

Distribution of epizootic haemorrhagic disease virus transmission in NSW: 2008/09 to 2017/18

Project Final Report to
Animal Health Australia

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Abbreviations

BTV	bluetongue virus
EHD	epizootic haemorrhagic disease
EHDV	epizootic haemorrhagic disease virus
ELISA	enzyme linked immunosorbent assay
NAMP	National Arbovirus Monitoring Program
NC	negative control
OD	optical density
PI	percentage inhibition
qRT-PCR	real-time polymerase chain reaction
SD	standard deviation
SGV	Simbu group viruses

Introduction and Objectives

Epizootic haemorrhagic disease (EHD) is an arboviral disease of wild and domestic ruminants that is transmitted between hosts by species of *Culicoides* biting midges. EHD virus (EHDV) belongs to the genus Orbivirus, family Reoviridae and shares many characteristics with other members of the genus including bluetongue virus (BTV). Seven serotypes (1-2 and 4-8) of EHDV are currently recognised of which six have been detected in Australia (1, 2, 5, 6, 7 and 8)[1]. The previously described EHDV-3 is now included in serotype 1[1].

While EHDVs are capable of infecting wild and domestic ruminants they have predominantly been associated with disease in wild cervids, in particular white-tailed deer of North America and in cattle in the Middle East region and sometimes in Japan. When observed, clinical signs are like those of severe bluetongue in sheep and may range from peracute illness with death often occurring within 36 hours (particularly white-tailed deer) to a more chronic illness with animals remaining unwell for several weeks. Overseas, severe clinical signs have also been observed in cattle infected with the Ibaraki strain (EHDV-2) and more recently due to serotypes 6 and 7[1]. In cattle, the disease is characterised by fever, anorexia, ulcerative stomatitis, swelling of eyelids, respiratory distress, nasal and ocular discharge, redness and scaling of muzzle and lips, lameness, erythema of the udder and difficulty swallowing (Ibaraki disease). Naturally occurring disease due to EHDVs have not been recorded in Australia[2,3].

As EHDV is an arbovirus, its distribution is limited to the ecological range of the competent *Culicoides* vectors. In NSW, the principal vector of bluetongue virus and the Simbu group viruses (SGV) is *C. brevitarsis*, and it is also likely to be the principal vector of EHDV. The vector status of *C. wadai*, for EHDV is unknown. It has the potential to be a vector species but has only been detected on the north coast of NSW. While the distribution varies from year to year *C. brevitarsis* is detected annually in NSW along the coastal plain from the Queensland border to Nowra on the South Coast, through the Hunter Valley, on the North Tablelands, and variably on the North-west Slopes of northern NSW. Like bluetongue virus, transmission of EHDV is expected to occur in NSW during summer or autumn within a similar geographical range to BTV and SGVs. While the SGVs are more efficiently transmitted than BTV by *C. brevitarsis*, similar data for Australian EHDVs is not available.

The emergence of EHD in EHDV-free zones and reports of clinical disease in cattle from EHDV-free zones prompted the inclusion of EHD in the OIE list of notifiable diseases in May 2008[1]. Standards for trade are included in the OIE Terrestrial Code. Concern has been raised at the national level in Australia that EHDV may become more important in the trade context, particularly given its relationship to bluetongue virus.

In February 2018 the Department of Agriculture and Water Resource submitted a discussion paper on surveillance options to the National Arbovirus Monitoring Program (NAMP) for consideration. This study is the outcome of that paper.

Issues that were identified in the paper included:

- Having achieved OIE listing, EHDV may become more widely considered a biosecurity concern for trade.
- If countries were to require that livestock are sourced from a zone free of EHDV transmission, how would Australia provide export certification to meet this requirement?

- In south-eastern Australia, the only recognised competent vector for BTVs, SGVs and EHDVs is *Culicoides brevitarsis*. The distribution of all three virus groups will vary from year to year. It is generally observed that the detection of SGV seroconversion in cattle sentinel herds is a much more sensitive indicator of the presence of *C. brevitarsis* compared with BTV seroconversion. In the BTV free zone immediately adjacent to the BTV buffer zone it is often observed that SGVs are still transmitted between susceptible livestock.
- The BTV free zone is likely to approximate the EHDV free zone.
- Additional sentinel herd surveillance in NSW could be used to test the proposal to use the bluetongue free zone to also provide export certification for EHDV.
- A proposal to establish a Simbu group line (that could also be used as an EHDV line) was discussed with industry but considered risky in case it became a default BTV line and excluded farms situated in the currently BTV free zone where SGV transmission occurs.

Subject to undertaking additional surveillance, an additional zoning strategy that could be considered was:

- If the EHDV free zone is determined to be significantly smaller than the BTV zone, use the data collected through additional surveillance to develop a conservative fixed distance between the bluetongue surveillance zone and the zone that is almost certainly free from EHDV transmission. The two parallel lines could move synchronously in response to ongoing NAMP bluetongue surveillance. This method could also be considered for an Akabane transmission free zone.

The recommendation of the discussion paper was that NAMP surveillance in NSW include additional EHDV testing to test the hypothesis that the BTV zone can be used to also to provide export certification for EHDV. The study was limited to NSW because the southern range of *C. brevitarsis* on the east coast is within NSW. Testing of samples from NSW would allow a cost-effective approach to test this hypothesis because there are up to 40 sentinel herds monitored in any one season. Rather than undertake this prospectively, because NSW holds a large serum bank of NAMP samples, a retrospective serological survey could be carried out and is the subject of this report.

Therefore, the aim of this project was to determine the transmission range of EHDVs each year in NSW over a 10-year period and to determine if the BTV transmission zone is suitable for defining the EHDV transmission zone.

Methods

Study design

Samples collected as part of the NAMP during the monitoring seasons 2008/09 to 2017/18 were utilised for testing. NAMP samples for NSW are collected seasonally from October to June/July the following year to reflect the transmission season of the arboviruses being monitored. Clotted blood samples are collected at each sampling and the serum removed from the clot on arrival of the sample at the laboratory. Serum samples are initially stored at 4°C for short term storage and later transferred to frozen storage at approximately -20°C for long term archival purposes.

For each monitoring year the 'end of season' samples for each herd were tested. Sometimes, herds were lost from the program prior to June/July. In this case the serum collected at the last sampling for the season was utilised. As testing was only performed on 'end of season' samples, data was uploaded to the NAMP Database as serosurvey rather than sentinel herd data.

EHDV transmission for each monitoring period was compared with that of BTVs and the SGVs by mapping. Only seropositive results for EHDV or seroconversions for BTVs and SGVs were mapped on a season by season basis for this report.

Serology

A previously developed and evaluated blocking EHDV antibody ELISA was used for this study (E6 ELISA based on the mAb C31 and antigen derived from EHDV-6)[4].

Results were interpreted based on the percentage inhibition (PI) of the sample, calculated using the following formula (NC = negative control; OD = optical density):

$$PI = (\text{Mean NC OD} - (\text{Mean Sample OD} / \text{Mean NC OD})) \times 100$$

Two geographically unrelated populations of cattle (A: n=300; B: n=166) from regions known to be free of *C. brevitarsis* and EHDV were tested to determine test specificity and optimise the test reference intervals. Additionally, the reactivity of the NAMP samples used in this study from 2009-10, 2010-11, 2011-12, 2015-16, 2016-17 and 2017-18 were plotted in a frequency histogram.

Based on results of this evaluation the EDHV ELISA results were interpreted as:

Negative:	PI < 50%
Inconclusive:	PI ≥ 50% to < 65%
Positive:	PI ≥ 65%

All serum samples were tested at a 1/10 dilution. Samples were initially screened for reactivity by testing in a mono-well format. Confirmatory testing in duplicate was performed for any sample with a PI ≥ 40%. Inconclusive results were not interpreted as evidence of previous infection for the purpose of this study.

Mapping

EHDV serology results were uploaded to the NAMP database as serosurvey results with the period of susceptibility determined by the number of months after 1st December in the monitoring season that the samples tested were collected.

The maps included in this report were produced from supplied latitude and longitude data for the PIC of the relevant sentinel herds. For each monitoring year the seropositive herds (EHDV) or herds where seroconversions were detected (BTV and SGV) were mapped to show the extent of transmission for each virus on a year to year basis.

Outlier results

For any results that appeared to be geographically different to the distribution of SGV seroconversions for a particular season or geographically remote from other EHDV seropositive herds, the NAMP database was probed to assess *C. brevitarsis* detections for that season both at that study site and nearby locations. Additionally, *C. brevitarsis* detections at selected sites between 1990 and 2021 were reviewed as required.

No molecular testing was performed as part of this study to clarify if these outlier results were true infections or false positive serological results. While a reference collection of NAMP sera is retained indefinitely, red blood cell samples (from clotted or EDTA treated blood) from animals not infected with BTV are not retained beyond the season in which they were collected

Results

Determination of test cut-off values

A marked difference was observed in the reactivity in the EHDV ELISA of samples from the two geographically distinct EHDV free populations. For Population A (n=300) the mean PI in the ELISA was 8.2% (SD=12.24), while for Population B (n=166) the mean PI in the ELISA was 31.3% (SD=18.69). Figure 1 is a frequency histogram of all results from these two populations. The positive cut-off for the EHDV ELISA was set at $PI \geq 65\%$ as this gave the optimal specificity outcome for the more 'reactive' samples in Population B (Figure 2). The negative cut-off was set at $PI < 50\%$ as 98.3% of all samples in population A gave a negative result at this value. Based on a cut-off of 50%, this ELISA has a specificity of 93.8%, or 97.2% if inconclusive reactors are included in the negative population.

