

## THE DIFFERENTIAL DIAGNOSIS OF EQUINE MORBILLIVIRUS INFECTION INCLUDING PATHOLOGY AND CLINICAL SIGNS

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### ABSTRACT

Equine morbillivirus (EMV) was identified as the cause of an outbreak of acute respiratory disease in a racing stable at Hendra, Brisbane, in September 1994 in which 14 horses and their trainer died. It has since been established that this outbreak was preceded by a more limited outbreak in which 2 horses died in early August 1994 on a property near Mackay in coastal north Queensland. Infection of these horses was confirmed in November 1995 by retrospective examination using histology, immunofluorescence and polymerase chain reaction of formalised tissues. This examination was undertaken following detection of EMV infection of the owner of the property, who died on 21st October 1995. He had an acute progressive encephalitis associated with a marked increase in serum neutralising (SN) antibody titre to EMV.

At Hendra, affected horses were anorexic, depressed, ataxic, some showed head pressing and facial oedema was marked in one. They were usually febrile, with temperatures as high as 41°C, and developed increasingly severe respiratory distress. A frothy nasal discharge was common terminally. Two of the 7 infected horses which survived showed myoclonic twitches similar to those seen in some dogs infected with canine distemper virus. No haematological or biochemical abnormalities were detected which could be considered specific for EMV infection.

Clinical signs reported in the Mackay horses included aimless pacing, ataxia, muscle trembling, respiratory distress, bloody nasal discharge and oedematous swelling of the head, particularly the cheeks and, in one case, the infraorbital fossa.

Gross pathological changes in both outbreaks were confined to the lungs which were very heavy, grossly oedematous and congested. The airways contained a thick tenaceous frothy exudate. Microscopically, the lung changes were those of an interstitial pneumonia with massive oedema and

haemorrhage, macrophage proliferation, alveolar necrosis, vasculitis and/or vascular necrosis. Syncytial giant cells which gave specific cytoplasmic immunofluorescence to EMV were present in the endothelium of lung capillaries and arterioles and also within glomerular vessels in the kidney.

A wide range of toxins, bacterial and other viral pathogens were eliminated in a comprehensive approach to the identification of the EMV as the aetiological agent of this new disease syndrome. Species susceptible to infection identified to date are man, horses, cats, guinea pigs and possibly fruit bats.

### INTRODUCTION

The first descriptions of disease due to infection with equine morbillivirus (EMV) in man (Selvey and Sheridan 1994; Selvey *et al.* 1995) and in horses (Murray *et al.* 1995) arose from an outbreak in a racehorse stable at Hendra, a suburb of Brisbane. The outbreak followed the introduction of an apparently EMV-infected pregnant mare (the index case) from a spelling paddock elsewhere in Brisbane. This mare died on 9th September 1994 and, by 27th September 1994, a further 12 horses and the trainer had died from an acute respiratory disease. A fourteenth death was a presumptive case which died in the spelling paddock prior to 9th September 1994.

Rogers *et al.* (1996) reported that the Hendra outbreak had, in fact, been preceded by the occurrence of fatal infections in 2 horses at Mackay one month earlier. This incident only came to light when the farmer who handled the sick and dead horses developed terminal illness and died 14 months later. Confirmation of EMV infection in the horses was made by retrospective examination of preserved tissues taken at the time of their death (Hooper *et al.* 1996).

When the Hendra outbreak occurred, EMV was an unknown pathogen, and a large amount of

exclusion testing was undertaken before it was identified as the aetiological agent. The approach taken to differential diagnosis has not been reported previously so it has been included in this article together with a description of the clinical signs and of the pathology of EMV in horses.

## **DISEASE DIAGNOSIS AND CHARACTERISTICS**

### ***Clinical signs***

At Hendra, affected horses were initially depressed, anorexic and hyperthermic with rectal temperatures ranging from 39°C–41°C. They commonly became ataxic, weak and reluctant to move, held their heads low and several stood with their heads pressed against the wall. The index case had oedema of the lips, mandibles and eyelids and oedema of the head and legs was seen in several other cases.

