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## ISOLATION OF EGG DROP SYNDROME VIRUS AND ITS MOLECULAR CHARACTERIZATION USING SODIUM DODECYL SULPHATE POLYACRYLAMIDE GEL ELECTROPHORESIS

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### ABSTRACT

Six isolates of egg drop syndrome (EDS) virus were recovered from five different outbreaks of EDS in commercial laying hens in and around Faisalabad. The aberrant eggs were fed to the susceptible laying hens for experimental induction of infection. The samples from infected birds (egg washing, cloacal swabs, oviducts and spleens) were collected, processed and inoculated into 11-day old duck embryos. The presence of virus in harvested allanto-amniotic fluid was monitored by spot and microhaemagglutination tests and confirmed by haemagglutination inhibition and agar gel precipitation tests. The EDS virus grew well in duck embryos and agglutinated only avian but not mammalian red blood cells. These isolates were purified through velocity density gradient centrifugation. Protein concentration was determined through Lowry method and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was conducted by loading 300 µg protein concentration on 12.5% gel using discontinuous buffer system. All the six isolates showed 13 polypeptides, which were identical to those described in the referral EDS-76 virus (strain-127). The molecular weights of the polypeptides ranged from 6.5 KDa to 126 KDa.

**Key words:** Egg drop syndrome, haemagglutination, sodium dodecyl sulphate polyacrylamide gel electrophoresis.

### INTRODUCTION

Livestock play an important role in the economy of Pakistan contributing about 11.4% to the total GDP. Poultry has emerged as a good substitute of beef and mutton. In Pakistan, there are about 22.1 million layers producing 8,247 million eggs annually (Economic Survey of Pakistan, 2003-04). Despite the efforts at improved management methods, feed formulation and disease preventive measures adopted in the public and private sector, overall poultry population remained susceptible to a number of infectious and non-infectious diseases. Among the infectious diseases, egg drop syndrome (EDS) is causing a serious threat to layer industry.

Since its initial description, egg drop syndrome 1976 (EDS-76) has become a major cause of loss of egg production throughout the world (Van-Eck *et al.*, 1976). EDS virus has close phylogenetic relationship with bovine adenovirus serotype 7 (BAV-7) and ovine adenovirus strain 287 (OAV-287) (Harrach *et al.*, 1997). Previously, EDS virus was classified under the genus *Aviadenovirus*. Now this virus is classified under the genus *Atadenovirus* along with BAV-7 and OAV 287 (Hess *et al.*, 1997; Regenmortel *et al.*, 2000). It is a nonenveloped, haemagglutinating DNA virus, 74-80 nm in diameter and replicates in the nucleus of the host

cells (Jordan, 1990). The infected birds lay soft-shelled, or shell-less, discoloured and miss-shaped eggs without any change in internal quality. In acute cases there may be mild depression, however, feeding, watering and general appearance of the affected birds remain normal (Yamaguchi *et al.*, 1980).

The information regarding type of samples from infected birds for virus isolation, haemagglutination (HA) potential, cultural and antigenic characters of the virus is limited. Similarly, the information about the antigenic variation among various field isolates of EDS virus has not been delineated in Pakistan. This study was carried out to isolate EDS virus from aberrant eggs laid by infected laying commercial birds and to characterize it using SDS-PAGE to confirm the molecular nature of local isolates and to find any antigenic variation among different field isolates.

### MATERIALS AND METHODS

#### Isolation of virus

#### Induction of experimental infection

Aberrant eggs were collected from five EDS infected commercial layer flocks located in and around Faisalabad city. Ten eggs from each source were broken and mixed with 2 Kg of feed separately.

Eighteen adult layers (White Leghorn), which were not previously vaccinated against EDS, were divided into six groups, having three birds in each group. The infected feed from each source was offered to the hens of first five groups (2 Kg to each group) for six consecutive days. The normal feed was given to the birds of 6<sup>th</sup> group.

