



Diagnosing

David Mussared
reconstructs the
events which led
to CSIRO's
identification of
the virus that
brought
Queensland's
horse-racing
industry to a
standstill.

Last September in suburban Brisbane a brand new disease popped up out of nowhere. It killed a man and 14 horses, then vanished. No one knows where it came from; no one can be certain it won't be back.

The disease was caused by a virus, one never been recorded before. It could have been the start of a human or animal health disaster. Instead it seems to have disappeared.

What happened? Where did the virus come from? Why did it stop? Behind the screaming media headlines, an enthralling scientific detective story unfolded in Australia, as researchers in two states battled to assemble a jigsaw of microscopic pieces.

The pressures were terrific, and the stakes could not have been higher. While the scientists worked a man lay dying in a Brisbane hospital, perhaps from the mystery disease, and most of the Queensland racing industry was paralysed by quarantine to reduce the risk of spread. At worst the scientists knew they might be dealing with the start of a new human and animal epidemic: at best it was an isolated outbreak with no wider human or animal consequences.

The strange new disease, which now boasts the unremarkable working title of 'acute equine respiratory syndrome',

first appeared in early September. A horse transported from Cannon Hill, in Brisbane's outskirts, to Queensland trainer Vic Rail's Hendra stable, fell critically ill. Subsequently, other horses at Hendra also started falling ill, dying after a few days. Antibiotics had no effect. Then Rail himself, who had extremely close contact with the horses, fell ill. So did one of his stablehands.

The Queensland Department of Primary Industries (QDPI) first heard about the disease on Thursday, September 22. The CSIRO's Australian Animal Health Laboratory (AAHL) in Geelong was alerted. To the alarm of the Geelong scientists — whose job it is to protect Australia from exotic disease outbreaks — the symptoms of the Queensland horse-killer had all the hallmarks of African horse sickness.

AAHL laboratory head, Dr Keith Murray, says the Queensland horses suffered a high fever, with temperatures reaching up to 41°C. And, in another classic symptom of African horse sickness, the respiratory attack they suffered produced a frothy nasal discharge.

African horse sickness, which can kill up to 50% of the horses it infects, rates high on Australia's list of least wanted animal diseases. Once in the country it could easily be spread by native Australian insects, and might be impossible to eradicate. The disease is

a deadly equine virus

endemic in southern Africa, periodically spreading into north Africa and southern Spain, and up the Nile Valley into the Middle East.

Maximum security

QDPI immediately sent lung, spleen and blood samples from two of the stricken horses to Geelong. For transport the samples were packed into high-security, CSIRO-designed eskies built to survive anything from rough handling to a plane crash.

The samples arrived at 1.30 on the Friday morning at the animal health laboratory, the only Australian laboratory cleared to hold diagnostic samples of such dangerous diseases. The diagnostic team led by Dr Laurie Gleeson ran the Brisbane samples through a battery of rapid diagnostic tests. They used polymerase chain reaction (PCR) to probe them for the tell-tale DNA sequence of African horse sickness, and for another feared exotic horse disease, Equine Influenza. They also used highly sensitive ELISA (enzyme-linked immunosorbent assay) tests for antibodies to known viruses. All were negative.

By four in the afternoon, AAHL was able to say neither of the feared viruses was likely to have caused the disease. AAHL microscopist Alex Hyatt also reported he could not see any evidence of another suspect, equine herpes virus,

on his electron microscope images. Because of the danger the virus might infect humans, the scientists had to work on the samples in special isolation rooms and cabinets deep inside the high-security facility.

Murray says the AAHL and QDPI teams worked around the clock during the weekend. On Saturday, two live horses were brought into the Geelong laboratory and infected with the Queensland tissue samples to see if they too would develop the killer disease. By the end of the weekend QDPI researchers in Brisbane had virtually eliminated most possible causes — tests for herbicides, plant toxins and bacterial infections all came up negative.

By Monday morning the scientists in Queensland and Victoria knew they were probably dealing with unknown virus, but what was it?

Two new developments provided clues. By Monday, cell cultures that Paul

Selleck had infected with the Queensland samples were showing dramatic signs that something was going on. Holes appeared in the tissue layer. Many cells were fused together, with multiple nuclei, into large cells called *syncidia*, characteristic of some virus infections.

