A Novel Morbillivirus Pneumonia of Horses and its Transmission to Humans

To the editor: On September 22 and 23, 1994, veterinary authorities in Queensland and at the CSIRO Australian Animal Health Laboratory were advised of an outbreak of acute respiratory disease in horses at a stable in the Brisbane suburb of Hendra. The trainer of the horses had been hospitalized for a respiratory disease and was in critical condition. At that time, the cause of the horses' illness was unclear and any link between equine and human disease was thought improbable. Poisoning, bacterial, viral, and exotic disease causes were investigated. The history of the horses on this property was considered important (Figure 1). Two weeks before the trainer's illness, on September 7, two horses had been moved to the Hendra stable from a spelling paddock in Cannon Hill (6 km). One of these, a pregnant mare, was sick and died within 2 days. The other horse was subsequently moved on and never became sick. By September 26, 13 horses had died: the mare; 10 other horses in the Hendra stable; one horse, which had very close contact with horses in the Hendra stable, on a neighboring property; and one which had been transported from the stable to another site (150 km). Four Hendra horses and three others (one in an adjacent stable, one moved to Kenilworth, and one to Samford) were later considered to have been exposed and recovered from the illness. Some of these horses were asymptomatic. Nine Hendra horses have remained unaffected. The sick horses were anorexic, depressed, usually febrile (temperature up to 41°C), showed elevated respiratory rates, and became ataxic. Head pressing was occasionally seen, and commonly, a frothy nasal discharge occurred before death.

On September 14, a stablehand at the Hendra stable developed an influenza-like illness characterized by fever and myalgia. The next day, the horse trainer also became ill with similar symptoms. Both had close contact with the dying mare, particularly the trainer who was exposed to nasal discharge while trying to feed her; he had abrasions on his hands and arms. The stablehand, a 40-year-old man, remained ill for 6 weeks and gradually recovered. Besides myalgia, he also had headaches, lethargy, and vertigo. The trainer, a 49-year-old man, was a heavy smoker and showed signs consistent with *Legionella* infection. He ultimately required ventilation for respiratory distress and died after 6 days (Selvey L, et al. A novel morbillivirus infection causing severe respiratory illness in humans and horses, submitted).

At the beginning of the diagnostic investigation in horses, African horse sickness, equine influenza, and hyperacute equine herpes virus were excluded as possible causes by antigen trapping enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), or electron microscopy. Tests for *Pasturella*, *Bacillus anthracis*, *Yersinia*, *Legionella*, *Pseudomonas*, and *Streptobacillus moniliformis* were negative, and poisons consistent with the clinical and gross pathology, such as paraquat, were excluded by specific testing.

However, within 3 days, a syncitial forming virus was detected in vero-cell cultures inoculated with diseased horse tissues and shortly thereafter was seen to grow in a wide range of cells. These included MDBK, BHK, and RK13 cells. Subsequently, a syncitial forming virus also was isolated in LLK-MK2 cells that had been inoculated with tissue from the deceased trainer's kidney. The isolation of these viruses and their preliminary characterization by electron microscopy, immunoelectromicroscopy, serology, and genetic analyses are described elsewhere (Murray PK, et al. A new morbillivirus which caused fatal disease in horses and man, submitted).

In summary, ultrastructural analysis showed that the virus is a member of the *Paramyxoviridae* family. It is enveloped, pleomorphic (varies in size from 38 nm to more than 600 nm), and is covered with 10 nm and 18 nm surface projections. It contains herringbone nucleocapsids that are 18 nm wide with a 5 nm periodicity. The presence of 'double-fringed' surface projections on this virus is considered unique. Immunoelectron microscopy showed that both the horse and the human virus react with convalescent-phase horse sera and with sera from the two human cases.

PCR primers were synthesized from consensus *Paramyxoviridae* matrix protein sequences and tested against the horse virus. Those specific for paramyxoviruses and pneumoviruses did not bind, but one pair of morbillivirus primers gave a 400 bp product. Determination of the sequence of this product enabled the synthesis of horse virus-specific primers. Phylogenetic analyses of the matrix protein sequence indicates that the virus is unique and distantly related to other known members of the group. A comparison of translated M protein sequence shows that it has a 50% homology with the morbillivirus group (80% if conservative amino acid substitutions are used). This distant relatedness is emphasized by our observations that neutralizing antisera to measles virus, canine distemper, and rinperest virus failed to neutralize the virus.

