °C and subjected to agarose gel electrophoresis.

Agarose gel electrophoresis

An amount of 1.2 gms of agarose was dissolved in 100 ml of TBE (0.0087 M Tris-HCl, 0.0089 M boric acid, 0.002 M EDTA) buffer and 5.0 µl ethidium bromide stain was added to make 1.2 % agarose gel. The samples having 5.0 µl of bromophenol blue (0.2 %) were loaded and electrophoresis was carried out at 70 volts electric current for two hours. Gels were visualized under UV light and photographed.

RESULTS

Eight polypeptides of different molecular weights were observed by comparing with protein markers of known molecular weights of 132, 66, 45, 29, 19 and 14.3 KDa. Results of HPS agent isolates electrophoresed along with markers are presented in Fig. 1 shows that eight polypeptides of molecular weights 119.0, 88.7, 66.6, 57.0, 39.6, 18.8, 17.0 and 15.7 KDa were present in lanes 1-4 i.e. field isolates of HPS agent. Whereas in the preliminary trials used for the nucleic acid typing a prominent band with molecular weight of 23 KDa was encountered in the gel (Fig. 2). In addition, a known discripted drag band was also visible at the base of the lane 1 in the gel.

DISCUSSION

SDS-PAGE applied to the pelleted materials procured on sucrose gradient cushions revealed comparable results. The study revealed eight polypeptide bands in the field isolates of the HPS agent. The simultaneous run of marker proteins with known molecular weights indicated that these polypeptides were of molecular weights ranging from 15.7 to 119.0 KDa as moving towards anode.In comparison to these molecular weights Laver *et al.* (1971) reported that an adenovirus has eight polypeptides in which four polypeptides were internal proteins, two of them were pentone base and fiber, one hexose and last one was unknown. The present study was also conducted for the nucleic acid determination. A band of 23.0 KDa was revealed on agarose gel but

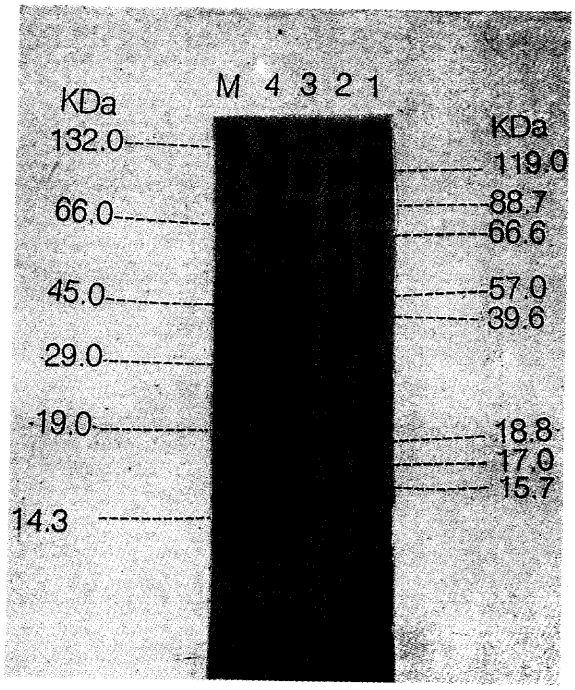

Fig.1 Polypeptide map of a filed isolate of hydropericardium syndrome agent. Lanes 1 through 4 contained filed isolates. Lane M contains protein markers of known molecular weight.

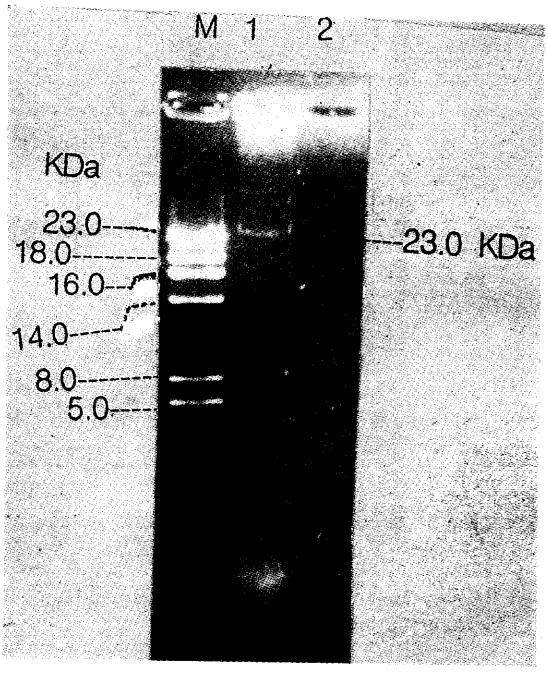

Fig.2 Nucleic acid characterization of hydropericardium syndrome (HPS) agent. Lane M contains Hind III digested lambda DNA marker. Lane 1 indicates nucleic acid of field isolate, lane 2 represents normal liver sample.

an other hazy band of low molecular weight was also present which could be RNA, some impurities of RNA or any other agent as suspected by Afzal *et al.* (1991).

Our results indicate that HPS is caused by a agent that has eight polypeptides and DNA of 23 KDa. These characteristics are comparable for avian adenovirus as reported by Green *et al.* (1967). However, in our studies, one low molecular weight band is yet to be characterized.

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