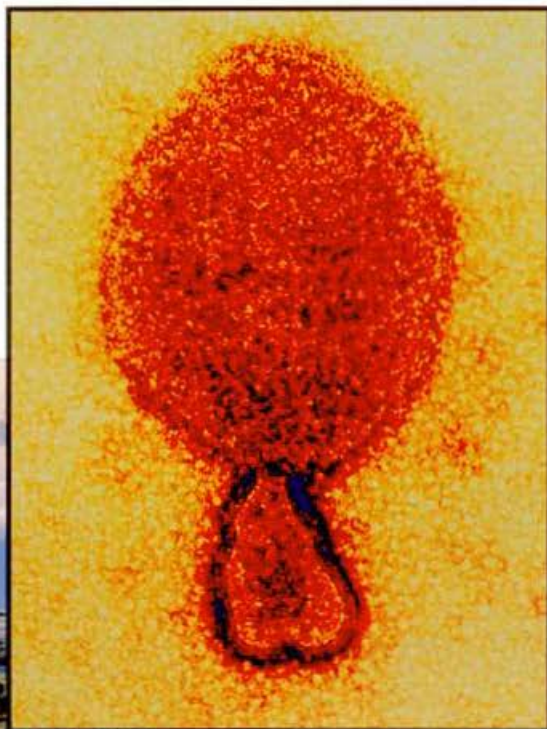
A virus revealed

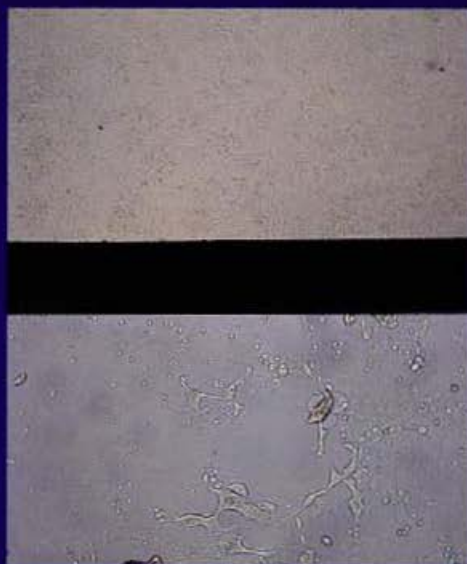
Meanwhile Hyatt kept up his 'very-small game hunting' with the electron microscope, searching the infected cells to try and catch sight of a virus. On Tuesday he saw one. He spotted small, plump blobs on the electron micrographs; blobs which he recognised as viruses wrapped in cell membrane after budding off from a cell.

Hyatt used detergent to wash away the envelopes, exposing the naked viruses. Under the enormous magnification of the electron microscope, the herringbone pattern of the virus's

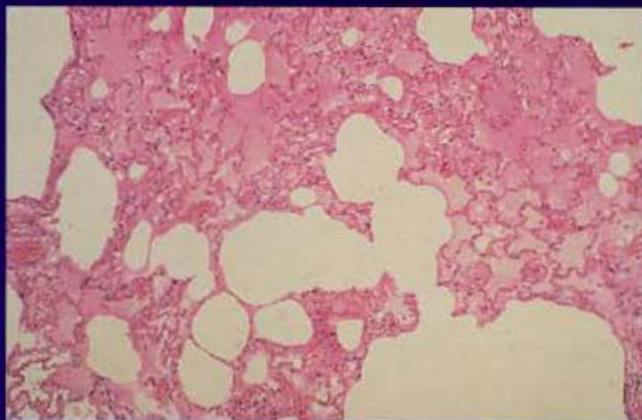
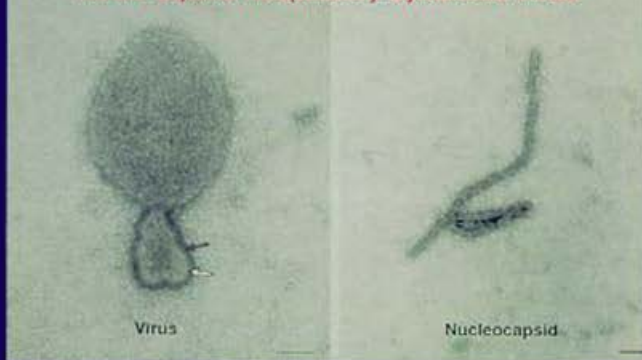
Right: A computer-enhanced image of Acute Equine Respiratory Syndrome Virus.