#### **Collection and processing of samples**

First three abnormal eggs laid by the EDS infected hens were collected and washed in tryptose broth. Cloacal swabs were collected on day 8-10 post-feeding and also placed in tryptose broth. The birds were sacrificed on day 15 post-feeding. Oviduct and spleen were collected, weighed, mixed with equal volume of tryptose broth, ground and centrifuged at 400 x g for 10 minutes. The supernatant was collected and antibiotics (Gentamycin @ 40 µg/ml) and antifungal (Nystatin @ 30 IU/ml) were added in each sample.

#### **Embryo inoculation**

A 0.2 ml of each processed sample was inoculated through allantoic cavity route in duplicate in 11-day-old embryonated duck eggs (Senne, 1989). The inoculated embryos were sealed, incubated at 37°C and allanto-amniotic fluid (AAF) was harvested 144 hours post-inoculation (Buxton and Fraser, 1977).

#### **Identification and confirmation of virus**

Presence of EDS virus in harvested AAF was initially determined by spot haemagglutination and then by microhaemagglutination tests (Allan *et al.*, 1978). Microhaemagglutination test was also performed by using the erythrocytes from buffalo, horse, goat and rabbit. The hyperimmune serum against EDS virus was raised in rabbits using imported oil based EDS virus vaccine (Izovac, EDS). The known hyperimmune serum was then used to perform haemagglutination inhibition and agar gel precipitation tests for the confirmation of the virus (Buxton and Fraser, 1977; Sulochana and Lalithakunjam, 1991).

#### **Molecular characterization**

##### **Purification**

Identified EDS virus isolates were purified through sucrose velocity density gradient centrifugation technique, as described by Swain *et al.* (1997). For this purpose, 20 and 30% sucrose solutions (W/V) were used. The virus material was centrifuged at 100,000 x g for 2.5 hours at 4°C in ultra-centrifuge machine (Hitachi Himac) with swinging drive rotor. The purified bands of virus were collected separately, dialyzed against normal saline and stored at -20°C.

##### **Determination of protein concentration**

Protein contained in each purified EDS virus isolate was determined using the Lowry method (Lowry

*et al.*, 1951). Bovine serum albumin (BSA) was used for standard assay method.

#### **SDS-polyacrylamide gel electrophoresis**

Discontinuous buffer system was used for the separation of EDS virus proteins, as described by Laemmli (1970). A 300 µg protein concentration of each virus isolate was loaded on 12.5% gel along with a protein marker. Electrophoresis was carried out at room temperature, using 200 volt current for 6 hours. Gel was stained with coomassie brilliant blue stain for overnight, then destained for 3 hours in destaining solution, dried in gel dryer under vacuum (Rapidry, Japan) and photographed through a digital camera. The molecular weights of different polypeptides were determined with the help of known protein marker on a computer software Kodak electrophoresis documentation and analysis system (KEDAS-290).

## **RESULTS AND DISCUSSION**

After feeding the aberrant eggs (soft-shelled, shell-less, miss-shaped) to experimental birds, half of them started laying abnormal eggs on day 8 post-feeding, while the others laid normal eggs. Six isolates were recovered (three from oviducts, one each from egg washing, cloacal swab and spleen). The isolated virus grew well on 11-day old embryonated duck eggs and produced high virus titer. The infected duck embryos were displaying blood vessels and were alive on 144 hours post-inoculation. Similar results have also been reported by Bartha (1984).

The haemagglutination titer of AAF harvested from the infected embryos ranged from 8192 to 32768. The maximum virus was recovered from oviduct samples (32768), which is the main predilection site for the replication of EDS virus. The virus titer was 8192 in spleen and 16384 in egg washings and cloacal swab. EDS virus replicates in duck and geese embryos producing high virus titer has also been reported by Zsak *et al.* (1982) and Bartha (1984).

