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Short Communication

TEARS - A SOURCE FOR NEWCASTLE DISEASE VIRUS IN DIAGNOSIS OF THE DISEASE IN CHICKEN

Newcastle disease produces a variety of clinical conditions viz., drop in egg production, respiratory distress, diarrhoea etc. The diagnosis of new castle disease is not always easy as a number other diseases simulate the symptoms. Thus the accurate diagnosis almost always involve isolation, identification and characterisation of the virus (Alexander, 1988). Successful isolation of Newcastle Disease Virus (NDV) has been made from tracheal swabs or respiratory discharge, cloacal swabs or faeces, trachea, lungs, spleen, proventriculus, intestine, brain, bone marrow and other organs. This paper discribes isolation of NDV from tears of infected chickens and the advantage of sampling tears for diagonsis.

Six weeks old sixty white leghorn chickens not exposed to NDV were taken for the experiment. Six field isolates of NDV which were characterised as velogenic based on mean death time (MDT), intracerebral pathogenicity index (ICPI) and intravenous pathogenicity index (IVPI) (Roy, 1995) were used for this experiment. Each of the isolates were inoculated into 10 days old embryonated hen's eggs. Embryos died within 60 hours after inoculation. Fresh allantoic fluid (AF) of each isolate was diluted (1:10) insterile phosphate bufferd saline (PBS) and 0.1 ml per chicken was injected intravenously into ten chickens.

Tears were collected after instillation of one drop of glycerol into the eye (Aitken *et al.*, 1975) by using a micropipette. Tears were collected before injection of virus and at 4th day after injection of virus. Tears collected from ten different chickens subjected to injection with a particular virus were pooled together and diluted 1:1 (v/v) in PBS (pH 7.2) containing antibiotics (penicillin - 10,000 IU/ml, streptomycin 10 mg/ml and gentamicin 250 µg/ml) and used for virus isolation in 10 days old embryonated eggs as per the method followed by Alexander (1988).

Haemagglutination (HA) and haemegglutination inhibition (HI) tests were done as described by Alexander (1988).

Harderian glands and lacrimal glands were collected as described by Survashe and Altken (1977) from the

infected chicken at the point of their sacrifice and also from uninoculated age matched healthy controls.

Injected birds were visibly sick by 3rd day post infection. By 4th day tear samples were collected for virus isolation. All the samples killed the inoculated embryos within 48 to 56 hours after infection. Clear AF harvested from the embryos showed HA with 1% chicken erythrocytes and the HA was inhibited with NDV specific antiserum in HI test. Tears collected from pre-inoculated chickens did not kill the inoculated embryos. Ninety six hours after inoculation the embryos were chilled and clear AF harvested did not show HA activity. Materials were passaged serially for two more times in embryonated hen's eggs. AF harvested from each passage did not show HA with 1 per cent chicken erythrocytes. Hence the pre inoculated tears were declared negative for NDV.

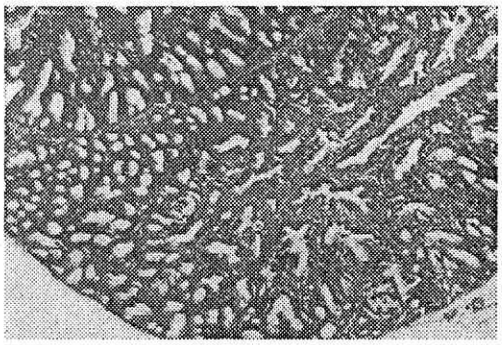

Fig.1 Harderian gland showing hyperaemia, depletion of plasma cells, marked distention of vessels, degenerative changes of lining epithelium and accumulation of debris in the lumen. H&E x 320

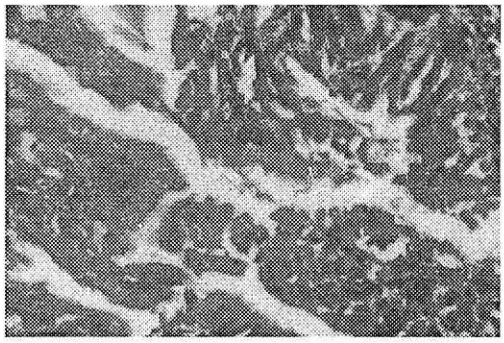

Fig.2 Lacrimal gland showing extensive heamorrhages and marked changes in the lining epithelium. H&E x 32

Histopathologically, Harderian glands of infected chickens showed hyperemia, depletion of plasma cells, marked distension of vessels, degenerative changes of lining epithelium and accumulation of debries in the lumen (fig. 1). The lacrimal glands showed extensive haemorrhages and marked changes in the lining epithelium (fig. 2). But no microscopic lesion could be observed in Harderian glands and lacrimal glands collected from uninfected control chicken. Commonly faecal swabs and tracheal swabs are used for isolation of NDV from the suspected flocks. In an experiment 90% success was observed using cloacal swabs (Roy *et al.*,1995). Sampling from dead birds usually include the affected

organs. Tears from the affected chicken are free from contaminents could be collected easily from the suspected flocks and the screening of NDV could be carried out early. It was reported that intraocular vaccination resulted in high titre of vaccine virus in the Harderian glands (Russel, 1953). In the present study experimental birds were infected intramascularly and it indicated that whatever may be the route of infection the virus get distributed in the Harderian and Lacrimal glands as histopathological lesions were observed in both the glands but no such changes were observed in unaffected chickens. The virus multiply in both Harderian and Lacrimal glands and enrich the tears. It is concluded that tears are suitable samples for NDV isolation from affected flocks.

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