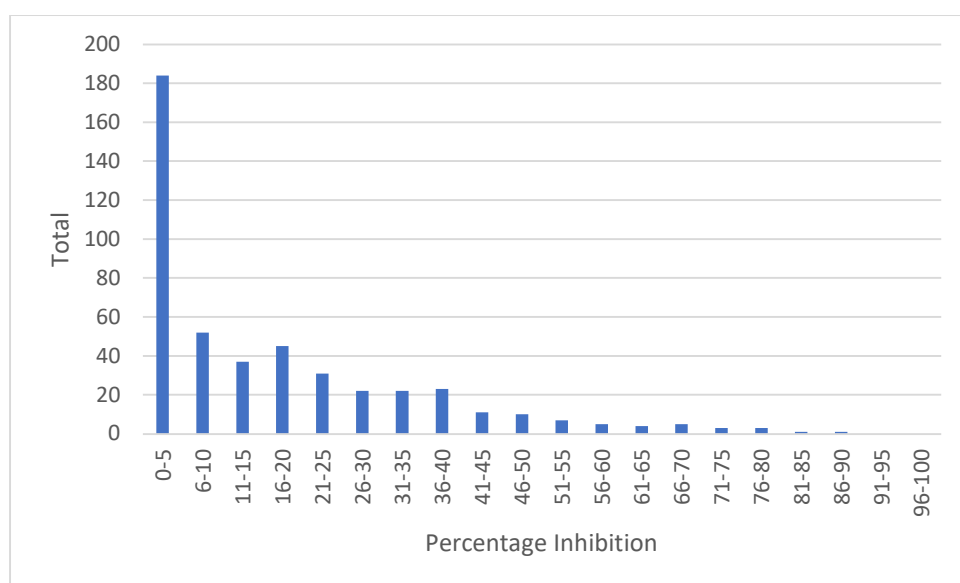


Figure 1: Frequency histogram of the PI for each sample tested from the two EHDV negative populations (n=466)

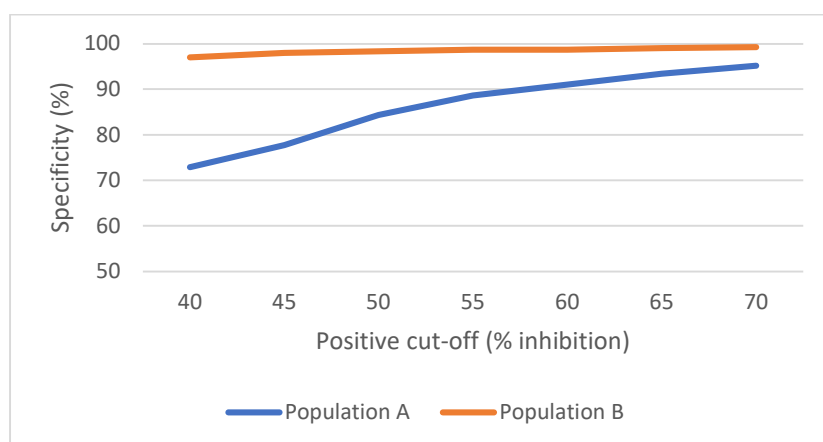


Figure 2: Comparison of the EHDV ELISA specificity comparing samples from two EHDV free populations.

When the reactivity of the NAMP samples used in this study from 2009-10, 2010-11, 2011-12, 2015-16, 2016-17 and 2017-18 (n=2493) was plotted in a frequency histogram, the samples split into two distinct groups, those with PIs <50 and those with PIs >75 (Figure 3), supporting the selected intervals for result interpretation.

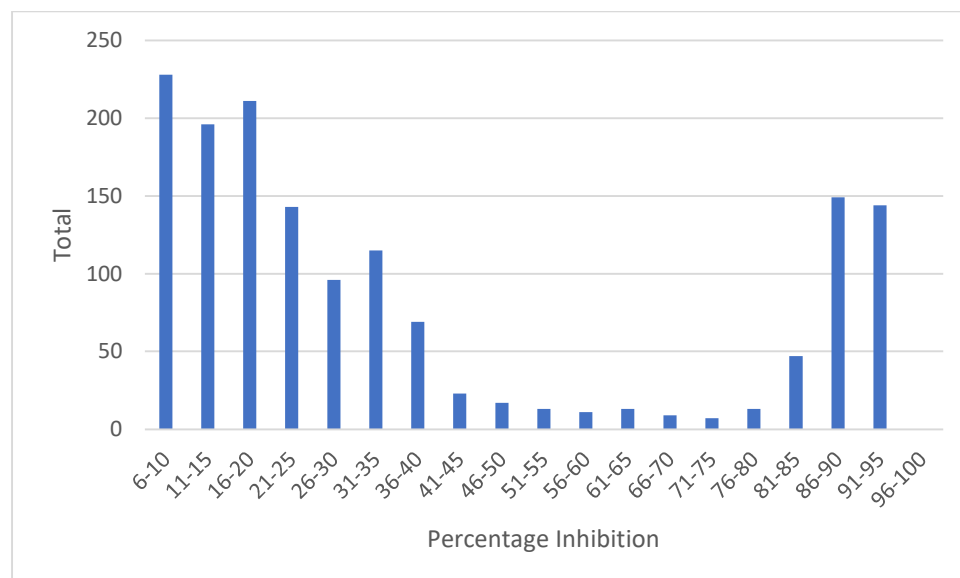


Figure 3: Frequency histogram of the PI values from all NAMP samples tested in this study from the years 2009-10, 2010-11, 2011-12, 2015-16, 2016-17 and 2017-18. Note that samples with PI values ≤ 5 (n=989) were excluded from the graph so that the frequency of the other results was more clearly illustrated,

Summary of samples tested

A total of 4172 samples were tested for the study (Table 1). The average number of herds and samples tested per year were 37 (range: 33 to 40) and 417 (range: 370 to 482) respectively.

Table 1: Summary of the number of herds and samples tested for each transmission season.

Year	Number of herds	Number of samples
2008/09	35	370
2009/10	33	372
2010/11	37	420
2011/12	36	400
2012/13	36	395
2013/14	36	417
2014/15	40	448
2015/16	40	439
2016/17	40	429
2017/18	40	482

Year by year summary of results

The full details of the number of samples tested and the number seropositive for EHDV by year and location are tabulated in the Appendix Tables A1 to A10. The number of animals in each sentinel herd monitored for BTV and SGV seroconversions is detailed. For BTV and SGV the cumulative number of seroconversions for each virus is documented. In some cases, the number of animals monitored for BTV and SGV seroconversions is greater than those tested for EHDV. This reflects animals that may have been removed or not sampled at the 'end of season' bleed.

2008-2009:

EHDV transmission was generally within the range of SGV transmission and limited to the coastal plain and Hunter Valley regions. EHDV was detected in the south near Sutton Forest adjacent to the southern-most detection of SGVs. While BTV transmission did not extend south of the Hunter Valley, EHDV was located at two sites, Richmond and Sutton Forest, ~135 and 250 km further south (Figure 4).

2009-2010:

Transmission of both EHDV and SGV was more extensive during 2009-2010 than the previous year (Figure 5). Evidence of EHDV transmission was detected along the coastal plain as far south as Milton on the South Coast, through the Hunter Valley and as far west as Bourke, Coonabarabran and Dubbo in inland NSW. BTV transmission was limited to the coastal plain, the eastern margins of the Great Dividing Range in the north, Hunter Valley and near Coolatai in the North-West Slopes region. Milton is ~320 km south of where the most southerly BTV transmission was detected at Paterson in the Hunter Valley. Bourke, where there was the most western detection of EHDV is ~460 km west of Coolatai.

SGV activity has not been recorded in sentinel herds near Bourke or Dubbo in the past 30 years and is uncommon at Coonabarabran. While *C. brevitarsis* has not previously been detected at the light trap site situated north of Bourke it has been detected ~150 km north at Cunnamulla in Queensland (1994, 1997, 2013 and 2019) and Akabane seroconversions were detected in the NAMP herd near Cunnamulla in October 2010, indicating that the EHDV detection may be a true seropositive. *C. brevitarsis* has only been detected on one occasion near Dubbo at Peak Hill in 2016. In 2010, *C. brevitarsis* was detected at Mudgee (approximately 100 km SE of Dubbo). *C. brevitarsis* has not been detected at Coonabarabran but has been detected nearby at Pilliga and Coonamble.

2010-2011:

BTV transmission in 2010-2011 was limited to the Far North Coast. EHDV transmission was more extensive and generally followed that of the SGV on the Northern Tablelands and North-west Slopes region. EHDV seropositive animals were also detected near Camden and Braidwood in the absence of SGV seroconversions and > 400 km south of the site of EHDV to the north on the Northern Tablelands (Figure 6). *C. brevitarsis* was not detected at the Camden monitoring site this season. Despite this, SGV transmission was detected about 50 km to the north at Richmond in the Sydney basin. No vector data is available at for the Braidwood site, but no significant vectors were detected nearby at Goulburn.

2011-2012:

EHDV transmission was limited to the Far North Coast, Northern Tablelands and North-west Slopes region, and, aside from the absence of BTV seroconversions on the Northern Tablelands, was similar to the distribution of BTV transmission for this season (Figure 7).

2012-2013:

EHDV transmission was detected at most coastal and Queensland border sites where BTV seroconversions were recorded (Figure 8) but was more extensive and consistent with the range of SGV than BTV transmission on the Northern Tablelands and North-west Slopes regions.

2013-2014:

There was extensive SGV, BTV and EHDV transmission during 2013-2014 (Figure 9) extending to South Coast herds. EHDV transmission mirrored the spread of BTV transmission except at Tamworth on the Northern Tablelands and at Bodalla on the Far South Coast.

2014-2015:

While there was extensive SGV transmission during this year, BTV and EHDV transmission was more limited (Figure 10). EHDV seropositive animals were detected in the North-west Slopes region, near Yarrowitch on the Northern Tablelands and then on the South Coast near Milton and Bega, while BTV transmission was only detected in a single herd on the Far North Coast.

The detection of EHDV in single animals at isolated sites on the South Coast at Milton and Bega during this season is consistent with the detections of *C. brevitarsis* and SGV seroconversions in this region. The distance (~475 km and) between these South Coast detections and transmission in the north of the state, suggests that there may have been persistence of virus from the previous season.

2015-2016:

EHDV seropositive animals were detected at most sites where BTV seroconversions were detected (Figure 11). In addition, EHDV seropositive animals were also detected further west (North-west Slopes) and south (Sydney basin and South Coast) than where BTV transmission was detected.

The detection of a single EHDV seropositive animal in each of the sentinel herds near Tamworth, Pilliga and Moree was not associated with detection of *C. brevitarsis* during this monitoring season.

2016-2017:

In all herds where there was detection of EHDV seropositive animals, SBV was also detected. These EHDV detections were mostly within the range of locations where BTV seroconversions were detected except for the Northern Tablelands where EHDV seropositive animals were detected independently of BTV activity (Figure 12).

2017-2018:

During 2017-2018 there was extensive SGV transmission and BTV seroconversions were limited to four sites in the north (Figure 13). EHDV seropositive animals were detected in three of four sentinel herds where BTV seroconversions were detected and also at selected sites across the full range of locations where SGV seroconversions were detected.

Single detections of EHDV seropositive animals were identified in the herds at Sutton Forest, Bodalla and Bega on the South Coast. These detections were significantly south of the next detections to the north in the lower Hunter Valley. No vector monitoring is performed at Sutton Forest but SGV transmission was detected at each of these three sites as was *C. brevitarsis* at Bodalla and Bega.

