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Cell Culture Adapted Duck Plague Virus Vaccine

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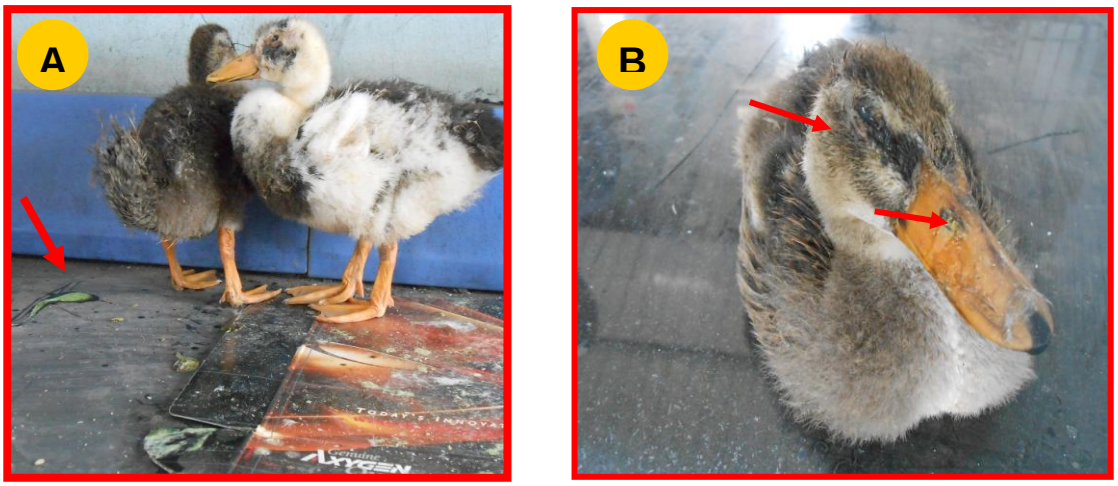
Asia is considered as the homeland for ducks holding 90 per cent of the world duck population. India occupies second position next to China with a duck population of 27.64 million in 2007 which registered an annual growth rate of 0.88 per cent (Livestock Census, 2007). Duck viral enteritis (DVE) commonly known as duck plague (DP) is an acute, fatal viral infection of ducks, geese, swans and other species of the order Anseriformes. Duck plague causes significant economic loss in duck production in many places of India including Assam. The disease is prevalent throughout the world causing heavy mortality in domestic ducks as well as in wild mallards. Rapid, easy, and preclinical diagnosis of DPV infection is the need of the hour. Again, effective vaccination is the only option to prevent and control the disease.



Health Care: Comparing to the chicken, ducks are generally resistant to most of the infectious diseases. Duck plague is one of the most important diseases of ducks causes high mortality in duck population. All ages of ducks are mostly affected by the duck plague virus. It is characterized by vascular damage with tissue hemorrhages and free blood in the body cavities. The lumen of the intestine and gizzard are filled with virus. There is no treatment of the disease. The birds can be protected only by vaccination. In India chicken embryo adapted duck plague vaccine is available.

Definition of vaccine: Duck plague vaccine is a suspension of modified living virus prepared from infected chicken embryo fibroblast primary cells.

Preparation of primary cells: Fresh fertile hens eggs obtained from salmonella free flocks are incubated in an incubator. Briefly, 9-11 day old chicken embryos were taken, candled and those showing good development of the embryo with prominent blood vessels were selected. The eggs were swabbed with 70% alcohol and allowed to dry. The area of the air sac was marked and broken gently with the help of forceps. Then the embryo was taken out and put into a dish containing balanced salt solution. The extremities of the embryos along with the internal organs were removed and washed several times with BSS, so that the blood and other debris get removed. With the help of a pair of sterile curved scissors embryos were properly minced and washed with BSS several times. Then the tissue pieces were transferred to a trypsinization flask containing a teflon-coated magnetic bar and sufficient amount of 0.25% trypsin was poured and trypsinized for 15 minutes in a magnetic stirrer at a moderate speed. Then supernatant was discarded to remove the toxic factors released from the tissue fragments. Fresh trypsin solution was poured into and trypsinization continued for 10 min and the tissue fragments were allowed to settle. Then cell suspension was passed through sterilized muslin cloth tied to the mouth of a beaker. The left over tissue was further trypsinized twice as done before, 15 minutes each time and passed through the muslin cloth. Then the cell suspension was centrifuged at 1000rpm for 10 minutes. The trypsin was poured off and washing with balanced salt solution was done twice and finally with the growth medium. The viability of the cell was tested using trypan blue (0.4%). The cell was then dispersed in growth medium containing 4 percent foetal calf serum (Hyclone, USA) by the packed cell volume (1:200) and dispensed in $25cm^2$ cell culture flask (Nunc, Denmark) giving 10 ml of growth media and incubated in presence of 5% Co2 at 37^oC for 48 hours. Culture bottles were examined under inverted microscope (Leica, Germany) for cell sticking and multiplication. The cell culture flask showing confluent monolayer (about 70 percent) were used for inoculation of the duck plague vaccine virus.

