# Evidence of a novel orthobunyavirus from the Simbu serogroup in New South Wales, Australia, from 2012

**T. Tung**[1, 2] (tiffany.tung@sydney.edu.au), D. Finlaison[2], P. Kirkland[2], C. Hornitzky[2], A. Ellliott[2], P. Hick[2], J.-A. Toribio[1]

[1] *Faculty of Science, Sydney School of Veterinary Science, The University of Sydney, Camden, Australia*
[2] *EMAI, Virology laboratory, NSW DPIRD, Menangle, Australia*

**Summary**

Orthobunyaviruses from the Simbu serogroup are arthropod-borne viruses that can cause congenital malformations and neurological disease in ruminants, leading to significant economic losses. In Australia, the National Arbovirus Monitoring Program (NAMP) conducts surveillance for veterinary-important arboviruses, including testing of a sentinel cattle herd network across the country. In 2017, a novel orthobunyavirus from the Simbu serogroup (K902) was isolated from sentinel cattle in northern New South Wales (NSW), Australia. This virus had not previously been detected in Australia, raising concerns about its emergence. To establish the timeline for the introduction of K902 into NSW, a two-stage survey of archived sera from sentinel cattle was carried out: first, a targeted presence survey; and, if no antibodies were found, testing was expanded to evaluate freedom using a design prevalence of 2.5% with 95% confidence. Evidence of K902 transmission could be detected in NSW since 2012. The serosurvey of archived NAMP samples from 2000 to 2011 also confirmed no evidence of K902 before 2012. This supports the absence of K902 transmission during the decade preceding its first detection in the NSW sentinel herd network. The undetected transmission of K902 for five years prior to its initial isolation underscores the importance of long-term surveillance systems for the detection of emerging arboviruses. These surveillance systems, together with an extensive sample archive, provide valuable opportunities and resources for the characterisation of novel and previously unidentified veterinary-important arboviruses.

**Introduction**

Several orthobunyaviruses in the Simbu serogroup are emerging and re-emerging teratogenic viruses that can severely impact livestock industries. Schmallenberg virus (*Orthobunyavirus schmallenbergense*) emerged in Europe in 2011-2012, resulting in widespread outbreaks of congenital malformations in cattle, sheep, and goats [1]. In addition to Schmallenberg virus, five other viruses in this serogroup have been recognised to cause congenital malformations or neurological disease in livestock [2]. Akabane (*Orthobunyavirus akabaneense*), Aino (*Orthobunyavirus ainoense*), Shuni (*Orthobunyavirus shuniense*), Peaton (*Orthobunyavirus peachesterense*) and Shamonda (*Orthobunyavirus schmallenbergense*) viruses have all been associated with disease in ruminants.

Surveillance for these veterinary-important arboviruses is critical to maintaining international trade in livestock and animal products [3]. To monitor virus transmission, surveillance programs employ strategies such as light trapping of insect vectors and sentinel herd surveillance [4]. Longitudinal testing through sentinel herd surveillance can detect the timing of infection within defined regions and timeframes. Across Australia, Akabane virus has been monitored using longitudinal sampling of naïve sentinel cattle herds since the 1980s [4]. Since 1993, the sentinel herd network has been formalised nationally, and the transmission patterns of important veterinary arboviruses have been monitored by the National Arbovirus Monitoring Program (NAMP) [4].

In 2017, an orthobunyavirus from the Simbu serogroup (K902) was isolated from cattle in a sentinel herd in northern NSW. The isolation of K902 prompted a critical question: How long has this virus been present in NSW?

**Materials and Methods**

*New South Wales sentinel herds and sample collection*
Across NSW, the NAMP recruits approximately 30–40 sentinel cattle herds annually over a 10-month period from October to July, corresponding with seasonal arbovirus vector activity [4]. Each herd comprises at least ten property-born cattle, typically around six months of age at initial sampling in October [4]. Herds are strategically selected across the state to define the Bluetongue virus transmission zone, with blood samples collected monthly to quarterly, depending on location and risk [5]. Following collection, clotted blood samples were transported to the NSW Department of Primary Industries and Regional Development (NSW DPIRD) Virology Laboratory. Serum was separated by centrifugation (3000 × g for 5 min at 4 °C), stored at 4 °C prior to testing, and archived at −20 °C for long-term storage [6].

*Virus neutralisation test for K902 antibodies*

Detection of K902 antibodies was performed using a virus neutralisation test (VNT) following a standard protocol [7]. Briefly, serum samples were heat-inactivated at 56 °C for 30 min and incubated with a working stock of K902 (1.8–2.5 $\log_{10}$ $TCID_{50}$ per 50 µL) at 37 °C for 1 h prior to the addition of HmLu-1 cells. Plates were incubated at 37 °C for 120 hours and subsequently examined for cytopathic effect.

*Stage 1: Targeted survey for K902 antibodies in archived samples from 2005-2018*
The samples from the archived collection were selected based on the following criteria to maximise the likelihood of detecting antibodies against an unknown orthobunyavirus from the Simbu serogroup: 1) Samples were from animals within a NSW sentinel herd where seroconversion was observed in at least one animal during the monitoring period using a Simbu serogroup-reactive ELISA; 2) The sample was collected on the final collection date of the monitoring period for the sentinel herd; and 3) The samples have 'unexplained' antibodies against an orthobunyavirus in the Simbu serogroup, testing positive in the group-reactive ELISA and negative for the known endemic viruses in this serogroup by virus neutralisation test (Akabane, Aino, Douglas, Peaton, and Tinaroo).