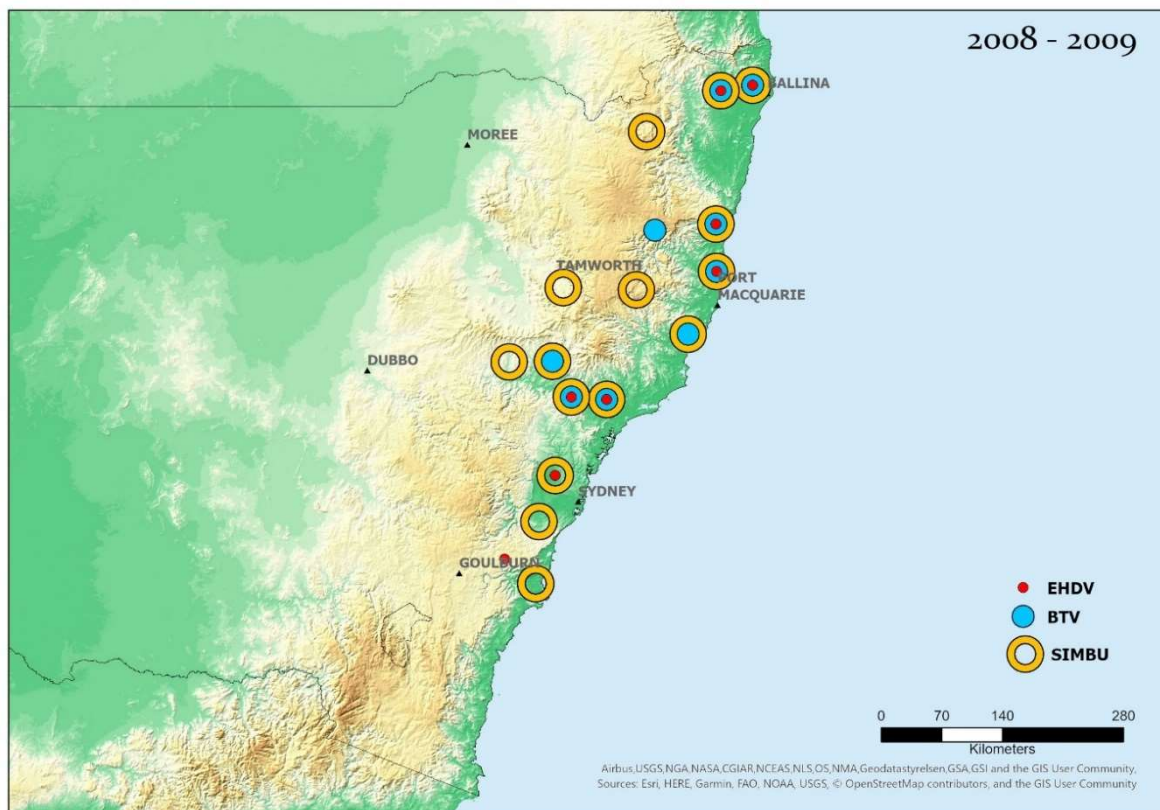


Figure 4: Location of NAMP sentinel herds with BTV and SGV seroconversions and EHDV seropositive animals for 2008-2009 monitoring season.

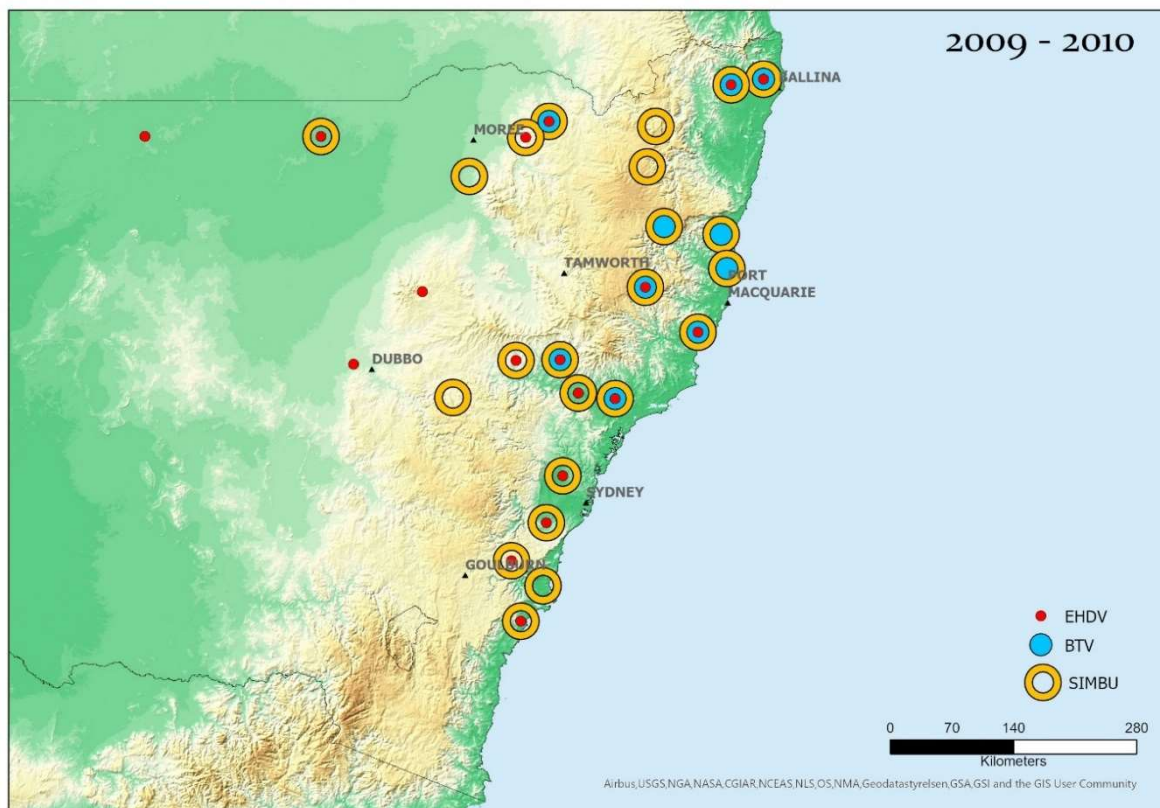


Figure 5: Location of NAMP sentinel herds with BTV and SGV seroconversions and EHDV seropositive animals for 2009-2010 monitoring season.

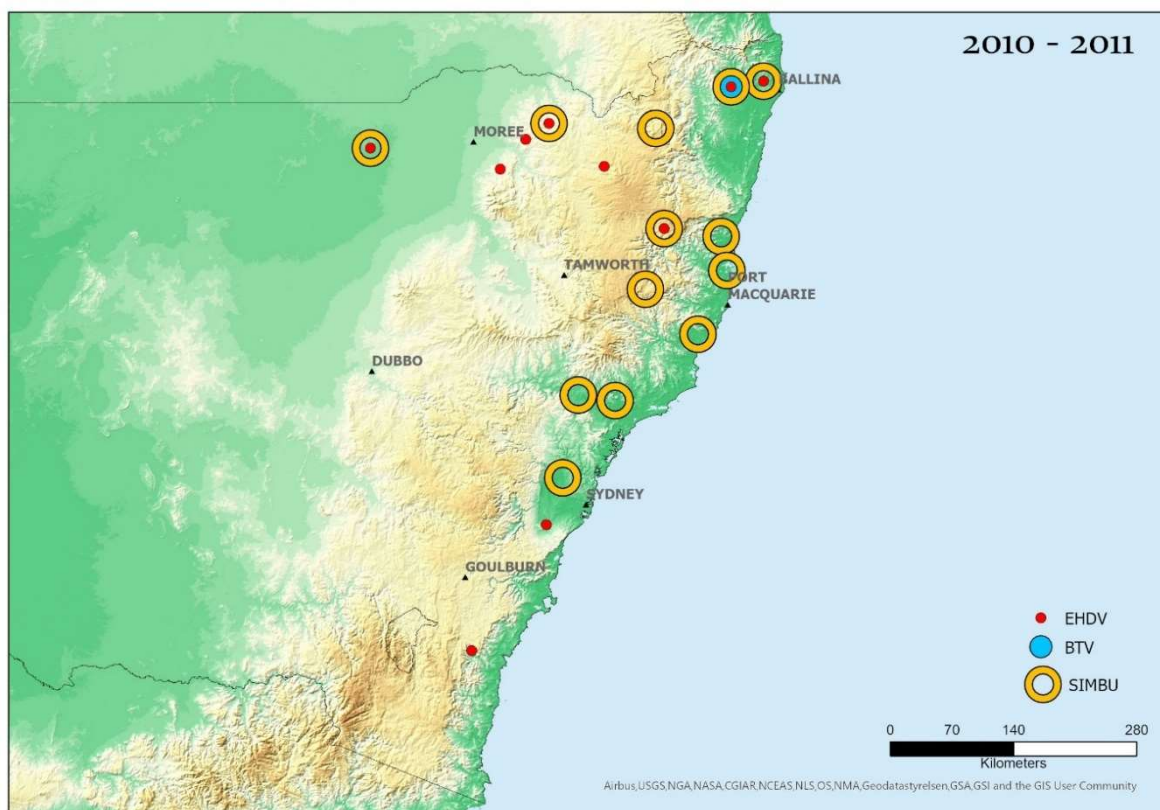


Figure 6: Location of NAMP sentinel herds with BTV and SGV seroconversions and EHDV seropositive animals for 2010-2011 monitoring season.