Duck plague vaccine virus grown in chicken embryo was adapted in the chicken embryo fibroblast cell culture. A 20 per cent suspension of the infected CAM in PBS was used as an inoculum for cell monolayer. Suspension was centrifuged at 3000 rpm for 30 minutes and supernatant was collected. To make bacteria free, supernatant was filtered through 0.45 µm membrane filter (Millipore) and treated with cocktail antibiotic solution (Sigma, USA) @ 10µl/ml of inoculum. After thorough washing 60-70 per cent complete cell monolayer was inoculated with 1 ml virus inoculum per $25mm^2$ cell culture flask (Nunc, Denmark) and kept at

37^{O}C for 1hour to facilitate proper adsorption of virus on to the cells. The unadsorbed viruses were washed with Ca^{2+} Mg^{2+} free PBS. Then, maintenance medium containing 2 % foetal calf serum was added into the flask and incubated at 37^{0}C for 3-4 days. Uninoculated healthy monolayer was kept as control. The cell monolayer was observed daily under inverted microscope (Leica, Germany) for any change in cell morphology.

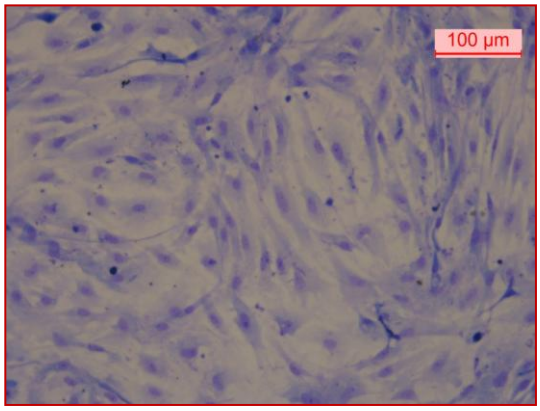


FIG.: 24 HOUR CONFLUENT MONOLAYER OF CEF CELLS (MAY GRUNWALD STAIN, 200X)

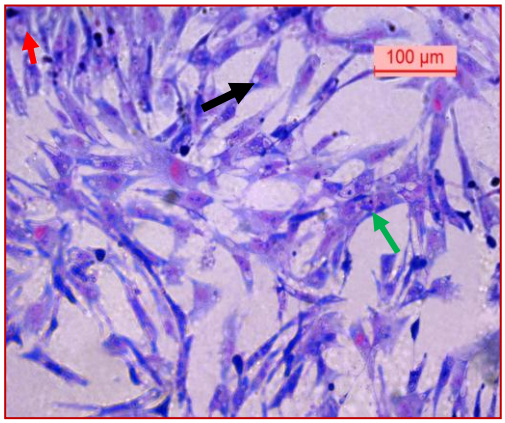


FIG **CEF CELLS SHOWING VACUOLATION (BLACK ARROW), SYNCYTIA (GREEN ARROW) AND INTRANUCLEAR INCLUSION (RED ARROW) BODIES (RED ARROW) AT 2 DPI (MAY GRUNWALD STAIN, 200X)**

