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ORIGINAL RESEARCH

# Incidence of egg drop syndrome – 1976 in Namakkal district, Tamil Nadu, India

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## Abstract

**Aim:** To know the magnitude of influence by Egg Drop Syndrome – 1976 (EDS –'76) virus infection in causing drop in egg production in and around Namakkal.

**Materials and Methods:** A total of 150 cloacal swabs and 15 pouch shell glands (uteri) homogenates from 15 poultry farms in and around Namakkal area were used for virus isolation. Three numbers of 10 –day- old embryonated duck eggs were used for the inoculation of each suspected material for virus isolation. The isolate was identified by HA property, by specific inhibition of HA and by AGPT using hyperimmune serum raised against reference EDS –'76 virus strain 127.

**Results:** Out of samples from 15 farms only one isolate (6.6%) was obtained from poultry farm No.5.

**Conclusion:** The results of the present study revealed that the EDS –'76 virus influence in causing drop in egg production in this area to be minimal.

**Key words:** cloacal swab, duck embryo, egg drop syndrome, identification, isolation

## Introduction

Egg drop syndrome – 1976 (EDS –'76) is a major cause for loss of egg production upto 40 per cent and laying of thin shelled and shell less eggs by apparently healthy birds. The syndrome is caused by an adenovirus with transmission occurring vertically and horizontally. It has been reported to affect a wide range of birds including turkeys and layers and is a major constraint to the profitability of egg production in both commercial and village laying birds [1]. It was first reported in Netherlands [2] and has since been reported from numerous countries world-wide including goslings in Hungary [3]. Serological evidence of EDS'76 virus in turkeys [4] and free-range chickens is also observed as a concern in Nigeria [5,6,7]. In India the disease was reported in the year 1984 [8]. The drop in egg production in layer birds has become a major concern in India [9] and particularly at Namakkal due to the enormous economic burden faced by the farmers. The loss incurred by poultry industry due to reduced productivity, culling and cost of medicine is considered to be often greater than loss due to mortality. Earlier workers studied the multiple etiology of "Egg drop syndrome" in this area. But the magnitude of influence by EDS –'76 virus alone has not been studied. Hence a comprehensive research study was formulated to assess the incidence of EDS –'76 virus infection by Isolation and identification of the virus from commercial layer farms at Namakkal.



## Materials and Methods

Samples: A total of 150 cloacal swabs and 15 pouch shell glands (uteri) [10] homogenates from 15 poultry farms were collected in Hank's balanced salt solution (HBSS) with antibiotics from apparently healthy birds showing sudden drop in egg production in Namakkal area. The samples were transported at 4°C to the laboratory for further work.

Ethical approval: The permission was accorded by the Institutional Animal Ethics Committee to collect the sample from live birds.

Embryonated duck Eggs: Duck eggs were purchased from the local farmers, cleaned with anti septic lotion (1% savlon) and incubated at 37°C with proper turning. Fertile eggs were selected on 10$^{th}$ day and the viral samples were inoculated in ten- day- old embryos through allantoic cavity route.

Processing of cloacal swabs: The cloacal swabs [11] were collected in HBSS directly from live birds manifesting the sign of drop in egg production. The collected samples were homogenized and centrifuged at 2500 rpm for 15 minutes at 4°C. The supernatant was collected and filtered through a disposable syringe filter of pore size 0.2m.

Inoculation into duck embryo: Three numbers of 10 –day- old embryonated duck eggs were used for the

inoculation of each suspected material for virus isolation. The eggs were inoculated with 0.2 ml of inoculum via allantoic cavity route, sealed and incubated at 37°C. The eggs were candled twice daily and deaths within 24 h were considered non-specific and were discarded. On the fifth day post inoculation, the eggs were chilled overnight at 4°C and opened to collect the allantoic fluid. The harvested allantoic fluid was clarified at 2500 rpm for 20 min and screened for haemagglutination (HA) activity. A minimum of 3 blind passages for each sample were made before declaring it as negative for the presence of viral agent [12].

