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Avian Encephalomyelitis

Virology and Serology

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1. Introduction

Avian encephalomyelitis (AE) is a widespread disease of poultry that is transmitted via the oral-faecal route (Calnek *et al.*, 1961). Avian encephalomyelitis virus (AEV) is the aetiological agent of the disease which is also known as 'epidemic tremor' and has been widely reported in Australia over many years (Albiston, 1966; Smart *et al.*, 1986; Shafren *et al.*, 1989). In young chickens AEVs induce paralysis, ataxia and muscular dystrophy (Jungheer and Minard, 1942), while in older chickens infection is usually subclinical, resulting in declines in egg production and hatchability (Taylor *et al.*, 1955). Most tests for AEV are serological and, until recently, depended upon the availability of large numbers of specific pathogen free (SPF) chick embryos. Enzyme-linked immunosorbent assays (ELISAs), which allow a diagnostic result within hours rather than 10–12 days (Garrett *et al.*, 1984; Smart and Grix, 1985; Shafren and Tannock, 1988), have now entirely replaced the earlier techniques and only ELISAs are considered in detail here.

2. Virus Isolation

The virus can be isolated in susceptible eggs but up to 20 passages may be required before the embryos show any obvious signs of infection. Shafren and Tannock (1990), using an AEV antigen ELISA, have detected viral antigen in the tissues of embryos inoculated with AEV field isolates that do not exhibit signs of infection. Earlier attempts to grow AEV field isolates in avian monolayer cell cultures from various organs of non-neural origin repeatedly met with failure (Mancini and Yates, 1968a, 1968b; Sato *et al.*, 1971). However, more recently there have been several reports of the cultivation of AEV strains in chick embryo brain cultures (Berger, 1982; Nicholas *et al.*, 1986).

The virus can also be isolated from chickens hatched from susceptible eggs after fumigation. Chicks with early neurological symptoms (see 3.) are killed and their brains are collected. Each brain is divided longitudinally and half is fixed for later histopathological examination, if necessary. The fresh halves of brain are pooled and homogenised as a 10% (w/v) suspension in diluent with added antibiotics. The suspension is lightly centrifuged and 30 μ L of the supernatant inoculated into each cerebral hemisphere of 12 one-day-old susceptible chicks.

The chicks should preferably be kept in an isolator and segregated from all other birds. They are observed for 28 days and any that develop symptoms of epidemic tremor from day 8 onwards are bled for serum and then sacrificed. The brains are removed and portions may be examined histologically. The remainder of the fresh brain should be stored at -70°C. Serum

should be collected from the survivors at 28 days when the transmission test is terminated. The pooled serums may be tested for the presence of antibodies by ELISA.

3. Clinical Signs of Epidemic Tremor

Usually chicks from one to five weeks of age are affected. There is a variable mortality although the morbidity may exceed 50%. Clinical signs usually seen are ataxia, paralysis, depression and fine tremors of the head, neck or tail; in some cases the tremors are not easy to detect and can best be felt by holding the chick off the ground with its two carpi between finger and thumb and the wings held vertically upwards (Smith, 1976). Death usually follows as a result of dehydration and starvation, with sick birds being unable to fend for themselves (Olitsky, 1939; Jungheer and Minard, 1942).

4. Pathology

The gross and microscopic pathology of field isolates of AEV has been adequately described (National Academy of Sciences, 1971). There are no obvious gross signs, apart from dehydrated tissues. The materials best collected for histopathological examination are the brain, pancreas, proventriculus and gizzard.

The brain lesions are characterised by widespread non-suppurative encephalomyelitis and tend to be more prominent when clinical signs have been evident for over a week. There is neuronal degeneration followed by gliosis in the cerebrum, cerebellum, brain stem and spinal cord. Mononuclear cell perivascular cuffing is usually severe. Meningeal involvement is not prominent. Although some workers report a sparing of the cerebellum, this is not a reliable feature.

Marked focal lymphoid hyperplasia is seen in the pancreas, the muscles of the gizzard and proventriculus. Such lesions combined with the presence of typical brain changes are regarded as diagnostic for avian encephalomyelitis.

5. Serological Diagnosis

Both the serum neutralisation test (Calnek and Jehnich, 1959) and the embryo susceptibility tests (National Academy of Sciences, 1971) are time consuming and expensive and require embryos from an accredited flock that is free from AEV, preferably an SPF flock. For these reasons, only ELISAs are used in Australia. Several formats of the ELISA procedure are available, but one commonly used throughout Australia (Shafren and Tannock, 1988) is as follows.

A 50 μ L amount of a 1:800 dilution of affinity-purified rabbit anti-AEV/IgG (available from Associate Professor G.A. Tannock, Faculty of Medicine, The University of Newcastle, Callaghan, NSW 2308, Australia) in 0.1 mol/L sodium carbonate (Na_2CO_3) (pH 9.5) is added to each well of an ELISA plate, which is incubated at 22°C (room temperature) overnight in a moist chamber. The plate is washed and 50 μ L of ELISA antigen is added to each well. It is then incubated at 22°C for one hour and washed a further three times and 50 μ L of a 1:100 dilution of test sera in ELISA diluent (1.5 mol/L NaCl, 1 mmol/L EDTA, 0.1 mol/L Tris pH 7.3, 3% rabbit serum, 3% goat serum and 0.1% Tween 20) is added to each well. After incubation at 22°C for one hour, the plate is washed as before and 50 μ L of a 1:500 dilution of horseradish peroxidase-conjugated goat anti-chicken IgG in ELISA diluent is added to each well. It is then incubated for one hour at 22°C and then washed three times with PBS-Tween (phosphate buffered saline pH 7.3 containing 0.1% Tween 20) and once with distilled water. A 100 μ L amount of 5-amino salicylic (5-AS) acid prepared according to the method of Ellens and Gielkens (1980) is then added to each well and the reaction allowed to proceed for 30 min at 22°C before being stopped with 50 μ L of 2.0 mol/L sodium hydroxide (NaOH). Test samples with an absorbance at 492 nm (A_{492}) greater than 0.200 are considered positive. Seroconversion occurs in most birds three weeks after the onset of clinical symptoms (Smart *et al.*, 1986; Shafren and Tannock, 1989).

6. Differential Diagnosis

Diagnosis should exclude nutritional encephalomalacia (crazy chick disease), Newcastle Disease, Marek's Disease and bacterial meningo-encephalitis caused by (*Salmonella pullorum*). The brain lesions of Newcastle Disease consist of neuronal degeneration and gliosis, but the perivascular cellular reaction is usually much less than for avian encephalomyelitis. Neuronal degeneration is not a feature of Marek's Disease.

7. References

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