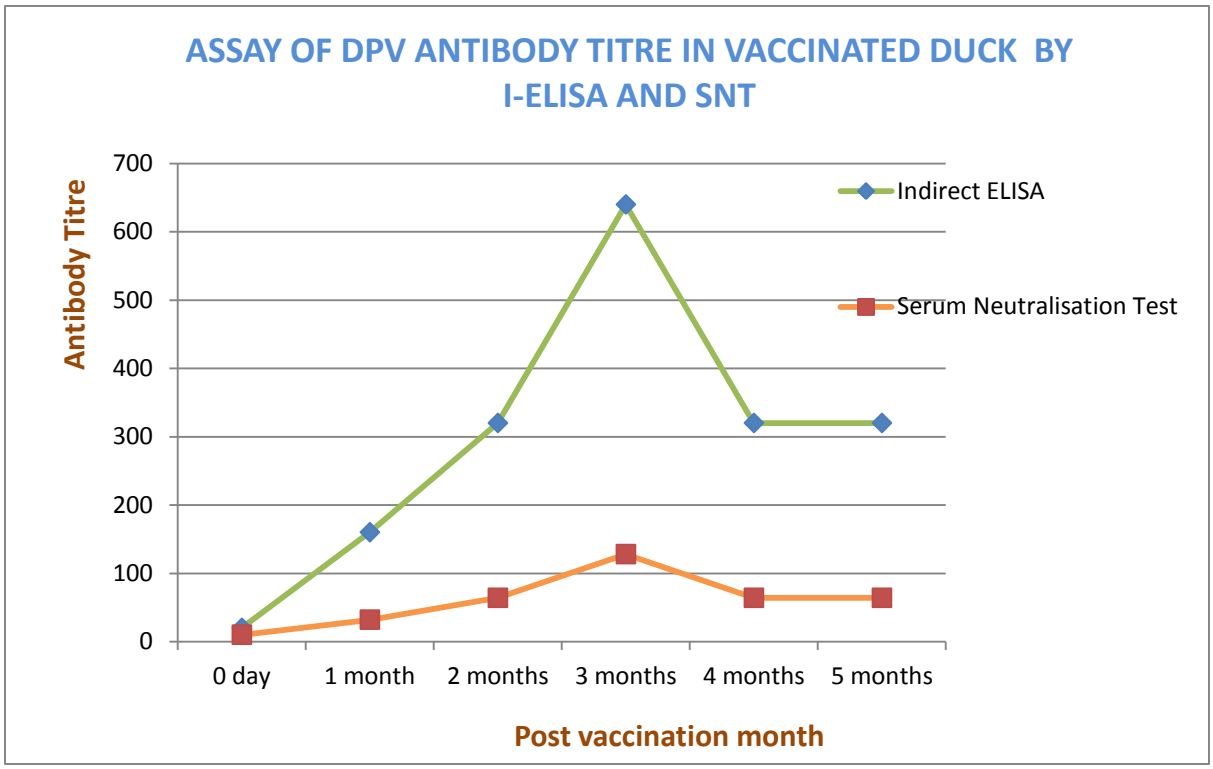
After completion of CPE, infected cell culture bottle along with control cell monolayer were kept at -20^{o}C. A repeated freezing-thawing was done 3-4 times and centrifuged at 5000rpm 30 minutes. Supernatant was collected as source of virus. Presence of DPV virus was confirmed by polymerase chain reaction (PCR) as well as by S-ELISA. Cell free supernatant was distributed in 1ml aliquot and preserved at -20^{o}C for further assay. CEF adapted DP vaccine virus was further passaged till desirable titre attained.$TCID_{50}$ of the propagated DPV vaccine strain was calculated at 40th passage level and estimated titre was $10^{6.88}$ $TCID_{50}$/ml.

Sterility test: Bacteriological sterility test showed no growth of contaminant after inoculation of the crude vaccine on thioglycolate bacteriological media and blood agar.

Safety test: The safety test carried out in ducklings. Subcutaneously 10 numbers of healthy ducklings of 1 month old free from DPV antibody were inoculated with 0.5 ml 10^{3} $TCID_{50}$/ ml of test vaccine and observed for 7-14 days for appearance of any adverse clinical sign. Birds

vaccinated with cell culture adapted DPV vaccine strain did not show any signs and symptoms of duck plague.

Potency test: In potency test 50 ducklings were vaccinated with 0.5 ml of 10^3 $TCID_{50}$/ml of cell culture adapted vaccine strain through subcutaneous route respectively. Unvaccinated control group injected with 0.5 ml sterile PBS following same route. After 2 months of primary vaccination booster dose was injected following the same route and dose. Accordingly blood samples were also collected from all ducklings on day 0, 30, 60, 90, 120 and 150 day after primary vaccination to evaluate the antibody titre by I-ELISA and Serum Neutralization Test (SNT). The present study showed a comparable increase of antibody and neutralizing titre upto 3 months of primary immunization and gradually declined after that. The relation between the antibody titre and neutralization titre is positively correlated and neutralization titre increased with the increasing titre of antibody and declined with the decreasing tire of antibody.



Challenge test: Ten numbers of representing vaccinated ducklings were challenged with virulent DPV strain @ 1 ml of 100 Duckling Infective $Dose_{50}$ (DID_{50}) per bird through intramuscular route after 90 days of primary vaccination. One unvaccinated control group was also injected

with the same dose of the virus through same route and observed for 7-10 days to see the onset of duck plague specific clinical sign and symptoms. There was onset of typical clinical and symptoms in ducklings of control group within 3 days of injection with the virulent duck plague virus and died within 6 days after onset of clinical sign. In contrary, there were no detectable clinical signs and symptoms after 8-10 post challenge days in vaccinated ducklings. The result showed that ducklings containing pre challenge antibody titre minimum 1:640 and neutralization titre 1:128 were found to be protected against challenge with a virulent duck plague virus. The results of challenge test showed 100% protection of ducklings vaccinated with 0.5 ml of $10^3 TCID_{50}$/ml dose of vaccine virus. Thereby 10^3 dose of vaccine virus can be considered as optimum (OIE recommended dose, OIE terrestrial manual, 2012) for protecting duck against virulent DPV. Further field trials may be required to validate the vaccine.

Storage: Vaccine stored at -15˚C to -80˚C should retain its potency for 1 year and if stored at -5˚C freezing chamber of refrigerator for about 3 months.

Dose: It is given @0.5ml subcutaneously.

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