Rapid shallow respiration was apparent in all cases with rates between 40–60 respirations/min. This elevated respiratory rate became marked in the terminal stages when affected horses became very distressed. Heart rates increased to 50–100 beats/min and cardiac arrhythmias were sometimes noted in terminal stages. Mucous membranes became congested and mild jaundice was seen in some cases as was cyanosis. There was a copious nasal discharge in most fatal cases of white or blood tinged stable foam. Respiratory sounds were generally not detectable at this stage due to obstruction of the airways.

In 2 cases, death occurred on the same day that clinical signs were noticed but the mean time to death was 2.1 days.

Two of the survivors from Hendra developed mild neurological signs consisting of myoclonic twitches similar to those seen in some dogs infected with canine distemper virus. These twitches were transient in one case but persisted until euthanasia in the other and involved the upper forelimb in the transient case and the face, lower lip and upper hind limb muscles in the persistent case (R. Wright, unpublished data).

### ***Clinical pathology***

Although some biochemical abnormalities such as hyperglycaemia, elevated urea nitrogen and total bilirubin, hypocalcaemia, hypophosphataemia and increased aspartate aminotransferase and creatine kinase were detected, these changes were not consistent or unique to EMV infection. Similarly, apart from haemoconcentration, there were no consistent changes in other haematological parameters (R. Wright, unpublished data).

## ***Gross pathology***

Although generalised yellowing of fascia and subcutaneous tissue was present in some cases, the major changes were restricted to the lungs. In the natural cases, subpleural oedema was marked as was pulmonary congestion and ventral consolidation. Affected lungs were swollen, wet and heavy. There were petechial haemorrhages on pleural surfaces and copious quantities of heavily blood stained oedema fluid gushed from cut surfaces. Patchy, recent focal haemorrhages of varying size were present in lung parenchyma. The airways were usually completely filled with thick, fine foam which was either white or blood tinged. Some excess thoracic and pericardial fluid was often present as was petechiation of the peritoneum and epicardial haemorrhage.

## ***Microscopic pathology***

The range of tissues examined included liver, heart, lung, brain, adrenal, kidney, spleen, skeletal muscle, duodenum, jejunum, colon and stomach. Major changes were restricted to the lungs and can be described as acute interstitial pneumonia with widespread oedema, fibrin exudation and haemorrhage. Alveolar capillary endothelium was severely damaged with necrotic cells being widely distributed. Mononuclear cells, macrophages and a few neutrophils were present in alveolar lumens. These tended to be more numerous in animals dying less acutely and these cases also showed necrosis of alveolar walls. Multinucleate syncytial cells were present in alveoli in some cases but more frequently involved the endothelium of arterioles and capillaries. In 2 cases endothelial cells in renal glomeruli showed occasional hypertrophy, necrosis and early syncytium formation (P. J. Ketterer, unpublished data).

Hooper *et al.* (1996) examined the kidneys from both Mackay horses but lung was available from only one. They considered the kidney and lung changes to be identical to those of the Hendra horses. However, one of the Mackay horses also had extreme proteinuria with tubules and Bowmans capsules distended with eosinophilic material. This had not previously been seen in cases of EMV infection. There was a mild skeletal muscle myopathy with oedema of blood vessel walls in one animal. Specific cytoplasmic immunofluorescence for EMV was demonstrated in renal glomeruli and adjacent tubules, small blood vessel walls in skeletal muscle, along alveolar walls and in pulmonary vascular syncytia.

Identical pulmonary changes were described by Murray *et al.* (1995) in cases of experimentally

reproduced disease together with the demonstration of specific cytoplasmic immunofluorescence in vascular endothelial syncytial cells.

### **Differential diagnosis**

Because of the acuteness of the disease, the severe respiratory distress and the frothy nasal discharge seen terminally, testing for African Horse Sickness by the Australian Animal Health Laboratory (AAHL) was given a high priority. However, testing for a comprehensive range of bacterial, toxic and other viral pathogens was also undertaken.