Reference virus: Reference positive EDS –'76 virus (Strain 127) obtained from Veterinary Research Laboratory, Stormount, Belfast, Northern Ireland and maintained at Department of Microbiology, Veterinary College and Research Institute, Namakkal was used in this study.

Hyper immune serum: The hyper immune serum against EDS –'76 (Strain – 127) virus was raised [13] in 15 – week - old healthy cockrels free from EDS –'76 virus antibodies. Six cockerels were inoculated with equal quantities of standard EDS –'76 (strain 127) virus infected duck embryo allantoic fluid with HA titre of log2 10 /25 l and Freund's complete adjuvant by intra muscular route at the dose rate of 0.6 ml / cockerel. The second injection of 0.5 ml. of infected allantoic fluid alone was administered by intravenous route on the tenth day and the serum was collected on the 28$^{th}$ day after the first injection. The serum samples from the 6 cockerels were pooled, inactivated at 56°C for 30 min and stored at –20°C until use.

Haemagglutination test (HA): The HA test was performed by the microtitre method in V bottom microplates. Two fold dilutions of the virus were made in 25 l volumes, starting from 1:2 dilution using normal saline. To each dilution of the virus, an equal volume of 0.8 percent washed chicken erythrocytes were added and incubated at room temperature for 30 min. The reciprocal of the highest dilution of the virus showing complete HA was taken as titre [12].

Table-1. HA titers of EDS –'76 isolate

| No. of passages | HA titer ( $log_2$ / 25 l) |
|---|---|
| 1 | 4 |
| 2 | 6 |
| 3 | 9 |
| 4 | 12 |
| 5 | 15 |

Haemagglutination–inhibition test: This test was conducted to find out the specificity of freshly collected allantoic fluid and supernatant of the processed tissue samples using EDS –'76 antiserum. This test was performed in V bottom microplates. Two fold dilutions of 25 l of known EDS –'76 antiserum ($log_2$ 9) were made in sterile physiological saline and incubated with 25 l of virus containing 4 HA units of the virus (HA positive samples). After 20 minutes 25 l of 0.8 per cent v/v suspension of chicken erythrocytes was added. The plates were left to stand for 45 min at room temperature before the results were read. The plates were observed for inhibition of the haemagglutinating activity by EDS –'76 specific antiserum. Those samples which showed specific HI was taken as positive for EDS –'76 [12].

Agar gel precipitation test: Glass slides were cleaned with alcohol and air dried. The slides were overlaid with 4 ml of 1 % agar gel and allowed to solidify at room temperature. After solidification, the slides were kept at 4°C for 30 min in a humid chamber. The wells were punched using single gel puncture (5mm diameter with 3 mm interspace). The central well was charged with antiserum raised against reference virus strain 127. The peripheral wells were charged with known positive control antigen (strain 127), uninfected allantoic fluid (AF) as negative control and the test antigens. The test antigens constituted the allantoic fluid harvested from infected embryonated duck eggs 4-5 days after inoculation. The charged slides were transferred to humid chambers and incubated at 37°C and observed at 24, 48 and 72 h by oblique by transmitted light [14,15].

Washing and staining: The gels were immersed in normal saline for 48 hours with three changes followed by distilled water for 24 hours to remove the unprecipitated proteins. The gels were covered with two layers of wet Whatman No.1 filter paper. Then finally the gels were dried in an incubator overnight. The dried gel was stained with coomassie brilliant blue stain for 30 min and then destained with destaining solution until the precipitation lines were clearly visible.

## Results

Isolation and Identification: A total of 150 cloacal swabs and 15 pouch shell glands (uteri) homogenates from 15 poultry farms in and around Namakkal area were used for virus isolation. Cloacal swabs and shell gland homogenates from poultry farm No.5 yielded haemagglutinating virus in embryonated duck eggs. The isolate was identified as EDS –'76 virus by specific inhibition of the HA and by AGPT using antiserum raised against reference EDS –'76 virus strain 127.

Lesions in duck embryos: There was no mortality observed during the incubation period of five days after inoculation. Petechial haemorrhages on CAM and haemorrhages over embryos were noticed during harvest.

Hyper immune serum: The hyperimmune serum raised against reference EDS –'76 virus strain 127 showed a HI titer of $log_2$ 9 per 25 l.