*Stage 2: Demonstration of the absence of transmission of K902*
The freedom survey was also completed on the archived samples from the NSW sentinel herds. The required sample size was calculated as 102 samples in each monitoring period to achieve 95% confidence at a design prevalence of 2.5% [8].

**Results**

The targeted survey indicates that K902 was present in the NSW sentinel herd network from 2012 to 2018 (Table 1). From 2005 to 2018, 362 (82.4%) of the selected samples were available for testing from the sentinel archive. From 2012 to 2018, K902 antibodies were detected in a mean proportion of 70.2% of tested samples per monitoring period (range: 22.2-85.4). No antibodies were detected in targeted samples collected between 2005 and 2012. Consistent with this finding, no K902 antibodies were detected by VNT in 1,409 samples collected between 2000 and 2011 as part of the freedom survey (Table 2).

**Discussion**

This study used archived sentinel herd sera to establish a serological timeline for the introduction of a novel orthobunyavirus, K902, into NSW. No serological evidence of K902 infection was detected from 2000 to 2011. The freedom survey followed parts of the study design previously used for Schmallenberg virus, with a prevalence threshold of 2.5% [9]. Although the number of samples required to fully meet this survey design could not be tested for 2011, the samples available were sufficient to support the absence of K902 in NSW sentinel herds at a prevalence of ≥3% with 95% confidence.

Overall findings supported the absence of K902 transmission prior to 2012. However, interpretation should consider two inconclusive reactors identified in 2000 and 2001 (Table 2). These inconclusive samples detected early in the study period are most plausibly explained by serological cross-reactivity with other endemic Simbu serogroup viruses, reflecting the limitations of VNT interpretation in regions where multiple orthobunyaviruses co-circulate [6, 10].

Testing targeted samples with a higher likelihood of demonstrating antibodies against an unknown orthobunyavirus enabled efficient detection once K902 was present, consistent with the risk-based design of the NSW sentinel herd network, in which herds are selected from locations optimised for arbovirus surveillance [4-6]. While the seroprevalence of K902 cannot be estimated from this study, these results provide the first serological evidence of a novel orthobunyavirus from the Simbu serogroup circulating in Australia from 2012. Continued detections from 2012 indicate several years of undetected K902 transmission prior to virus isolation in 2017. These findings highlight the challenges of identifying newly introduced orthobunyaviruses from the Simbu serogroup in the absence of reported disease and reinforce the importance of long-term surveillance systems for detecting emerging arboviruses.

Table 1. Results from the virus neutralisation tests (VNT) for antibodies against K902 as part of the detection survey using targeted samples from sentinel herds in New South Wales during each monitoring period (2005-2018).

| Year | No. of eligible samples | No. tested in the VNT | VNT results | | | |
|---|---|---|---|---|---|---|
| | | | Positive | Inconclusive | Negative | No result |
| 2005 | 7 | 7 | 0 | 0 | 7 | 0 |
| 2006 | 29 | 21 | 0 | 0 | 21 | 0 |
| 2007 | 38 | 29 | 0 | 0 | 29 | 0 |
| 2008* | 0 | 0 | | | | |
| 2009 | 57 | 46 | 0 | 0 | 46 | 0 |
| 2010 | 24 | 22 | 0 | 0 | 22 | 0 |
| 2011 | 13 | 12 | 0 | 0 | 12 | 0 |
| 2012 | 37 | 9 | 7 | 2 | 0 | 0 |
| 2013 | 55 | 48 | 41 | 6 | 1 | 0 |
| 2014 | 35 | 34 | 20 | 6 | 7 | 1 |
| 2015 | 20 | 18 | 10 | 2 | 5 | 1 |
| 2016 | 32 | 27 | 6 | 5 | 14 | 2 |
| 2017 | 46 | 43 | 22 | 7 | 11 | 3 |
| 2018 | 46 | 46 | 15 | 9 | 14 | 8 |
| *Total* | 439 | 362 | 121 | 37 | 200 | 15 |

* For this detection survey, samples from the monitoring period ending in 2008 could not be included because VNT results were unavailable to identify eligible samples.

Table 2. Results from the virus neutralisation tests (VNT) for antibodies against K902 as part of the freedom survey to demonstrate the absence of this virus at a design threshold of ≥2.5% prevalence with 95% confidence using archived samples from sentinel herds in New South Wales during each monitoring period (2000-2011).

| Year | No. of eligible samples | No. tested in the VNT | VNT results | | | |
|---|---|---|---|---|---|---|
| | | | Positive | Inconclusive | Negative | No result |
| 2000 | 196 | 120 | 0 | 1 | 119 | 0 |
| 2001 | 274 | 117 | 0 | 1 | 116 | 0 |
| 2002 | 237 | 119 | 0 | 0 | 119 | 0 |
| 2003 | 312 | 111 | 0 | 0 | 111 | 0 |
| 2004 | 259 | 113 | 0 | 0 | 112 | 1 |
| 2005 | 247 | 121 | 0 | 0 | 121 | 0 |
| 2006 | 216 | 136 | 0 | 0 | 131 | 5 |
| 2007 | 251 | 121 | 0 | 0 | 113 | 8 |
| 2008 | 273 | 111 | 0 | 0 | 111 | 0 |
| 2009 | 347 | 108 | 0 | 0 | 92 | 16 |
| 2010 | 393 | 135 | 0 | 0 | 112 | 23 |
| 2011 | 381 | 97 | 0 | 0 | 97 | 0 |
| *Total* | 3386 | 1409 | 0 | 2 | 1354 | 53 |

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**Keywords:** Arbovirus surveillance, Disease freedom, Orthobunyavirus, Serosurvey, Simbu group viruses.

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