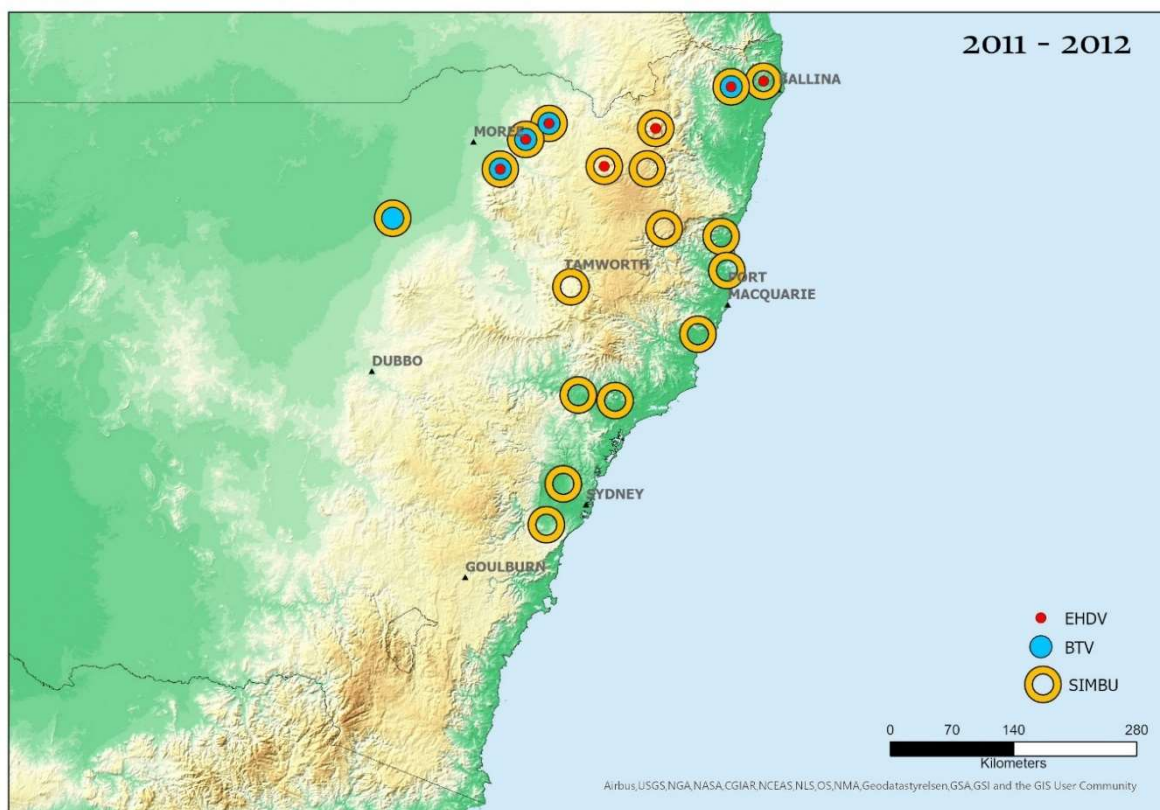


Figure 7: Location of NAMP sentinel herds with BTV and SGV seroconversions and EHDV seropositive animals for 2011-2012 monitoring season.

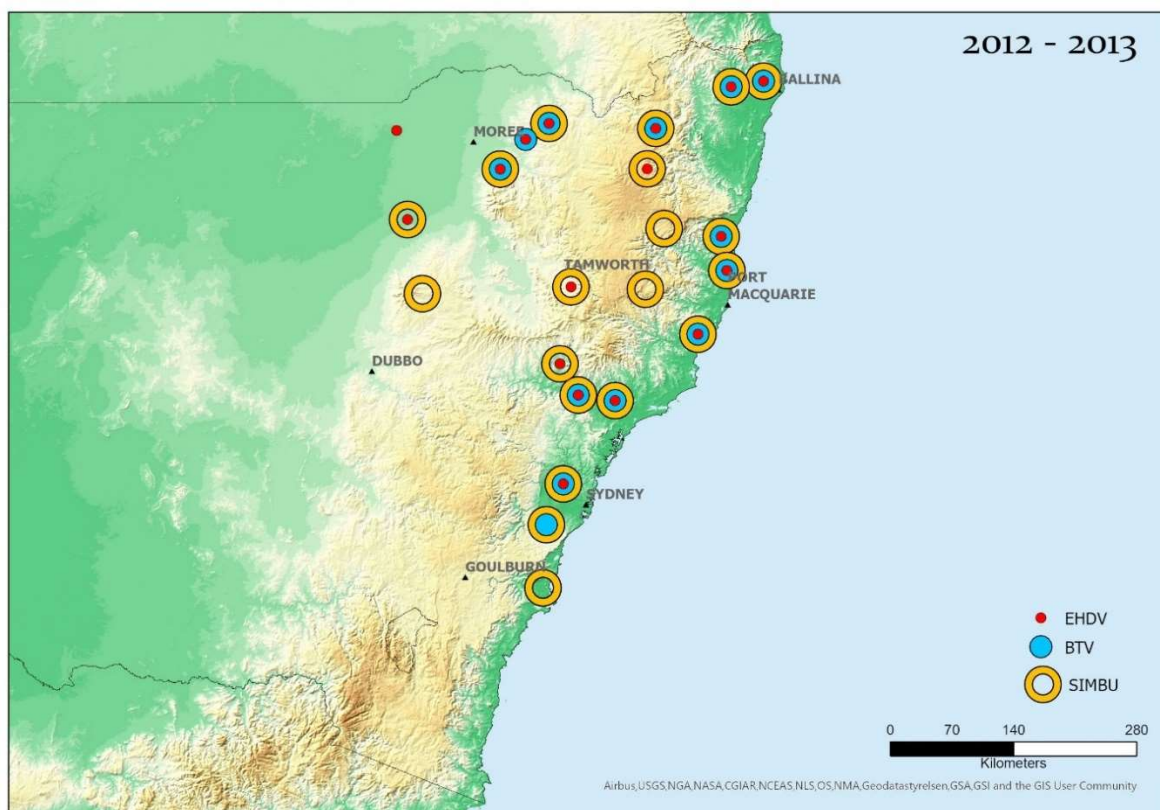


Figure 8: Location of NAMP sentinel herds with BTV and SGV seroconversions and EHDV seropositive animals for 2012-2013 monitoring season.

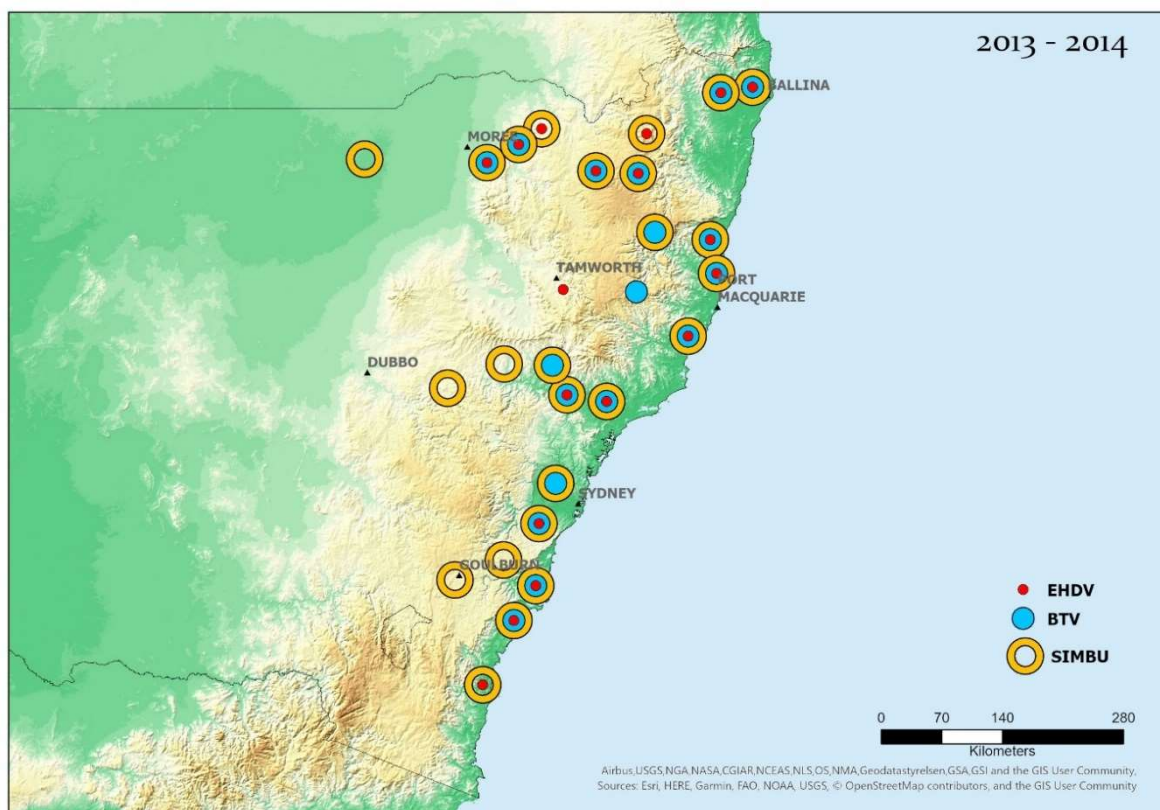


Figure 9: Location of NAMP sentinel herds with BTV and SGV seroconversions and EHDV seropositive animals for 2013-2014 monitoring season.

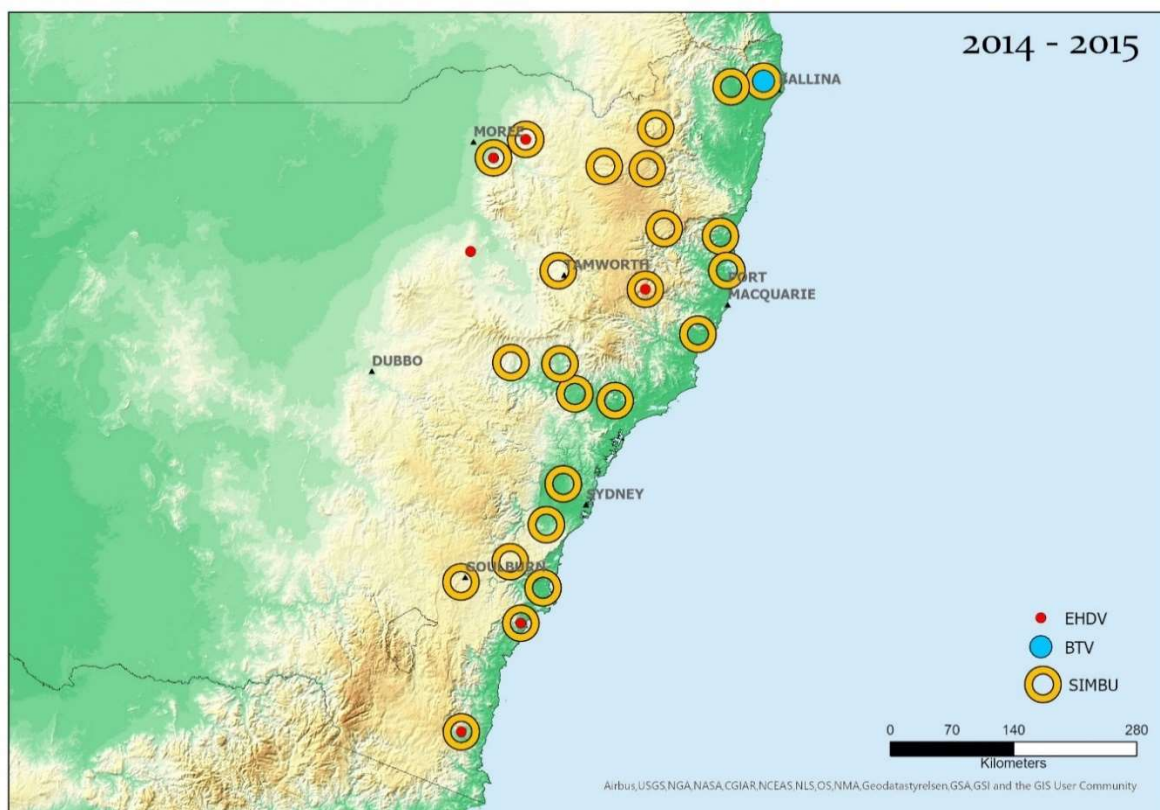


Figure 10: Location of NAMP sentinel herds with BTV and SGV seroconversions and EHDV seropositive animals for 2014-2015 monitoring season.