Haemagglutination test (HA): Micro HA test were performed for the EDS –'76 isolate and the HA titer for each subsequent passage is tabulated in table I. HA titer at fifth passage level was $log_2$ 15 per 25l.

Haemagglutination inhibition test: The isolate was confirmed as EDS –'76 by specific inhibition of HA using hyper immune serum. The isolate was subjected to HI at the level of second passage.

Agar gel precipitation test (AGPT): The isolate showed one intense precipitation line against hyper immune serum after 24 h of incubation at 37° C as that of a line observed against reference antigen (positive control). Known negative control in the form of uninfected duck allantoic fluid and suspected samples did not reveal any precipitation line.

## Discussion

The syndrome of egg drop is caused by multiple etiologies such as Newcastle disease virus (NDV), Infectious bronchitis virus (IBV), Fowl pox virus, *E. coli*, *Pasteurella multocida* and aflatoxicosis. Recent development in disease management, absence of specific symptoms associated with these diseases as well as protective vaccination of the flocks with improved vaccines and vaccination schedules ruled out their occurrence considerably. Several workers later found that EDS –'76 virus was a major etiological agent for reduced egg production during the peak production period in commercial birds. Hence a study was undertaken to investigate the magnitude of egg drop syndrome associated with EDS –'76 virus in and around Namakkal.

Attempts were made to isolate the EDS –'76 virus from apparently healthy birds with the history of drop in egg production because there is no marked clinical signs except egg abnormalities in this disease [16]. The samples used for isolation in the present study included cloacal swabs and uteri [17]. The fact that the virus is excreted in faeces for considerable period [18], cloacal swabs constituted the main source in this study for virus isolation from affected birds [19].

The duck embryo was used for the isolation of the virus in the present study since it has been established to support the growth of the virus to a high titer than chicken embryos [20]. The EDS –'76 virus grows well in 11 – day – old duck embryos inoculated via allantoic sac route [21,22,23]. The HA titer after fifth passage in duck embryo even reached upto $\log_2$ 15/ 25 l [20]. No mortality of the embryos was noticed but haemorrhages over the embryo were observed [24].

The virus isolation was successful to many workers when they attempted to isolate within 15 days after onset of drop in production [17,20,25]. The virus excretion in faeces is maximum during the first 15 days of infection after that the development of antibody renders the isolation difficult [25]. In present study the materials were collected at right time and placed in 1X tissue culture growth medium with antibiotics and transported to the laboratory at 4° C [20,26].

The HA titer of the isolate initially was $\log_2$ 4/25 l, but later after every successive passages in duck embryo the HI titer increased rapidly [27]. The HA activity of the local EDS –'76 virus isolate was specifically inhibited by reference antiserum (strain 127) but not with hyperimmune serum to NDV, thus confirming the isolate as EDS –'76 virus. In AGPT there was one intense precipitation line between the isolate and hyperimmune serum as that of positive control indicating the EDS –'76 isolate [13].

Only one isolate was obtained after screening the samples from 15 poultry farms in and around Namakkal. This result is in agreement with the findings of earlier workers [28] who had stated that the New Castle disease (29.71%), aflatoxicosis (3.62%) and Infectious Bronchitis Virus (2.17%) as the major cause of drop in egg production rather than EDS –'76 (1.45%) in this area.

## Conclusion

One isolate of EDS –'76 virus was isolated and identified by its HA property, by specific inhibition of the HA and by AGPT using anti serum raised against reference EDS –'76 virus strain 127. The HA titer of the isolate initially was $\log_2$ 4 per 25 l and increased rapidly during every successive passage in duck embryo. Agar gel precipitation test revealed one intense precipitation line between the isolate and hyper immune serum. The present study revealed that the EDS –'76 virus influence in causing drop in egg production in this area to be minimal. This conclusion is on the basis of materials that are collected in and around Namakkal.

## Authors' contribution

PS and KS designed the research work. JJ extended help in final manuscript formation. All authors read and approved the final manuscript.

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## Competing interests

Authors declare that they have no competing interest.

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