The viruses isolated from the horses and the trainer are ultrastructurally identical. Serum from

September 1994								
	7	**9**	**13**	**14**	**15**	**16**	**17**	**19-26**
Horses								
Cannon Hill (Paddock)	2 horses moved							
Hendra (Stables)		Mare died				2 horses moved		10 horses dead 4 recovered
Hendra (Neighboring property)		1 horse moved						1 horse dead 1 recovered
Kenilworth (150 km distant)								1 horse dead 1 recovered
Samford (Paddock)								1 recovered
			New South Wales					
Humans								
Stablehand				Becomes ill				Slow recovery
Trainer					Becomes ill		Hospitalized	Died

Figure 1. A chronology of the development of cases of acute equine respiratory disease and associated illness in humans.

the horses and the two human cases specifically cross-neutralize the virus, and the horse virus-specific PCR primers provide a positive reaction with the human virus isolate. Therefore, the horses and the trainer were infected with the same virus.

At the beginning of the diagnostic investigation, tissues from the lungs and spleens of diseased horses were injected into two recipient horses. After 6 and 10 days, the recipient horses became ill with high fever and severe respiratory signs, demonstrating that the disease was transmissible. Two days later the horses were destroyed. The equine morbillivirus was isolated from tissues from both of these horses. To document that the isolated horse virus was pathogenic, experimental transmission tests were also conducted. Two additional horses received a total of 2×10^7 TCID50 of tissue culture virus by intravenous inoculation and by intranasal aerosol. Both horses became seriously ill, and after a short, severe clinical episode, were destroyed 4 and 5 days after exposure. At necropsy, they showed gross and histopathologic lesions that were primarily respiratory and consistent with the natural disease. Virus was reisolated from their lungs, liver, spleen, kidney, lymph nodes, and blood.

The pathology of this infection is interesting. In horses, the dominant gross pathology is lesions in the lungs. These are congested and edematous with prominent lymphatic dilation in the ventral margins. In natural cases, the airways were usually filled with thick, fine, stable foam which was occasionally blood-tinged; this was not seen in the experimental cases. Histologically, in horses, there is interstitial pneumonia, proteinaceous edema with pneumocyte, and capillary degeneration. Virus can be located in endothelial cells by immunofluorescence and syncitial cells also could be seen in blood vessel walls, confirming the vascular of trophism of this virus (Murray PK, et al, submitted). The trainer's post-mortem findings showed similarities to those of the horses (Selvey L, et al, submitted).

No further clinical cases of disease have been seen in horses or humans since this outbreak. Serologic surveillance of people who had close contact with the sick horses, mostly stable workers, veterinary pathologists, animal health field staff, or people who lived in the vicinity of the affected stables, was negative (Selvey L, et al, submitted).

Serologic testing of all horses on quarantined properties and within 1 km of the Hendra stable, and a sample of horses from the rest of Queensland was undertaken (Table 1). A total of 1,964 horses were tested from more than 630 premises. The negative results from this testing also indicate that the infection has not spread. In the entire horse survey, only seven horses, all from the Hendra property and the adjoining stables, were positive. Four of these animals had been clinically affected, but three were asymptomatic. Because of the potential risk and the difficulty in establishing freedom from infection, these seven recovered horses were later destroyed.

Although persistent virus excretion or carrier states are not known to occur in other morbillivirus infections, this equine virus is unique and it cannot be presumed to behave similarly. Australian veterinary authorities are now satisfied that the incident is over.

We have described a newly recorded disease of horses with an obvious zoonotic potential; moreover, the causative agent was previously unrecorded and is significantly different from other members of its genus, morbillivirus. Infection seems to have spread from the mare that first showed the now characteristic clinical signs, to other horses in the same stables, to a horse in close contact from an adjacent stable, and also to two human attendants. Clearly, this outbreak was not highly contagious and it rapidly resolved. However, the virus is highly pathogenic with 65% of naturally infected horses and all four experimental horses dying.

Further investigations of the virus and the disease are now warranted since it could reemerge in Australia or elsewhere. Investigations of its origin, its replication, its pathogenesis, and its possible occurrence elsewhere in connection with equine respiratory disease are merited.

Table 1. The premises and horses surveyed by serologic testing for equine morbillivirus, after the disease outbreak

	Premises	Horses
Quarantine Premises*	13	107
1 (within 100 m of Hendra stables)	7	54
2 (100 m to 200 m of Hendra stables)	21	122
3 (200 m to 1 km of Hendra stables)	93	730
4/5 (remainder of Queensland)	>500	963
Total	**>630**	**1,964**

*Quarantine premises included those with clinical cases, holding properties associated with the Hendra stables, and other premises where horses under investigation were kept.

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