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Isolation and Characterisation of Egg Syndrome Virus

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ABSTRACT

Two strains (PAK-CVS-1 & PAK-CVS-2) of Egg Drop Syndrome virus were isolated from infected commercial laying chickens. Aberrant eggs from the infected commercial laying flocks were mixed with feed. The infected feed was fed to the susceptible laying chickens which incepted laying abnormal eggs on eight days post-feeding. Only cloacal swabs and egg shell pouch area of oviduct of the infected chickens yielded the virus in duck embryos. The isolated virus strains grew well in nine days old duck embryos, agglutinated only avian but not mammalian erythrocytes and induced infection in the susceptible laying chickens without any obvious signs. Egg drop syndrome virus specific rabbit antiserum inhibited the haemagglutination potential of the virus isolates.

Key words: Egg Drop Syndrome; Duck Embryo; Haemagglutination

INTRODUCTION

Egg Drop Syndrome (EDS) virus is a common problem in laying birds, causing reduced egg production (Van Eck *et al.*, 1976; Yamaguchi *et al.*, 1980; Brugh *et al.*, 1983). The infected birds lay soft shelled, or shell-less, discoloured and mis-shaped eggs without any change in the internal quality. In acute cases, there may be mild depression ; however, feeding, watering and general appearance of the affected birds remain normal (Van Eck *et al.*, 1976; Yamaguchi *et al.*, 1980). The causative agent is a non-enveloped and haemagglutinating virus belonging to adenoviridae (Jordan, 1990). The disease is reproducible in healthy non-vaccinated laying birds which start laying soft shelled eggs from day 8-10 post-feeding of the infected eggs (Brugh *et al.*, 1983). The antibodies against EDS are passed from hen to the chicks through yolk and are detectable in their sera (Naeem, 1994).

The information regarding type of samples from infected birds for virus isolation, haemagglutination (HA) potential, cultural and antigenic characters of the virus is limited. This study was carried out to isolate and characterise EDS virus from the aberrant eggs laid by infected laying commercial birds.

MATERIALS AND METHODS

Infected eggs. Aberrant eggs were collected from two infected commercial flocks i.e. one from Faisalabad district (PAK-CVS-1 strain) and the other from Lahore district (PAK-CVS-2 strain). Five eggs from each source were broken and mixed with 1 kg feed separately. The infected feed was offered to a group of three birds for three consecutive days for induction of experimental infection. All the birds were free from EDS-specific haemagglutination inhibiting antibodies. First three abnormal eggs laid by the infected birds were collected. Cloacal swabs were collected from each bird on day 8-10 post feeding and placed directly in the tryptose broth (Difco). Oviduct and spleen of each bird were also collected from the sacrificed birds on day 15 post feeding.

Preparation of samples. Each of the infected samples i.e. oviducts and spleens were mixed with equal volume of the sterilised tryptose broth, ground and centrifuged (400 g for 10 minutes). The supernatant of each sample was collected. The cloacal swabs were suspended in 5 ml of the tryptose broth. The eggs were washed in 10 ml of the tryptose broth. The antibiotics were added (gentamycin 40 µg/ml; 30 units of nystatin/ml) to each sample. The mixture was left undisturbed for 30 minutes at 37°C.

Duck embryos. Twenty duck embryos (nine days old) were marked and divided into five equal groups. Each sample (0.2 ml) was inoculated through allantoic cavity in each of the four embryos of each group (Bartha, 1984) while the embryos of 5th group served as control. The embryos were incubated for 120 hours and allantoic-amniotic fluid (AAF) was harvested. HA activity of the AAF against chicken erythrocytes was determined. The AAF was reinoculated in each of the four duck embryos (nine days old) and incubated at 37°C for 96 hours. The AAF of each of these embryos was pooled. The HA potential of each of the pooled AAF against mammalian and avian erythrocytes was determined (McFerran *et al.*, 1978).

Characterisation of the virus. The imported oil-based EDS vaccine (Izovac EDS) was inoculated in five rabbits (0.3 ml/bird subcutaneously in lumber

region). The blood samples were collected on 28 days post-injection and the sera were separated and titrated in 96 well round bottom immunoplate using 4 HA of the AAF (Allan & Gough, 1974).

RESULTS AND DISCUSSION

The eggs collected from both the outbreaks of reduced egg production were soft shell, mis-shaped, thin shell but showed normal albumin quality. Two of the experimental birds fed on feed mixed with Faisalabad source of eggs started laying soft shelled eggs on day 8 post feeding. None of the birds fed on Lahore source of the eggs laid aberrant eggs, but the virus was detectable in the cloacal swabs. The eggs laid by experimental birds were shell-less, soft shell followed by thin shell. These eggs fell through the holes of the cages wire on the ground and were soiled with dirt and dust in contrast to normal eggs which did not pass through the holes of cage wire. A mucoid discharge was sticking to the laid abnormal eggs. The infected birds were apparently healthy and were having normal feeding. The disease introduction to susceptible layers ingesting infected eggs from the naturally or experimentally infected birds has been reported previously (McCrocken & McFerran, 1978; Smyth & Adair, 1979; Brugh *et al.*, 1983).

The isolated virus strains replicated in the duck embryos. The infected duck embryos were displaying blood vessels and were alive on 144 hours post inoculation. Similar results have been reported by Bartha (1984). HA titre of AAF harvested from the infected embryos was 1:8192. EDS virus replicates in duck and geese embryos producing high virus titre (McFerran *et al.*, 1978; Adair *et al.*, 1979; Zsak *et al.*, 1982; Bartha, 1984). Isolation of EDS virus was possible from the cloacal swabs and shell-gland pouch area of oviduct of the infected birds. Using duck embryo inoculation technique, the virus was not recovered from spleen, normal or abnormal eggs laid by the experimentally infected birds. However, McFerran *et al.* (1978) succeeded in isolating EDS from mucosae of nasopharynx, spleen, oviduct, uterine discharge, and infected eggs. The cultivated EDS virus strains agglutinated chicken, sparrow, parrot and pigeon erythrocytes but did not agglutinate the horse, buffalo and rabbit erythrocytes. This feature was in agreement with Jordan (1990). The HA potential of the isolated virus was inhibited by rabbit antibodies to EDS virus vaccine while HA potential of the ND virus was not inhibited by these antibodies. This feature indicated that the isolated virus strains were EDS virus. The EDS virus isolates through out the world are antigenically indistinguishable (McFerran *et al.*, 1978).

CONCLUSIONS

It is concluded that normal or abnormal eggs from the infected birds contained EDS virus that can induce disease in susceptible chickens. EDS virus from cloacal swabs or oviduct of experimentally infected birds may be recovered in the duck embryos. The virus replicates to high titre, agglutinate avian but not mammalian erythrocytes. The results unveiled the prospects of preparation of EDS antigen (diagnostic reagent) and oil-based EDS vaccine from the local strains.

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