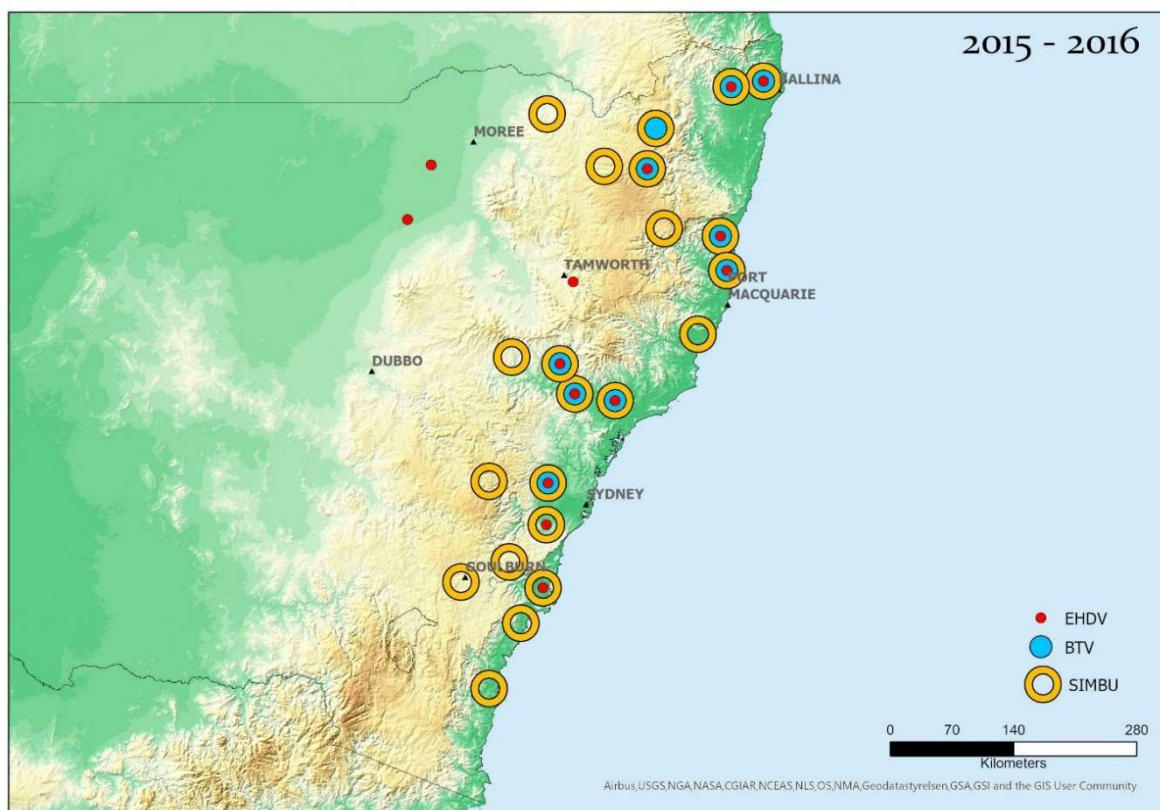


Figure 11: Location of NAMP sentinel herds with BTV and SGV seroconversions and EHDV seropositive animals for 2015-2016 monitoring season.

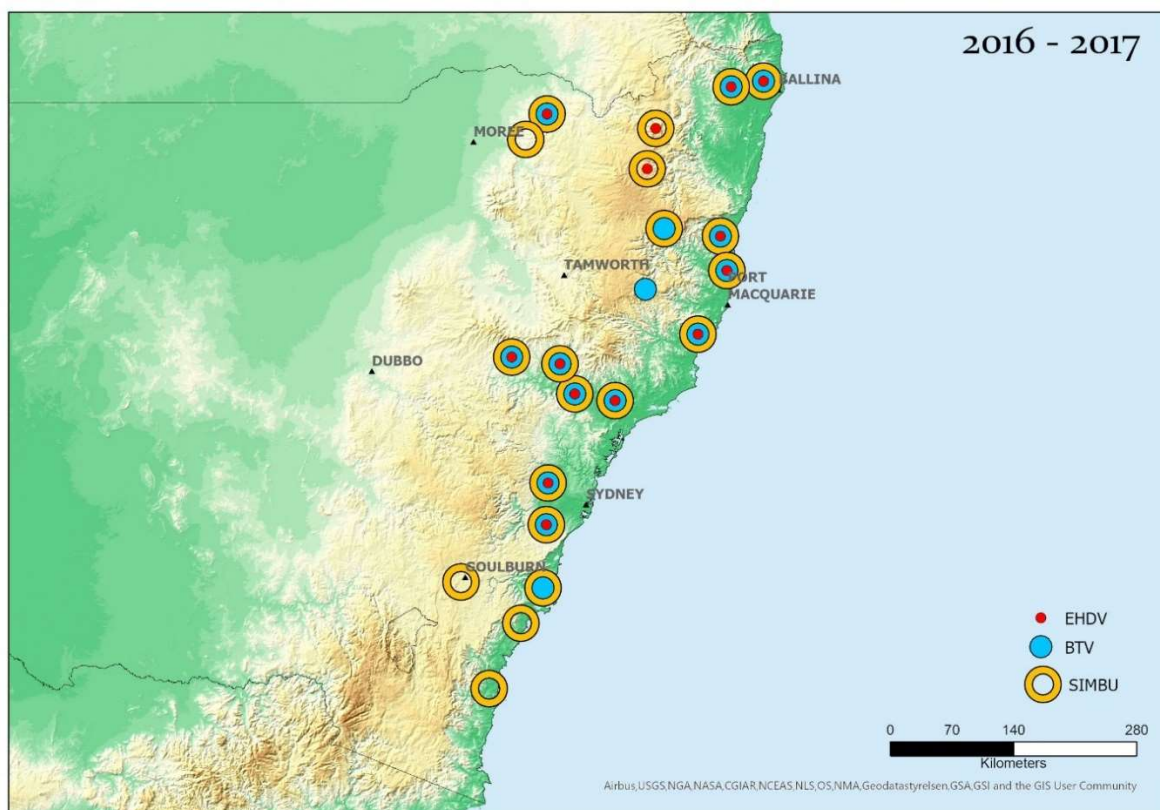


Figure 12: Location of NAMP sentinel herds with BTV and SGV seroconversions and EHDV seropositive animals for 2016-2017 monitoring season.

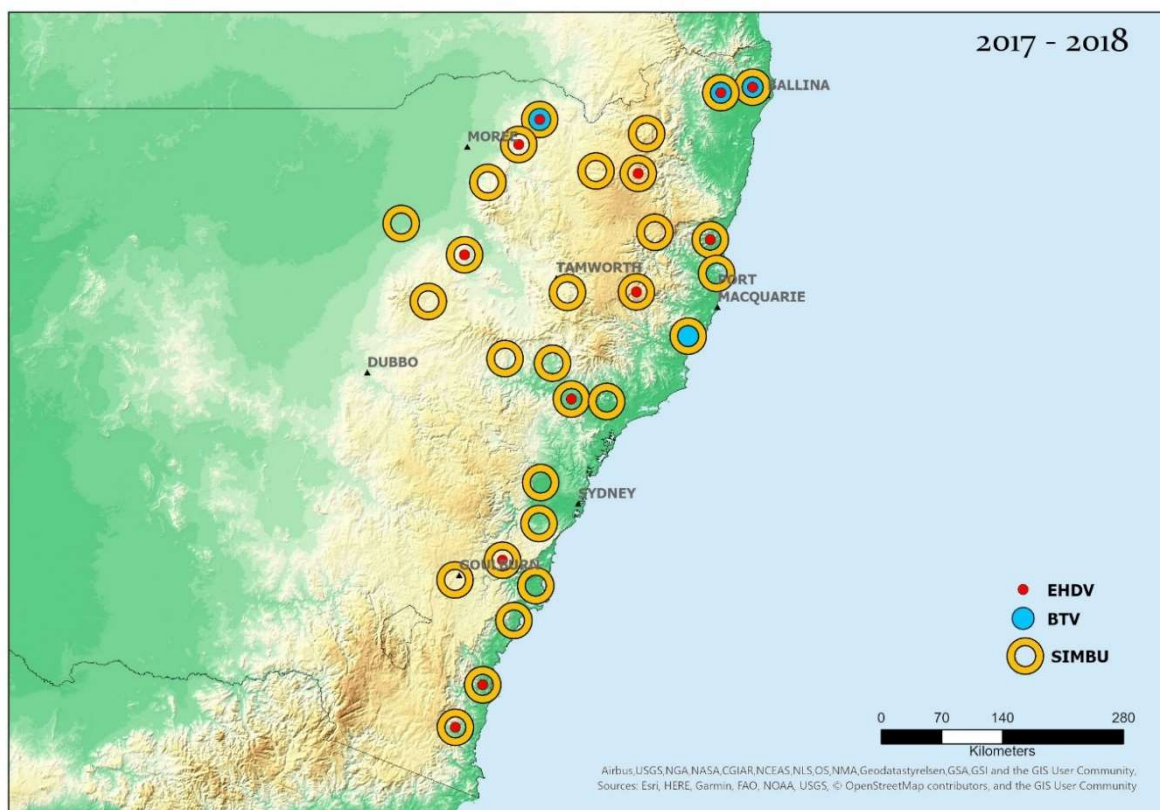


Figure 13: Location of NAMP sentinel herds with BTV and SGV seroconversions and EHDV seropositive animals for 2017-2018 monitoring season.

