An outbreak of Avian Encephalomyelitis in Tamil Nadu State of India

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Abstract

Avian encephalomyelitis (AE) was recorded in 7-day old Ross strain of broiler chickens showing clinical signs of ataxia, lateral recumbency and mild tremors. In a flock of 10,000 birds, 12% died without producing gross pathological lesions. Diagnosis of the disease was done based on virus isolation in embryonated chicken's eggs, demonstration of hexagonal virus particles in purkinje cells of cerebellum by electron microscopy and confirmation by agar gel immunodiffusion test using AE virus specific antiserum.

Key words: Avian encephalomyelitis, agar gel immunodiffusion, haemagglutination, haemagglutination inhibition, electron microscopy, diagnosis

Introduction

Avian encephalomyelitis (AE) is a disease of young chickens, pheasants, quails and turkeys caused by avian encephalomyelitis virus (AEV), a picornavirus. In young chickens AEV induces paralysis, ataxia and muscular dystrophy and in older chickens the infection is usually subclinical, resulting in reduced egg production and hatchability. In natural outbreaks the disease usually appears in 1-2 week old chickens with a morbidity rate of 40-60% and an average mortality of 25%. The disease was initially reported in the USA in 1932 and subsequently in Europe, Canada, Japan and Australia. The disease is also prevalent in different parts of India but the present paper describes an outbreak of AE and confirmation of the disease in young chicks in Tamil Nadu state of India.

Materials and Methods

History

Clinical signs of ataxia, lateral recumbency, blindness, and mild tremors in the head and neck were observed in 7-day old broiler chickens (Ross strain) at a commercial farm. Mortality was approximately 12% in a flock of 10,000 chickens.

Necropsy and Histopathology

Dead chickens were necropsied and observed for gross pathological changes. Brain samples were collected aseptically for virological study. Brain samples were also collected in 10% neutral buffered formalin (NBF) processed and embedded in paraffin. Sections were cut at 4-5 µ thickness, stained with haematoxylin and eosin for histopathological studies. Similarly samples of vital organs such as the brain, proventriculus, liver, lung and kidney were collected from 17-day old chicken embryos, inoculated with suspected brain material from affected chickens and subjected along with age matched uninfected control embryo tissues to histopathological study. Tissues from chicken embryos were collected from each passage during five serial passages of the suspected specimen.

Virological Examination

The brain samples collected from the outbreak were processed as described by Alexander and tested for haemagglutination (HA) with 1% chicken erythrocytes. The samples were also treated with antibiotics (Penicillin – 10, 000 Unit/ml, Streptomycin 10mg/ml and Gentamicin 250 µg/ml). A pooled brain sample was inoculated into 5 nine day old embryonated chicken's eggs as described by Alexander. Three blind passages were given and checked for HA activity with chicken erythrocytes at each passage level.
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Pooled brain sample was prepared and inoculated into 5 embryonated seven day old chickens eggs of through the yolk sac route. Five un-inoculated chicken embryos were kept as controls. The embryos were chilled for 17 days and growth and development of the infected embryos were compared with uninfected controls. Brain tissues were harvested from each passage during five serial passages and observed for similar changes.

These samples from each passage level were also homogenized and tested for AEV specific antigen by the agar gel immunodiffussion (AGID) test using AEV specific antiserum procured from The Charles River SPAFAS Laboratory, CT, USA. AGID was done by placing antiserum in the central well and brain homogenates in peripheral wells11.

**Electron Microscopy**

Pieces of brain collected from embryos at 3rd passage level were fixed in 3% glutaraldehyde solution for electron microscopical study. Epoxy resin blocks were prepared and ultra thin sections were cut at 60 nm. The sections were stained with uranyl acetate and lead citrate and screened under Philips-Teknai –10-transmission electron microscope (Holland) at 100 K.V. and the size of the virus particles was measured.

**Results**

**Necropsy and Histopathology**

No specific gross lesions were observed in dead birds. Histopathologically, brains of affected chickens showed disseminated giosis, slight perivascular cuffing of lymphocytes, infiltration of lymphocytes and plasma cells in meninges and grey matter. Microscopic lesions in the brain of infected embryos were appreciable from 2nd passage onwards. The lesions included congested blood vessels, focal giosis, poorly developed granular cell layers and nuclei cerebralis in the cerebellum and mildneuronal degeneration. The Proventriculus revealed focal aggregation of lymphocytes in the muscular layer. No lesions could be detected in other organs.

**Virology examination**

Brain samples did not reveal any HA activity. Fresh allantoic fluid did not show any HA activity even after three serial passages in embryonated chicken’s eggs. Brain samples inoculated via the yolk sac route and embryos were harvested at day 17. No significant changes were observed in growth compared to the unoinoculated control embryos at the 1st passage level. But from the 2nd passage to 5th passage, dwarfism was observed in inoculated chicken embryos compared to unoinoculated embryos. No mortality was seen at any passage level.

In the AGID test, no line of precipitation was seen by 72hrs. The slides were soaked in 1% tannic acid for 1 minute to increase resolution and again observed under diffuse light. Although lines of precipitation were not readily visible, soaking in 1% tannic acid improved the resolution (Hudson and Hay, 1980) and precipitation lines were then visible. The precipitation lines were observed with brain homogenate samples from passage level 2 to 5. These were therefore positive for AEV specific antigen.

**Electron Microscopy**

Electron microscopy of the brain revealed crystalline arrays of hexagonal virus particles measuring about 30 nm in the cytoplasm of Purkinje cells (Fig. 1). Semilunar shaped condensation of heterochromatin was observed in the nuclei of Purkinje cells. Cell shrinkage and bebbing of the perinuclear membrane were also observed. Some cells showed fragmentation of chromatin and apoptotic bodies.

![Figure 1. Electron micrograph of crystalline arrays of hexagonal virus particles in the cytoplasm of Purkinje cells.](image-url)
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Discussion

The clinical signs of ataxia, tremor of head and neck followed by death in young chicks at 7 days of age were highly suggestive of AE.

Histopathological lesions of the brain revealed non-suppurative encephalomyelitis. Samples were HA negative. Brain samples from infected embryos were positive for AEV by AGID test and thus involvement of AEV was confirmed. However brain collected from 1st passage was not positive by AGID and possibly this is due to low concentration of the virus in the 1st passage level.

Further confirmation was done by demonstration of the virus particles in the cytoplasm of Purkinje cells. It has been reported that AEV causes apoptosis of Purkinje cells in inoculated chicken embryos via the major structural protein VP3. In the present study mortality was only 12% compared to reported average mortality of 25% following AE infection. Possibly the breeder hens were harboring AEV and the newborn chicks were infected by embryo transmission since the outbreak was observed when they were 7 days old. However, the source of infection remains unknown.

References