The EDS virus agglutinated only chicken erythrocytes but did not agglutinate the mammalian (buffalo, horse, goat and rabbit) erythrocytes. This feature was in agreement with Jordan (1990). It was also observed that EDS virus mediated haemagglutination did not show elution on 24 hours post-incubation at 25°C. This property might be due to the lack of neuraminidase molecule on the virus surface, which is responsible for elution (Muhammad *et al.*, 1999)

Positive results of haemagglutination Inhibition and Agar Gel Precipitation tests (AGPT) using known hyperimmune serum against vaccinal strain of EDS virus raised in rabbits further confirmed the virus. Identified EDS virus isolates were purified through velocity density gradient centrifugation. The 30 and 20% sucrose solutions were used and virus material was centrifuged at 100,000 x g for 2.5 hours at 4 °C. A clear

band of purified virus was obtained at a density of 1.32g/ml. Similar method was also used by Swain *et al.* (1997) for purification of EDS virus. The values of protein concentration ranged from 11.66 to 18.17 µg/µl. A 300 µg protein concentration of each sample was loaded on 12.5% gel.

The protein analysis by SDS-PAGE of six-haemagglutinating virus isolates revealed 13 polypeptides, and their molecular weights ranged from 6.5 to 126 KDa (Fig. 1). This polypeptide pattern was similar to that of standard strain of EDS virus (strain-127). These results are in agreement with those of Chandramohan *et al.* (1998). They recovered five virus isolates from different parts of Tamil Nadu and characterized them through SDS-PAGE. They also reported 13 polypeptides ranging between 11 to 126 KDa. However, Swain *et al.* (1992) reported 12 polypeptides when they screened their local isolates by SDS-PAGE.

It was concluded that normal and abnormal eggs from EDS infected birds contained EDS virus that can induce disease in susceptible chickens. EDS virus from cloacal swabs, oviducts and spleens of experimentally infected birds may be recovered in duck embryos. The virus can be identified by its stable and differential haemagglutination properties and confirmed through HI and AGPT. Results of SDS-PAGE revealed that six EDS virus isolates were identical to each other in terms of number of proteins regardless of their source. These isolates were also comparable to those of referral EDS-

76 virus (strain-127). More work on immunogenic polypeptides of local isolates is needed to be done in the future in other parts of the country to find any variation at molecular level for the implementation of better disease control programme in the country.

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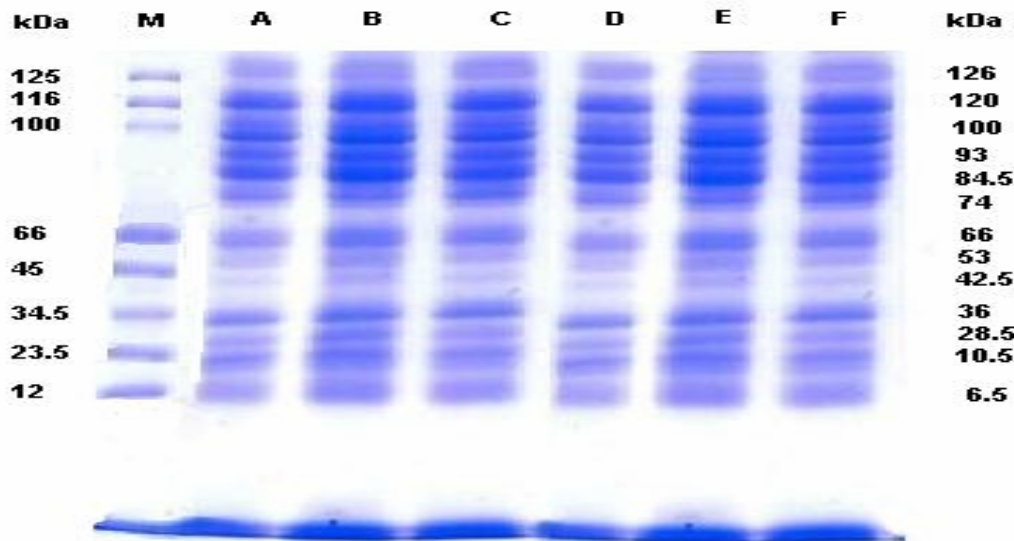


Fig. 1: Polypeptide pattern and molecular weights of six isolates of EDS virus separated by SDS-PAGE. M =Marker A =Oviduct B =Oviduct C =Egg washing D =Oviduct E =Cloacal swab F =Spleen

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