LETTER TO THE EDITOR

PURIFICATION OF EGG DROP SYNDROME-76 VIRUS BY VELOCITY DENSITY GRADIENT CENTRIFUGATION. A COMPARATIVE STUDY

P. SWAIN[1], J.M. KATARIA[2], K. DHAMA[1], K.C. VERMA[2]

[1]Division of Virology, Indian Veterinary Research Institute, Mukteswar, Nainital, Uttar Pradesh 263138; [2]Divison of Avian Diseases, Indian Veterinary Research Institute, Izatnagar, India

Received July 3, 1997

EDS-76 virus (family *Adenoviridae,* genus *Aviadenovirus*) has been purified and characterized by several workers using cesium chloride (*1,2,3*) and potassium tartarate density gradient centrifugation (*4*). The virus purification using cesium chloride causes a breakdown of virions and separation of haemagglutinin from them resulting in loss and lowering of haemagglutination (HA) and infectivity activities, respectively, which makes it difficult to assay the virus and the assay is time consuming. In this study we report the effects of cesium chloride, sodium potassium tartarate and sucrose (Sigma) as media in velocity density gradient centrifugation on the purity and biological activities of the EDS-76 virus.

The EDS-76 virus used in our study was obtained from Viral Diseases Laboratory of Division of Avian Diseases, Indian Veterinary Research Institute, Izatnagar. The virus was initially isolated from Japanese quail flock experiencing severe drop in egg production (*5*). The virus was grown in 12- to 14-day-old embryonated duck eggs and the allantoic fluid was harvested after 5 days of infection. The allantoic fluid having HA activity and infectivity of 2^{12} HAU/ml and $10^{8.5}$ EID_{50}/ml, respectively, was used in this study. It was clarified at 10,000 x g for 30 mins at 4°C and concentrated using 6% polyethylene glycol (MW 6,000). The concentrated virus stock (2^{16} HAU/ml) was purified by velocity centrifugation in continuous density gradients of cesium chloride, sodium potassium tartarate and sucrose at the density of 1.05 to 1.45 g/ml. The virus material after loading on the gradients was centrifuged at 100,000 x g for 2.5 hrs at 4°C. Visible virus bands were collected and dialysed against saline and checked for their purity, infectivity and HA activity (*4*). The purity was checked by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE, (*2*)).

Details of the results are given in the table. Two bands were obtained at the density of 1.30 (band 1) and 1.34 g/ml (band 2) with cesium chloride, whereas only a single band was obtained with sodium potassium tartarate and sucrose at densities of 1.30 and 1.32 g/ml, respectively. The band obtained with sodium potassium tartarate had highest infectivity and HA activity. Sodium potassium tartarate was followed in this respect by sucrose and cesium chloride. In case of cesium chloride the heavier band at density 1.34 g/ml (band 2) had poor HA activity but higher infectivity than that of the band 1 which showed higher HA activity and very poor infectivity. This indicated a fragile nature of virus haemagglutinin and corresponded to the earlier findings by other authors (*2,3*). In case of the supernatants, a higher HA activity was obtained with cesium chloride followed by sucrose and sodium potassium tartarate. This indicated a less harmful effect of sodium potassium tartarate on the virus particle as compared to cesium chloride. By SDS-PAGE, 13 polypeptides of M_r of 10 – 126 K were recorded in the bands in case of cesium chloride and sodium

Abbreviations: HA = haemagglutination; SDS-PAGE = polyacrylamide gel electrophoresis in presence of sodium dodecyl sulphate

Density gradient	No. of bands in SDS-PAGE	Density of bands (g/ml)		HA activity (log_2 HAU/ml)			Infectivity (EID_{50}/ml)		No. of polypeptides
		Band 1	Band 2	Band 1	Band 2	Supernatant	Band 1	Band 2	
Cesium chloride	2	1.30	1.34	11	7	12	4.5	11.5	13
Sodium potassium tartarate	1	1.30	–	18	–	4	14.5	–	13
Sucrose	1	1.32	–	15	–	8	12.5	–	15

was obtained with sodium potassium tartarate and sucrose at densities of 1.30 and 1.32 g/ml, respectively. The band obtained with sodium potassium tartarate had highest infectivity and HA activity. Sodium potassium tartarate was followed in this respect by sucrose and cesium chloride. In case of cesium chloride the heavier band at density 1.34 g/ml (band 2) had poor HA activity but higher infectivity than that of the band 1 which showed higher HA activity and very poor infectivity. This indicated a fragile nature of virus haemagglutinin and corresponded to the earlier findings by other authors (*2,3*). In case of the supernatants, a higher HA activity was obtained with cesium chloride followed by sucrose and sodium potassium tartarate. This indicated a less harmful effect of sodium potassium tartarate on the virus particle as compared to cesium chloride. By SDS-PAGE, 13 polypeptides of M_r of 10 – 126 K were recorded in the bands in case of cesium chloride and sodium potassium tartarate. Fifteen polypeptides detected in case of sucrose might be due to presence of some proteins of duck allantoic fluid.

We can conclude that the effect of sodium potassium tartarate on the purity and activity of the virus was more moderate as compared to those of sucrose and cesium chloride. Moreover, the purification procedure used is time saving and the virus material obtained in this way with sodium potassium tartarate retains its biological activities. Therefore, it can be recommended for further biochemical and molecular studies of the EDS-76 virus and other avian adenoviruses too.

References

1. Takai S, Higashihara M, Matumoto M, *Arch. Virol.* **80**, 59–67, 1984.

2. Todd D, MCNulty MS, *J. Gen. Virol.* **40**, 63–75, 1978.

3. Zsak L, Kisary J, *Arch. Virol.* **68**, 211–219, 1981.

4. Swain P, Kataria JM, Verma KC, Kumar S, *Indian J. Virol.* **8**, 8–14, 1992.

5. Kataria JM, Swain P, Dash BB, Verma KC., *12th Annual Conference of Indian Association of Veterinary Microbiologists, Immunologists and Specialists in Infectious Diseases.* Tirupati, Andhra Pradesh, India, September 12-14, 1991.