Discussion and conclusions

This study has compared the geographic distribution of BTV, EHDV and the SGV transmission over a ten-year period. As expected EHDV transmission was generally within the range of the SGV transmission and on some occasions paralleled BTV transmission. Despite this there were differences on a year to year basis where the geographic distribution of EHDV seropositive herds was more extensive than BTV particularly on the Northern Tablelands, North-west Slopes and South Coast regions. On this basis, the NAMP bluetongue virus transmission zone cannot be considered robust enough from a trade perspective to reflect all EHDV transmission that may have occurred in the 2-year period covered by the zone.

Where differences in transmission were identified between BTV, EHDV and SGV, this was typically observed at the margins of the ecological range of the vector (*C. brevitarsis*) – i.e. western parts of the North-west Slopes region, on the Northern Tablelands, upper Hunter Valley and South Coast. In general, the transmission range of EHDV appears to be more extensive than BTV. While SGV seroconversions were detected at more sites each year, these results suggest that the vector range may be better understood by the combined detections of SGV seroconversions and EHDV seropositive sentinel herds.

Failure to detect both SGVs and EHDV at the same site perhaps reflects the sensitivity of detection of lower levels of transmission at the margins of the vector distribution. It should also be noted that over this 10-year period, there have been changes to the assays used for SGV serology that may have impacted on SGV detections.

We do not believe that an EHDV transmission line should be used to define the BTV transmission zone. Based on the historical data presented, this approach would likely exclude properties situated in a BTV free zone. If an EHDV transmission zone becomes important to trade, we recommend that, at a minimum, sentinel herd monitoring be implemented at locations likely to be significant to trade. This should cover the range of the probable margins of the transmission zone and the free zone.

During the project EHDV seropositive animals were detected at some unexpected locations due to their distance from BTV and SGV seroconversions or from other EHDV seropositive herds. Based on the specificity of the EHDV ELISA these may reflect false positive results. Testing by EHDV PCR to resolve the infection status of these animals would have been optimal but clotted blood samples from the required animals in this time period are no longer available. Like BTV, EHDV is closely associated with circulating red blood cells. As a consequence, EHDV nucleic acid can be detected by PCR for as long as 6 months after initial infection. Confirmation of unexpected EHDV seropositive results by EHDV qRT-PCR is warranted as part of any future serological monitoring strategy. A similar approach as defined in the NAMP operations plan for BTV detections that occur in the surveillance or free zones that result in expansion of the transmission zone would be appropriate.

In conclusion, this report captures the transmission range of EHDV in NSW between 2008-09 and 2017-18 and indicates that the BTV transmission zone is not a suitable alternative for an EHDV transmission zone to meet international trading standards. Depending on the degree of precision required, a cost-effective monitoring strategy utilising strategic monitoring near the southern boundary of a potential EHDV transmission zone could be adopted.

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Appendix

Table A1: Summary of EHDV, BTV and SGV serology results for 2008-09.

Year: 2008-09			EHDV			BTV	SGV	No. of animals sampled for BTV/SGV
General Location	Site Num	Sampling date	No. of samples tested	Number seropositive	Monitoring period (months)	Total seroconversions	Total seroconversions	
Lismore	N200	11/06/2009	11	7	6	10	10	12
Casino	284	1/06/2009	10	3	6	8	7	10
Bellingen	N153	11/06/2009	10	10	6	9	7	10
Kempsey	N280	11/06/2009	10	10	6	6	10	10
Taree	N214	1/07/2009	10	0	7	6	9	10
Paterson	N011	29/06/2009	10	2	6	7	7	10
Singleton	N131	16/06/2009	10	9	6	10	8	10
Scone	N282	23/06/2009	10	0	6	1	10	10
Merriwa	N091	6/07/2009	10	0	7	0	1	10
Richmond	N193	29/07/2009	8	1	7	0	4	8
Camden	N078	12/06/2009	9	0	6	0	3	9
Sutton Forest	N284	2/07/2009	10	1	7	0	0	10
Goulburn	N087	30/07/2009	12	0	7	0	0	12
Nowra	N283	17/07/2009	11	0	7	0	1	11
Milton	N195	9/07/2009	10	0	7	0	0	10
Bodalla	N179	15/07/2009	10	0	7	0	0	10
Bega	N206	8/07/2009	10	0	7	0	0	10
Tenterfield	N135	27/10/2009	10	0	10	0	2	10
Glen Innes	N137	13/07/2009	10	0	7	0	0	10
Inverell	N213	13/05/2009	7	0	5	0	0	7
Tamworth	N209	8/09/2009	10	0	9	0	1	10
Armidale - Wollomombi	N149	23/07/2009	12	0	7	1	0	12
Yarrowitch	N173	1/07/2009	13	0	7	0	2	13
Coolatai	N120	24/07/2009	14	0	7	0	0	14
Warialda	N183	20/07/2009	11	0	7	0	0	11
Collarenebri	N210	1/07/2009	10	0	7	0	0	10
Narrabri	N199	11/06/2009	12	0	6	0	0	12
Coonabarabran	N285	10/06/2009	10	0	6	0	0	10
Bourke	N208	21/05/2009	15	0	5	0	0	15
Dubbo	N197	26/08/2009	9	0	8	0	0	9
Mudgee	N142	14/07/2009	10	0	7	0	0	10
Bathurst	N286	16/09/2009	10	0	9	0	0	10
Wagga Wagga	N105	9/06/2009	11	0	6	0	0	11
Yanco	N111	5/02/2009	15	0	2	0	0	15
Hay	N281	28/09/2009	10	0	9	0	0	10
TOTAL	35		370					

Table A2: Summary of EHDV, BTV and SGV serology results for 2009-10.

Year: 2009-10			EHDV			BTV	SGV	No. of animals sampled for BTV/SGV
General Location	Site Num	Sampling date	No. of samples tested	Number seropositive	Monitoring period (months)	Total seroconversions	Total seroconversions	
Lismore	N200	2/06/2010	12	12	6	1	11	12
Casino	284	5/07/2010	10	7	7	1	5*	10
Bellingen	N288	2/06/2010	10	0	6	7	10	10
Kempsey	N280	1/06/2010	10	0	6	2	10	10
Taree	N214	6/07/2010	10	5	7	5	7*	10
Paterson	N011	21/07/2010	10	10	7	2	10	10
Singleton	N291	8/07/2010	10	6	7	0	7	10
Scone	N282	7/06/2010	9	9	6	1	6	10
Merriwa	N091	29/06/2010	10	5	6	0	2	10
Richmond	N193	2/07/2010	10	9	7	0	4*	10
Camden	N078	8/07/2010	9	9	7	0	11	11
Sutton Forest	N284	21/06/2010	10	3	6	0	4	10
Goulburn	N087	2/06/2010	10	0	6	0	0	10
Braidwood	N287	28/05/2010	10	0	5	0	0	10
Nowra	N283	17/06/2010	11	0	6	0	1	11
Milton	N195	27/07/2010	10	1	7	0	2	10
Bodalla	N179	14/07/2010	10	0	7	0	0	10
Bega	N206	16/07/2010	10	0	7	0	0	10
Tenterfield	N135	15/07/2010	10	0	7	0	10	10
Glen Innes	N137	21/07/2010	10	0	7	0	5	10
Armidale - Wollomombi	N149	24/06/2010	12	0	6	8	12	12
Yarrowitch	N173	21/06/2010	13	1	6	3	11	13
Coolatai	N120	15/09/2010	11	10	9	3	11	11
Warialda	N183	2/07/2010	10	3	7	0	10	10
Moree	N155	8/04/2010	9	0	4	0	6	9
Collarenebri	N210	27/09/2010	18	2	9	0	1	18
Narrabri	N199	22/09/2010	18	0	9	0	0	18
Coonabarabran	N285	21/06/2010	10	1	6	0	0	10
Bourke	N208	9/02/2011	28	1	14	0	0	15
Dubbo	N197	24/06/2010	9	1	6	0	0	9
Mudgee	N142	30/06/2010	11	0	6	0	5	10
Wagga Wagga	N105	2/07/2010	12	0	7	0	0	12
Hay	N293	29/06/2010	10	0	6	0	0	10
TOTAL	33		372					

* all at risk animals seroconverted

Table A3: Summary of EHDV, BTV and SGV serology results for 2010-11.

Year: 2010-11			EHDV			BTV	SGV	No. of animals sampled for BTV/SGV
General Location	Site Num	Sampling date	No. of samples tested	Number seropositive	Monitoring period (months)	Total seroconversions	Total seroconversions	
Lismore	N200	9/06/2011	12	5	6	0	12	12
Casino	284	9/06/2011	13	6	6	11	13	13
Bellingen	N288	6/06/2011	8	0	6	0	10	9
Kempsey	N280	7/06/2011	10	0	6	0	10	10
Taree	N214	7/06/2011	10	0	6	0	10	10
Paterson	N011	27/06/2011	9	0	6	0	10	9
Singleton	N291	8/06/2011	9	0	6	0	10	9
Scone	N282	19/04/2011	10	0	4	0	0	10
Merriwa	N091	12/07/2011	10	0	7	0	0	10
Richmond	N193	24/06/2011	9	0	6	0	5	9
Camden	N078	23/06/2011	12	2	6	0	0	12
Sutton Forest	N284	15/06/2011	11	0	6	0	0	10
Goulburn	N087	21/07/2011	13	0	7	0	0	13
Braidwood	N287	11/01/2011	9	1	1	0	0	10
Nowra	N283	22/06/2011	11	0	6	0	0	11
Milton	N195	3/08/2011	10	0	8	0	0	10
Bodalla	N179	2/06/2011	10	0	6	0	0	10
Bega	N206	9/06/2011	10	0	6	0	0	10
Tenterfield	N135	11/07/2011	10	0	7	0	6	10
Glen Innes	N137	7/06/2011	10	0	6	0	0	10
Inverell	N213	6/05/2011	10	1	5	0	0	10
Tamworth	N209	17/06/2011	9	0	6	0	0	9
Armidale - Wollomombi	N149	28/06/2011	13	1	6	0	5	13
Yarrowitch	N173	4/07/2011	15	0	7	0	4	15
Coolatai	N120	4/07/2011	12	7	7	0	4	12
Warialda	N183	2/06/2011	12	5	6	0	0	12
Moree	N300	23/06/2011	17	4	6	0	0	17
Collarenebri	N170	3/06/2011	15	1	6	0	6	15
Narrabri	N199	27/06/2011	20	0	6	0	0	20
Coonabarabran	N285	28/06/2011	10	0	6	0	0	10
Bourke	N208	4/07/2011	13	0	7	0	0	13
Dubbo	N197	4/07/2011	10	0	7	0	0	10
Mudgee	N142	12/07/2011	10	0	7	0	0	10
Bathurst	N286	17/06/2011	12	0	6	0	0	12
Wagga Wagga	N105	2/06/2011	13	0	6	0	0	13
Yanco	N299	21/06/2011	14	0	6	0	0	14
Hay	N293	6/07/2011	9	0	7	0	0	9
TOTAL	37		420					

Table A4: Summary of EHDV, BTV and SGV serology results for 2011-12.

