Avian Encephalomyelitis Virus in Sudan

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Abstract: Avian encephalomyelitis (AE) virus was studied in young (2 wks-broilers) and (adult 18 wks) layer chickens at Khartoum State. Nervous signs, paresis with 3% morbidity and 1.5% mortality were recorded in young affected broiler flock. The adult layer flock showed only 7.5% drop in egg production. AE virus was isolated from the two affected flocks and confirmed by means of AGID test. Day-old chicks experimentally inoculated with the isolated viruses (T139 or Sh36) showed clinical signs typical to that of AE. This is the first report of AE virus isolated from field cases in Sudan.

Key words: Encephalomyelitis, virus, avian, Sudan.

INTRODUCTION

Avian encephalomyelitis (AE) is a viral disease of young chickens caused by a hepatovirus of the family Picornaviridae, characterized by central nervous system signs. It occurs worldwide in all seasons of the year. Primarily AE is a viral infection of chickens, turkeys, pheasants and quail, first reported in 1930 and its natural history parallel closed that of poliomyelitis of human and polio encephalomyelitis of swine. The virus grows in the yolk sac and brain of the embryonated chicken eggs from non immune hens.

AE is most prevalent in chickens 1-5 weeks of age. Susceptible chickens more than 5 weeks old will develop antibodies to AE but do not show clinical signs at the time of infection. Clinical signs appears at 7 to 10 days of age. Tremors of the head and neck are presumptive of the disease in the flock hence the name (Epidemic tremors). Affected chickens first may show dull expression of the eyes, followed by progressive in coordination, sitting on hocks, tremors of the head and neck and finally paralysis or prostration. Muscular tremors are best seen by exercising the bird. Affected birds are inactive, some may refuse to walk or walk on their hocks. Mature birds may experience a temporary drop in egg production (5-10%) but do not develop neurological signs. Morbidity from the naturally occurring disease has been observed in young flock and about 40-60% while mortality average 20% and may exceed 50%.

This paper deals with isolation of avian encephalomyelitis virus from two farms at Khartoum State, Sudan, and development of similar clinical signs on chicks experimentally inoculated with the isolated viruses.

MATERIALS AND METHODS

On July 2005, 10 layer chickens (18 weeks old) were received in the Department of Avian Pathology and Diagnosis at CVRL. They were from Tayba-Elhasanab farm, southern of Khartoum State. Birds were reared on a closed system. Chickens were apparently healthy but 7.5% drop in egg production was recorded. The total number of the flock was 22000.

On March 2006, another field case (16 days --old broiler chickens) was received in the Department, also from Shegalab farm southern of Khartoum. The main clinical signs of the affected chickens were nervous signs, paresis with 3% morbidity and 1.5% mortality. The total number of the affected birds was 2000. Affected birds from the two farms were killed, necropsied. Brains and intestine were collected, kept at -20°C for virus isolation. Proventriculus and part of the brains were fixed in 10% formal saline for histological examination.

Virus Isolation: According to Dennis, a 10% homogenate (w/v) of the brains or intestine in antibiotic solution containing penicillin, streptomycin and mycostatine was prepared. Following centrifugation, 0.2 ml of the supernatant was inoculated into the yolk sac of 7-day old chicken embryos and incubated at 37°C for 7-11 days. Brain and whole tissues of the inoculated embryos were collected and homogenated, part of the homogenate was used as antigens in AGID test against positive antiserum for virus identification. The other part was used to reproduce the disease experimentally in one day-old chicks. The isolated viruses were designated as T139 and Sh36 for Tayba-
ElHasnab and Shegelab respectively.

**Reference Antigens and Antiserum:** AE virus vaccine (Nobilis 1143) was kindly supplied by the Viral Vaccine Production Unit, CVRL, passaged up to three times into yolk sac of 7-day's chicken embryos. Brains were collected from the third passage, homogenated, centrifuged and used as a positive control antigen in the AGID test. AE virus positive antiserum was obtained from Biocheck Company, Holland.

**Agar Gel Immunodiffusion Test:** The test was conducted as described by Ikeda[1], in brief; each outer well was filled with 25 µl of the homogenated brains or whole tissues and positive control antigen. Positive AE antiserum (in the central well).

Plates were placed in a humidified chamber at room temperature until the final result was read within 24 to 72 hours.

**Experimental Infection:** Fifty, day-old chicks were divided into three groups. Group 1 and 2 each was made up of 20 chicks and inoculated with AE virus field isolates T139 or Sh 36 each bird received 10^4 EID₅₀ of the virus, intracerebrally[4]. The third group was left as un inoculated control; Birds were housed separately and observed daily for clinical signs. From 2-7 days post inoculation, brains and proventriculus were collected from birds showed clinical signs and preserved in 10% formal saline for histological examination.

**RESULTS AND DISCUSSIONS**

Following inoculation of the fertile eggs, mortality was detected on the fifth to eighth day of inoculation. Dead embryos were darkened red showing extensive hemorrhage.

On AGID test a clear precipitin line was formed between the positive (reference) AE antiserum and control positive AE antigen and the harvested, homogenated brains and whole tissues of (T139 and Sh36).

The histopathological lesions of the proventriculus were: necrosis, mild congestion, hemorrhage, degeneration of muscle layer and mononuclear cells infiltration while the brain showed vaculation, necrosis of neuron cells with karyolysis of nuclei and congestion of the capillaries.

**Experimental Infection:** During the first week of inoculation more than 92% of the inoculated birds show nervous signs, depression paresis and blindness Fig. 1(a,b) .All birds in group 3 were apparently healthy.

**Fig. 1:** Clinical signs in chicks experimentally inoculated with AE field isolate T139 and Sh 36.

On histopathology, microscopic lesions in the brains were vaculation, necrosis of neuron cells and congestion of blood capillaries, retraction of muscle fiber were seen on proventriculus.

**Discussion:** In the present study two flocks at Khartoum state were investigated for the presence of AE virus .The first was an adult layer flock about 18 weeks of age ,show only drop in egg production (7.5%). The second flock was broiler; 2weeks old .The main clinical signs were nervous signs, paresis, dullness, blindness with 1. 5% mortality rate.

Using embryonating chicken eggs and via yolk sac inoculation, AE virus was isolated from these flocks. The isolate viruses were confirmed as AE virus by means of AGID test.

When day-old chicks were experimentally inoculated Intracerebrally with the isolated viruses, the clinical signs and histopathological lesions observed 7
days post inoculation were in consistent with those mentioned for AE\textsuperscript{(1)}. The clinical signs of AE are very much age related as older birds show no neurological signs after infection\textsuperscript{(5)}.

In adult, the infection is usually sub-clinical. The virus can cause slight reduction of egg production in susceptible laying hens and can be transmitted to embryos resulting in reduce hatchability\textsuperscript{(5)}. The infected chicks which do hatch show characteristic signs of the disease between 1 to 7 days of age and act as a major source of infection for other chicks\textsuperscript{(5)}.

This is the first report of isolation of AE virus in Sudan. Other epidemiological studies were needed to investigate the prevalence of this disease so as to adopt specific control measured.

REFERENCES