

The discovery of Bungowannah virus – an example of the need for conventional and new technologies



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A novel disease in pigs and another new virus – so where is the flying fox connection? This was one of the first questions that many observers asked from the sidelines. In this instance there was no known connection with flying foxes, no suggestion of human illness but, as the investigation unravelled, a probable cause was identified – an apparently new virus that had close connections to an important pig pathogen that is exotic to Australia. This virus was, however, so genetically different from its relatives that pan reactive polymerase chain reaction-based assays would not detect it. It was so different antigenically that pan reactive monoclonal antibody panels would react with it. Nevertheless, a combination of simple agar gel immunodiffusion tests for antibodies and sophisticated nucleic acid amplification and sequencing methods proved to be the keys to the recognition of this virus.

In June 2003, a company veterinarian contacted a microbiologist at the Elizabeth Macarthur Agriculture Institute (EMAI) to discuss a problem with an increasing incidence of deaths in 3 to 6-week-old piglets. The caller was referred to the Virology Laboratory as the problem did not appear to involve a bacterial aetiology. Infection with encephalomyocarditis (EMC) virus (a cardiovirus that causes outbreaks of sudden death) was considered a likely cause. Although EMC virus is readily isolated in cell culture, no agent was identified in tissues from affected pigs. Over the next 8 months, the problem escalated, with the disease progressively moving through different production modules on the first affected farm and later to a second farm owned by the same company. These two farms were quarantined and the disease was

not observed anywhere else in New South Wales. The disease was evident as elevated numbers of stillborn foetuses, increased deaths in piglets prior to weaning at about 3-4 weeks of age and, to a lesser extent an increase in the number of mummified foetuses following death earlier in gestation. At the peak of the outbreak, up to 50% of piglets were lost¹. Pathological changes in tissues of affected piglets were confined predominantly to the heart and included acute to subacute multifocal, nonsuppurative myocarditis and myonecrosis. A wide range of recognised porcine viral and bacterial pathogens were excluded during diagnostic investigations and numerous attempts to isolate a virus in cell culture were unsuccessful. However, elevated immunoglobulin G (IgG) levels were identified in almost 50% of stillborn piglets indicating an *in utero* infection, probably of viral aetiology, was the likely cause.

Although not a natural route of infection for transmission studies, direct foetal inoculations were undertaken, utilising tissue extracts of lung and heart tissue taken from field cases of porcine myocarditis syndrome (PMC). This approach was adopted to maximise the chances of foetal infection and to bypass the placental barrier and any possible effects of the maternal immune response. A number of infected piglets were identified, with evidence of transmission of an agent to uninoculated foetuses in the same litter. Serum collected from foetuses in one infected litter was utilised for random amplification of nucleic acid sequence independent single primer amplification (SISPA) and sequences of a ribonucleic acid (RNA) virus, related to flaviviruses from the *pestivirus* genus, were detected. Using 'primer walking' techniques, the entire sequence of a novel pestivirus was identified with a proposal that it be recognised as a new species in the genus *pestivirus*². The name Bungowannah virus was adopted, after the location where the first clinical cases were observed. Phylogenetic analysis of the 5'UTR, N^{pro} and E2 coding regions showed this virus to be the most divergent pestivirus identified to date, with its closest relative Classical swine fever virus, which is a major pathogen of pigs in many countries but is exotic to Australia.

Extensive studies, based on the examination of material collected from field cases, support the hypothesis that Bungowannah virus is the causative agent of PMC³. Following the identification of a pestivirus, it was thought that ongoing detection of the virus and antibodies would be uncomplicated, due to the availability of pan

reactive assays for pestivirus RNA and pan reactive monoclonal antibodies. The genetic and antigenic diversity of this virus soon proved that this would not be the case and an inability to detect the virus in cell culture proved an additional complication. Nevertheless, using firstly the agar gel immunodiffusion (AGID)-based IgG assay to detect total foetal IgG and later an AGID to detect antibodies with limited cross-reactivity with other pestiviruses, it was possible to undertake basic antibody assays. Serological studies demonstrate a strong temporal association between the presence of disease on an affected unit and the detection of seropositive animals. Bungowannah virus-specific real-time RT-PCR and *in situ* hybridisation assays were developed and Bungowannah virus RNA was identified in stillborn pigs from the affected farms. A peroxidase-linked serological assay demonstrated that the elevated IgG in stillborn pigs is against Bungowannah virus.

Subsequently, it has been possible to amplify this virus in cell culture and detect antigens by immunoperoxidase staining using polyclonal antisera. Virus neutralisation assays are also available. Current research efforts are directed at the development of recombinant antigens for Bungowannah virus, together with appropriate antisera and their use in diagnostic enzyme-linked immunosorbent assays (ELISAs). These serological tools will allow studies to be initiated in the future to address the most intriguing and outstanding question – where did this virus come from? A series of animal transmission studies are progressing to definitively prove that Bungowannah virus is the cause of this high impact disease in pigs.

In conclusion, there are many aspects of this disease outbreak that send important messages. Like many of the emerging diseases, it has highlighted the need to have scientists equipped to undertake basic investigations of epidemiology, disease pathogenesis and transmission. There is also another salutary warning not to abandon the basic skills of diagnostic virology and immunology but on the other hand shows how these can be combined exquisitely with molecular biology tools to help unravel a problem. However, without simple AGID assays that provided key leads, in this instance, it is quite likely that the cause of this disease would remain undetected.

References

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