Year: 2011-12		EHDV				BTV	SGV	No. of animals sampled for BTV/SGV
General Location	Site Num	Sampling date	No. of samples tested	Number seropositive	Monitoring period (months)	Total seroconversions	Total seroconversions	
Lismore	N200	4/07/2012	11	2	7	0	11	11
Casino	284	10/07/2012	3	2	7	1	10	10
Bellingen	N288	4/07/2012	10	0	7	0	10	10
Kempsey	N280	12/07/2012	10	0	7	0	9	10
Taree	N214	13/07/2012	10	0	7	0	9	10
Paterson	N011	26/06/2012	10	0	6	0	6	10
Singleton	N291	14/06/2012	10	0	6	0	7	10
Merriwa	N307	10/08/2012	9	0	8	0	0	10
Camden	N078	27/06/2012	11	0	6	0	1	11
Richmond	N304	29/06/2012	12	0	6	0	1	12
Sutton Forest	N284	22/06/2012	12	0	6	0	0	12
Goulburn	N087	4/07/2012	12	0	7	0	0	10
Braidwood	N287	10/05/2012	8	0	5	0	0	10
Nowra	N283	28/06/2012	12	0	6	0	0	12
Milton	N195	18/07/2012	10	0	7	0	0	10
Bodalla	N179	3/07/2012	10	0	7	0	0	10
Bega	N206	24/07/2012	11	0	7	0	0	10
Tenterfield	N135	26/07/2012	10	1	7	0	3	10
Glen Innes	N137	1/06/2012	11	0	6	0	1	11
Inverell	N213	28/06/2012	8	1	6	0	1	8
Tamworth	N209	19/06/2012	10	0	6	0	1	10
Armidale - Wollomombi	N149	12/07/2012	12	0	7	0	5	12
Yarrowitch	N173	18/06/2012	12	0	6	0	0	12
Coolatai	N120	6/06/2012	15	15	6	15	9	15
Warialda	N183	19/06/2012	12	12	6	12	9	12
Moree	N300	14/06/2012	17	16	6	17	15	17
Narrabri	N305	14/06/2012	23	0	6	2	2	23
Coonabarabran	N285	12/06/2012	9	0	6	0	0	10
Wanaaring	N215	8/08/2012	14	0	8	0	0	14
Bourke	N208	23/05/2012	15	0	5	0	0	15
Dubbo	N197	22/06/2012	10	0	6	0	0	10
Mudgee	N142	15/08/2012	10	0	8	0	0	10
Bathurst	N286	17/07/2012	11	0	7	0	0	11
Wagga Wagga	N105	2/07/2012	10	0	7	0	0	10
Yanco	N299	14/06/2012	10	0	6	0	0	11
Hay	N293	30/05/2012	10	0	5	0	0	10
TOTAL	36		400					

Table A5: Summary of EHDV, BTV and SGV serology results for 2012-13.

Year: 2012-13		EHDV				BTV		SGV
General Location	Site Num	Sampling date	No. of samples tested	Number seropositive	Monitoring period (months)	Total seroconversions	Total seroconversions	No. of animals sampled for BTV/SGV
Lismore	N200	1/07/2013	11	11	7	11	10	11
Casino	284	9/07/2013	10	10	7	10	9	10
Bellingen	N288	16/07/2013	9	9	7	4	8	10
Kempsey	N280	8/07/2013	10	10	7	9	10	10
Taree	N214	4/07/2013	10	9	7	6	10	10
Paterson	N011	1/08/2013	10	6	8	2	11	10
Singleton	N291	10/07/2013	10	10	7	2	10	10
Scone	N181	15/07/2013	10	10	7	0	9	10
Richmond	N304	7/06/2013	12	12	6	2	12	12
Camden	N078	11/07/2013	10	0	7	1	5	10
Sutton Forest	N284	6/06/2013	11	0	6	0	0	11
Goulburn	N087	14/06/2013	12	0	6	0	0	12
Braidwood	N287	7/03/2013	10	0	3	0	0	10
Nowra	N283	5/07/2013	12	0	7	0	4	12
Milton	N195	2/07/2013	10	0	7	0	0	10
Bodalla	N310	2/07/2013	10	0	7	0	0	10
Bega	N206	4/07/2013	11	0	7	0	0	11
Tenterfield	N135	6/06/2013	10	2	6	3	4	10
Glen Innes	N137	5/06/2013	9	1	6	0	3	10
Inverell	N213	1/02/2013	10	0	2	0	0	10
Tamworth	N209	13/06/2013	10	8	6	0	10	10
Armidale - Wollomombi	N149	17/06/2013	14	0	6	0	11	14
Yarrowitch	N173	24/06/2013	13	0	6	0	5	13
Coolatai	N120	12/07/2013	15	2	7	3	8	15
Warialda	N183	12/07/2013	12	4	7	2	0	12
Moree	N300	11/07/2013	19	13	7	10	9	19
Collarenebri	N306	23/01/2013	8	4	13	0	0	9
Narrabri	N199	15/08/2013	12	2	8	0	13	12
Coonabarabran	N285	8/04/2013	10	0	4	0	2	10
Bourke	N208	4/08/2013	10	0	8	0	0	10
Dubbo	N197	3/07/2013	10	0	7	0	0	10
Mudgee	N142	5/08/2013	10	0	8	0	0	10
Bathurst	N286	17/05/2013	11	0	5	0	0	11
Wagga Wagga	N105	3/05/2013	15	0	5	0	0	15
Yanco	N299	25/06/2013	10	0	6	0	0	10
Hay	N293	11/06/2013	9	0	6	0	0	9
TOTAL	36		395					

Table A6: Summary of EHDV, BTV and SGV serology results for 2013-14.

Year: 2013-14		EHDV				BTV		SGV
General Location	Site Num	Sampling date	No. of samples tested	Number seropositive	Monitoring period (months)	Total seroconversions	Total seroconversions	No. of animals sampled for BTV/SGV
Lismore	N200	1/07/2014	12	9	7	7	12	12
Casino	284	1/07/2014	10	10	7	10	9	10
Bellingen	N288	3/07/2014	10	6	7	10	9	10
Kempsey	N280	2/07/2014	10	2	7	10	10	10
Taree	N214	8/07/2014	10	1	7	10	8	10
Paterson	N011	10/07/2014	10	3	7	10	11	10
Singleton	N291	30/06/2014	10	7	6	10	10	10
Scone	N181	22/07/2014	10	0	7	9	7	10
Merriwa	N307	17/07/2014	10	0	7	0	3	10
Richmond	N304	18/07/2014	12	0	7	12	9	12
Camden	N078	18/07/2014	12	6	7	11	12	12
Sutton Forest	N284	8/07/2014	11	0	7	0	1	11
Goulburn	N087	3/07/2014	13	0	7	0	2	13
Nowra	N283	7/07/2014	13	1	7	5	10	13
Milton	N195	2/07/2014	9	1	7	1	7	9
Bodalla	N310	30/06/2014	9	2	6	0	6	9
Bega	N312	23/06/2014	9	0	6	0	0	9
Tenterfield	N135	3/07/2014	10	1	7	0	3	10
Glen Innes	N137	29/07/2014	10	1	7	1	6	10
Inverell	N213	9/07/2014	9	1	7	2	2	9
Tamworth	N209	31/07/2014	10	1	7	0	0	10
Armidale - Wollomombi	N149	24/06/2014	12	0	6	1	3	12
Yarrowitch	N173	23/06/2014	13	0	6	5	0	13
Coolatai	N120	8/07/2014	12	5	7	0	6	12
Warialda	N183	14/07/2014	11	11	7	6	9	11
Moree	N300	9/07/2014	30	27	7	3	11	16
Collarenebri	N170	7/07/2014	8	0	7	0	6	8
Narrabri	N199	11/08/2014	16	0	8	0	0	16
Bourke	N208	29/07/2014	16	0	7	0	0	16
Wanaaring	N215	28/07/2014	15	0	7	0	0	15
Dubbo	N197	31/07/2014	10	0	7	0	0	10
Mudgee	N311	1/08/2014	10	0	8	0	2	10
Bathurst	N286	19/06/2014	12	0	6	0	0	12
Wagga Wagga	N105	16/06/2014	13	0	6	0	0	13
Yanco	N299	16/06/2014	10	0	6	0	0	10
Hay	N293	3/07/2014	10	0	7	0	0	10
TOTAL	36		417					

Table A7: Summary of EHDV, BTV and SGV serology results for 2014-15.

Year: 2014-15			EHDV			BTV	SGV	No. of animals sampled for BTV/SGV
General Location	Site Num	Sampling date	No. of samples tested	Number seropositive	Monitoring period (months)	Total seroconversions	Total seroconversions	
Lismore	N200	7/07/2015	11	0	7	1	10	11
Casino	284	7/07/2015	10	0	7	0	8	10
Bellingen	N288	15/07/2015	10	0	7	0	10	10
Kempsey	N280	14/07/2015	9	0	7	0	9	9
Taree	N214	2/06/2015	10	0	6	0	10	10
Paterson	N011	23/06/2015	11	0	6	0	10	10
Singleton	N291	3/06/2015	10	0	6	0	10	10
Scone	N181	26/06/2015	11	0	6	0	11	11
Merriwa	N307	1/07/2015	10	0	7	0	2	11
Richmond	N304	26/06/2015	9	0	6	0	10	8
Camden	N078	14/07/2015	11	0	7	0	12	12
Sutton Forest	N284	6/07/2015	11	0	7	0	2	11
Goulburn	N087	10/08/2015	9	0	8	0	4	9
Braidwood	N323	24/06/2015	10	0	6	0	0	10
Nowra	N283	3/06/2015	12	0	6	0	11	12
Milton	N195	16/07/2015	12	1	7	0	4	12
Bodalla	N310	30/07/2015	10	0	7	0	0	10
Bega	N312	29/07/2015	10	1	7	0	1	10
Tenterfield	N135	3/07/2015	8	0	7	0	3	8
Glen Innes	N137	23/06/2015	11	0	6	0	7	11
Inverell	N213	2/07/2015	10	0	7	0	1	10
Tamworth	N316	22/07/2015	11	0	7	0	1	11
Armidale - Wollomombi	N149	23/07/2015	14	0	7	0	10	14
Yarrowitch	N173	7/07/2015	11	1	7	0	5	11
Coolatai	N319	24/06/2015	15	0	6	0	0	15
Warialda	N183	24/06/2015	16	6	6	0	1	16
Moree	N300	24/06/2015	17	6	6	0	1	17
Collarenebri	N170	9/07/2015	12	0	7	0	0	12
Narrabri	N199	23/06/2015	14	0	6	0	0	14
Willala	N322	24/06/2015	16	1	6	0	0	16
Coonabarabran	N320	15/07/2015	9	0	7	0	0	9
Coonamble	N313	30/06/2015	10	0	6	0	0	8
Bourke	N208	1/09/2015	8	0	9	0	0	15
Wanaaring	N215	2/03/2015	15	0	3	0	0	15
Dubbo	N197	6/08/2015	10	0	8	0	0	10
Mudgee	N321	24/07/2015	9	0	7	0	0	9
Bathurst	N286	26/08/2015	12	0	8	0	0	12
Wagga Wagga	N105	22/06/2015	14	0	6	0	0	14
Yanco	N299	1/06/2015	12	0	6	0	0	12
Hay	N293	4/05/2015	8	0	5	0	0	8
TOTAL	40		448					

Table A8: Summary of EHDV, BTV and SGV serology results for 2015-16.