### **Bacterial pathogens**

Bacteria for which specific cultural examination was undertaken were *Bacillus anthracis*, Pasteurella spp, Yersinia spp, Legionella spp, Pseudomonas spp and *Streptobacillus moniliforme*. No such organisms were isolated.

### **Toxins**

A range of possible toxins producing pulmonary oedema was drawn up and divided on the basis of whether the oedema was the result of direct damage to the lung vasculature (Group 1), or secondary to impaired cardiac or renal function (Group 2). Other systemic toxins comprised Group 3. Because lung is the main target organ for EMV, compounds in Group 1 were the only likely toxins. Of these, paraquat was most likely but tests on stomach contents and urine were negative. Other compounds included in this group were ANTU, zinc phosphide, galegine, fumosins, furans, L-tryptophan and glucosinolates, but each was eliminated for a variety of reasons.

Compounds in the second group were ionophores, gossypol, cardiac glycosides, Argemone spp, fluoroacetate, chlorophenoxy herbicides, organochlorine insecticides, ivermectins, avermectins, potassium iodate, nitrogen dioxide and ethylene glycol. Of these the only possibility was monensin, an ionophore, and no traces of this compound were found in selected feed components.

Group 3 substances tested for with negative results were cyanide, arsenic, cadmium, cobalt, chromium, copper, iron, manganese, nickel, lead, titanium and zinc. Only normal background levels of these metallic analytes were detected.

In addition a botanical survey of the spelling paddock used for the Hendra horses was undertaken. The only known toxic species with significant populations in the pasture were *Cestrum parqui*, Solanum spp, *Gomphocarpus physocarpus*

and *Bryophyllum tubiflorum* (R. A. McKenzie, unpublished data). None of these species have been associated with a predominantly respiratory disease such as EMV.

### **Viruses**

Negative test results were obtained by the AAHL for African horse sickness, equine influenza, equine viral arteritis, and for hantavirus. The equine encephalitides were eliminated on the basis of negative brain histology (P. J. Ketterer, unpublished data). However, the virus now known as EMV was isolated in cell culture from lung and spleen from 4 of the 6 horses on which virus isolation was attempted. Isolates from 2 of these horses were made by AAHL and 2 by the Animal Research Institute, Yeerongpilly, Brisbane. An identical virus was isolated from the kidney of the first human fatality (Selvey *et al.* 1995).

Positive transmission tests (Murray *et al.* 1995), presence of SN antibodies in 7 exposed and recovered horses and in 2 human contacts with infected horses confirmed EMV as the aetiological agent. High titres of SN antibody to EMV were also detected in the fatal human case at Mackay (Allworth *et al.* 1995), which led to the retrospective study of horse fatalities on the property 12 months previously.

### **DISCUSSION**

EMV has, to date, presented as an acute respiratory disease of horses. A similarly acute disease has been reproduced experimentally in cats and guinea pigs (Westbury *et al.* 1995). However, 2 of the Hendra horses developed neurological signs and the farmer from Mackay died from an acute severe encephalitis 12 months after his apparent exposure to EMV. Because EMV is classified, at this stage at least, as a morbillivirus it is not unreasonable to postulate that it will also have a predilection for neural tissue as it is in the same group as measles and distemper viruses.

The 3 known cases of human infection which have occurred all had close contact with acutely ill horses and it has been assumed that this is when transmission took place. However, the limited spread in the Hendra stables clearly suggests that EMV is a lowly transmissible disease. Because the virus multiplies in pulmonary vascular endothelium leading to increased vascular permeability and pulmonary oedema, it is likely that the foamy nasal discharge contains high titres of virus. Virus has also been detected in the urine of experimentally infected cats (Westbury *et al.* 1996), but it is not known if this also occurs in horses. Clearly further

work is necessary to elucidate transmission mechanisms for this disease.

The source of EMV is also a matter for conjecture at this stage. It seems likely that it has a natural reservoir host which extends over at least 800 km of the Queensland coastline. Recent evidence of SN antibodies to EMV in *Pteropus* species of fructivorous bats with an antibody prevalence up to 20% (Young *et al.* 1996) supports this theory.

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