Below: The CSIRO Australian Animal Health Laboratory is the national centre for foreign animal disease testing, research and training. Staff from the laboratory worked around the clock to identify the cause of Queensland's mystery horse illness.





Acute Equine Respiratory Syndrome Virus



Top: The first signs of the virus in tissue culture. Top: normal tissue layer. Bottom: holes in the tissue layer and signs of cells joining together into large cells with multiple nuclei. Something was going on . . .

Middle: . . . Under the magnification of the electron microscope, the tell-tale herringbone pattern of the virus's protein coat was revealed. It was one of the Paramyxovirus family, related to the virus which causes human diseases such as measles and mumps.

Above: Four horses were deliberately infected with the virus at the AAHL laboratory. A week later, they showed unmistakable signs of the disease. In lung sections from the infected horses, some normal air spaces can be seen. Others are congested, full of a pink, bloody fluid. The horses die as this fluid fills their lungs.

protein coat told him what he needed to know. It was one of the Paramyxovirus family; related to the virus which causes human disease like measles and mumps, and animal diseases such as Newcastle disease and distemper. As a further piece to the puzzle, syncytia are a hallmark of Paramyxovirus infections.

Immediately, another AAHL scientist, Dr Allan Gould, set about cloning the virus, and sequencing its RNA.

The same Tuesday morning, September 27, Vic Rail died in a Brisbane hospital, but there was still no proven link with the horse deaths.

On Wednesday QDPI announced that AAHL had found a virus, but it was still not clear whether it was one of the 20 to 30 known Paramyxovirus viruses, or something altogether new. Nor could the scientists say for certain that the virus had killed the horses, or whether it was just there by coincidence. Two fresh horses were brought into the AAHL laboratory and deliberately infected with the isolated virus.

By Saturday — a week after they had been infected — the first two AAHL horses showed unmistakable signs of the Queensland disease. Within a few days the second pair of test horses also fell ill. Undoubtedly the mystery virus was responsible. Meanwhile Queensland health authorities, after analysing the CSIRO results, changed their verdict and announced that the horse virus had contributed to Rail's death.

In just 10 days, most of the pieces had fallen into place. The cause — a virus — had been confirmed and no more cases reported. But were there more cases yet to be found?

With the virus identified, AAHL was able to develop quick tests both for the virus and for antibodies to the virus using serum neutralisation and indirect fluorescent antibody tests. During the following week the QDPI sent more than 2500 horse blood samples and 150 human blood samples. There were no surprises, no new cases. The good news is that the virus, however virulent, was infectious, but not highly contagious.

Only one piece of the mystery remains to be solved: where did the virus come from? Gould's sequencing work showed that it was a morbillivirus, one of the three Paramyxovirus genera. Other morbilliviruses cause diseases like measles, distemper, rinderpest and — interestingly — phocid distemper.

Phocid distemper is a new virus, infecting seals, which emerged suddenly just a few years ago, causing huge numbers of seal deaths in the northern hemisphere. Some scientists believe the virus might have jumped somehow from dogs to northern hemisphere marine mammals. Others say it was an existing, low-level seal disease which suddenly spread and turned deadly.

But the Brisbane virus wasn't just a slightly altered version of distemper or rinderpest; it was quite different. So different that it must have come from somewhere else.

QDPI epidemiologist Chris Baldock now has the unenviable task of trying to track down the source of the virus. The original stricken horse came from Cannon Hill, where it might have picked up the virus either from natural or human contact. The virus might be endemic in some Australian native species, from which by a freak accident it could have crossed into the horse. Comprehensive testing of the animals in the Cannon Hill area will hopefully provide some answers.

The truth might never be known. But if the virus ever appears again the scientists now have the tools they need to identify it quickly. The incident has shown that Australia has the capability to quickly and comprehensively handle unused animal disease occurrences. It has also served as a warning for everyone who works with horses to be aware of unusual disease signs in their animals. Think the worst first, and call a vet.