Year: 2015-16			EHDV			BTV	SGV	No. of animals sampled for BTV/SGV
General Location	Site Num	Sampling date	No. of samples tested	Number seropositive	Monitoring period (months)	Total seroconversions	Total seroconversions	
Lismore	N200	5/07/2016	11	11	7	11	11	11
Casino	284	20/07/2016	10	10	7	10	8	10
Bellingen	N288	7/07/2016	10	10	7	8	10	9
Kempsey	N280	6/07/2016	10	2	7	1	9	10
Taree	N214	7/07/2016	10	0	7	0	9	10
Paterson	N011	13/07/2016	10	8	7	12	12	11
Singleton	N291	7/07/2016	10	8	7	3	10	10
Scone	N181	21/07/2016	10	5	7	1	10	10
Merriwa	N329	28/07/2016	9	0	7	0	4	10
Richmond	N015	18/07/2016	15	10	7	4	8	15
Camden	N078	25/07/2016	11	1	7	0	12	11
Sutton Forest	N328	13/07/2016	9	0	7	0	6	9
Goulburn	N087	27/06/2016	15	0	6	0	2	15
Braidwood	N330	14/07/2016	4	0	7	0	0	4
Nowra	N283	5/07/2016	11	1	7	0	10	11
Milton	N195	11/07/2016	10	0	7	0	12	10
Bodalla	N310	21/06/2016	9	0	6	0	5	9
Bega	N312	21/06/2016	10	0	6	0	0	10
Tenterfield	N135	21/06/2016	10	0	6	2	5	10
Glen Innes	N137	14/06/2016	11	1	6	3	9	11
Inverell	N213	3/08/2016	8	0	8	0	1	10
Tamworth	N209	20/07/2016	10	1	7	0	0	10
Armidale - Wollomombi	N149	6/07/2016	13	0	7	0	1	13
Yarrowitch	N173	4/07/2016	10	0	7	0	6	10
Coolatai	N319	28/07/2016	13	0	7	0	1	13
Warialda	N183	4/07/2016	10	0	7	0	0	10
Moree	N300	30/06/2016	11	0	6	0	0	10
Collarenebri	N331	21/04/2016	15	1	4	0	0	15
Narrabri	N199	25/07/2016	13	1	7	0	0	13
Willala	N322	8/07/2016	19	0	7	0	0	19
Coonabarabran	N320	1/08/2016	12	0	8	0	0	12
Coonamble	N313	8/09/2016	9	0	9	0	0	9
Bourke	N208	20/07/2016	16	0	7	0	0	16
Wanaaring	N215	18/08/2016	11	0	8	0	0	11
Peak Hill	N332	4/07/2016	10	0	7	0	0	10
Mudgee	N321	7/07/2016	9	0	7	0	0	9
Bathurst	N286	28/06/2016	12	0	6	0	1	12
Wagga Wagga	N105	8/06/2016	12	0	6	0	0	12
Yanco	N299	1/06/2016	11	0	6	0	0	11
Hay	N293	21/07/2016	10	0	7	0	0	10
TOTAL	40		439					

Table A9: Summary of EHDV, BTV and SGV serology results for 2016-17.

Year: 2016-17			EHDV			BTV	SGV	No. of animals sampled for BTV/SGV
General Location	Site Num	Sampling date	No. of samples tested	Number seropositive	Monitoring period (months)	Total seroconversions	Total seroconversions	
Lismore	N200	4/07/2017	10	10	7	9	10	10
Casino	284	11/07/2017	10	10	7	10	10	10
Bellingen	N288	2/08/2017	9	2	8	8	10	9
Kempsey	N280	28/07/2017	10	3	7	10	10	9
Taree	N214	11/07/2017	10	10	7	10	10	10
Paterson	N011	5/07/2017	10	10	7	9	10	10
Singleton	N291	5/07/2017	10	10	7	10	10	10
Scone	N181	17/07/2017	10	8	7	10	10	10
Merriwa	N329	14/07/2017	10	1	7	1	5	10
Richmond	N015	10/07/2017	13	5	7	9	13	13
Camden	N078	20/07/2017	12	1	7	4	12	12
Sutton Forest	N328	6/07/2017	10	0	7	0	0	10
Goulburn	N087	11/07/2017	11	0	7	0	1	11
Braidwood	N330	25/07/2017	12	0	7	0	0	12
Nowra	N283	26/06/2017	11	0	6	1	11	11
Milton	N195	5/07/2017	12	0	7	0	9	12
Bodalla	N310	12/07/2017	10	0	7	0	6	10
Bega	N312	12/07/2017	10	0	7	0	0	10
Tenterfield	N135	20/07/2017	10	1	7	0	10	10
Glen Innes	N137	1/06/2017	10	4	6	0	2	10
Inverell	N213	20/04/2017	10	0	4	0	0	10
Tamworth	N209	22/08/2017	10	0	8	0	0	10
Armidale - Wollomombi	N149	3/07/2017	14	0	7	1	1	14
Yarrowitch	N173	27/06/2017	5	0	6	2	0	5
Coolatai	N319	20/07/2017	12	3	7	1	11	12
Warialda	N183	24/07/2017	10	0	7	0	1	10
Moree	N300	10/07/2017	12	0	7	0	0	12
Walgett	N334	19/06/2017	10	0	6	0	0	10
Narrabri	N199	4/07/2017	16	0	7	0	0	16
Willala	N322	22/06/2017	13	0	6	0	0	13
Coonabarabran	N320	14/08/2017	10	0	8	0	0	10
Coonamble	N333	20/07/2017	10	0	7	0	0	10
Bourke	N208	11/07/2017	12	0	7	0	0	12
Wanaaring	N215	6/03/2017	12	0	3	0	0	13
Peak Hill	N332	7/06/2017	7	0	6	0	0	7
Mudgee	N321	6/07/2017	10	0	7	0	0	10
Bathurst	N286	18/07/2017	12	0	7	0	0	12
Wagga Wagga	N105	20/06/2017	13	0	6	0	0	13
Yanco	N299	14/06/2017	11	0	6	0	0	11
Hay	N293	21/06/2017	10	0	6	0	0	10
TOTAL	40		429					

Table A10: Summary of EHDV, BTV and SGV serology results for 2017-18.

Year: 2017-18		EHDV			BTV		SGV	
General Location	Site Num	Sampling date	No. of samples tested	Number seropositive	Monitoring period (months)	Total seroconversions	Total seroconversions	No. of animals sampled for BTV/SGV
Lismore	N200	3/07/2018	10	9	7	9	10	10
Casino	284	3/07/2018	10	10	7	10	10	10
Bellingen	N288	5/07/2018	10	7	7	0	10	10
Kempsey	N280	5/07/2018	12	0	7	0	8	12
Taree	N214	10/07/2018	11	0	7	1	11	11
Paterson	N011	10/07/2018	9	0	7	0	10	9
Singleton	N291	4/07/2018	10	1	7	0	10	10
Scone	N181	27/07/2018	10	0	7	0	8	10
Merriwa	N329	4/05/2018	12	0	5	0	1	12
Richmond	N015	13/07/2018	12	0	7	0	12	12
Camden	N078	8/06/2018	14	0	6	0	14	14
Sutton Forest	N328	29/06/2018	12	1	6	0	1	12
Goulburn	N087	17/07/2018	15	0	7	0	4	15
Braidwood	N330	17/08/2018	11	0	8	0	0	11
Nowra	N283	9/07/2018	12	0	7	0	11	12
Milton	N195	10/07/2018	12	0	7	0	12	12
Bodalla	N310	11/07/2018	9	1	7	0	8	9
Bega	N312	11/07/2018	10	1	7	0	5	10
Tenterfield	N135	4/09/2018	10	0	9	0	1	10
Glen Innes	N137	26/09/2018	11	1	9	0	5	11
Inverell	N213	20/04/2018	10	0	4	0	9	10
Tamworth	N337	10/04/2018	11	0	4	0	7	11
Armidale - Wollomombi	N149	9/07/2018	14	0	7	0	5	14
Yarrowitch	N173	9/04/2018	15	1	4	0	7	15
Coolatai	N319	25/07/2018	13	9	7	2	7	13
Warialda	N183	6/07/2018	10	2	7	0	9	10
Moree	N155	6/04/2018	20	0	4	0	3	20
Walgett	N338	4/07/2018	15	0	7	0	0	15
Narrabri	N199	21/06/2018	14	0	6	0	1	14
Willala	N322	5/06/2018	15	1	6	0	2	14
Coonabarabran	N320	10/04/2018	15	0	4	0	1	15
Coonamble	N336	11/07/2018	10	0	7	0	0	10
Bourke	N208	6/09/2018	15	0	9	0	0	15
Wanaaring	N215	17/03/2018	15	0	3	0	0	15
Peak Hill	N332	17/05/2018	10	0	5	0	0	10
Mudgee	N321	25/07/2018	11	0	7	0	0	11
Bathurst	N286	24/07/2018	12	0	7	0	0	12
Wagga Wagga	N105	23/07/2018	14	0	7	0	0	14
Yanco	N299	4/06/2018	11	0	6	0	0	11
Hay	N293	20/06/2018	10	0	6	0	0	10
TOTAL	40		482					