Appendix A: CSIRO ACDP – AAHL NAMP Activities Report FY2024/25

Contents

NAMP Testing Summary

Herd Sites

During the reporting period, CSIRO ACDP – AAHL received clinical samples and virus isolates originating from the following herds/locations:

Queensland
Alligator Creek
Allora
Burketown
Capella
Chinchilla
Cooktown
East Palmerston
Gatton
Good Night
Goondiwindi
Hervey Range
Normanton
Quilpie
Springsure
Windorah

Western Australia
Kalumburu Mission
East Kimberley
Gibb River, Kimberley
West Kimberley

Northern Territory
Costello
Coomalie
Douglas Daly Research Farm
Beatrice Hill Farm
Katherine Research Station
Kalkarindji
Gunbalanya
Victoria River Research Station

Victoria
Goon Nure

Herd locations, and the serotypes detected by VP2 gene or whole genome sequencing for submissions received in FY24-25 are shown in Figure 1. For comparison, data from FY2023-2024, FY2022-23 & FY2021-22 are shown in Figures 2 & 3 & 4.

48

Number of NAMP Submissions received in the time period

501

Number of NAMP Samples received in the time period

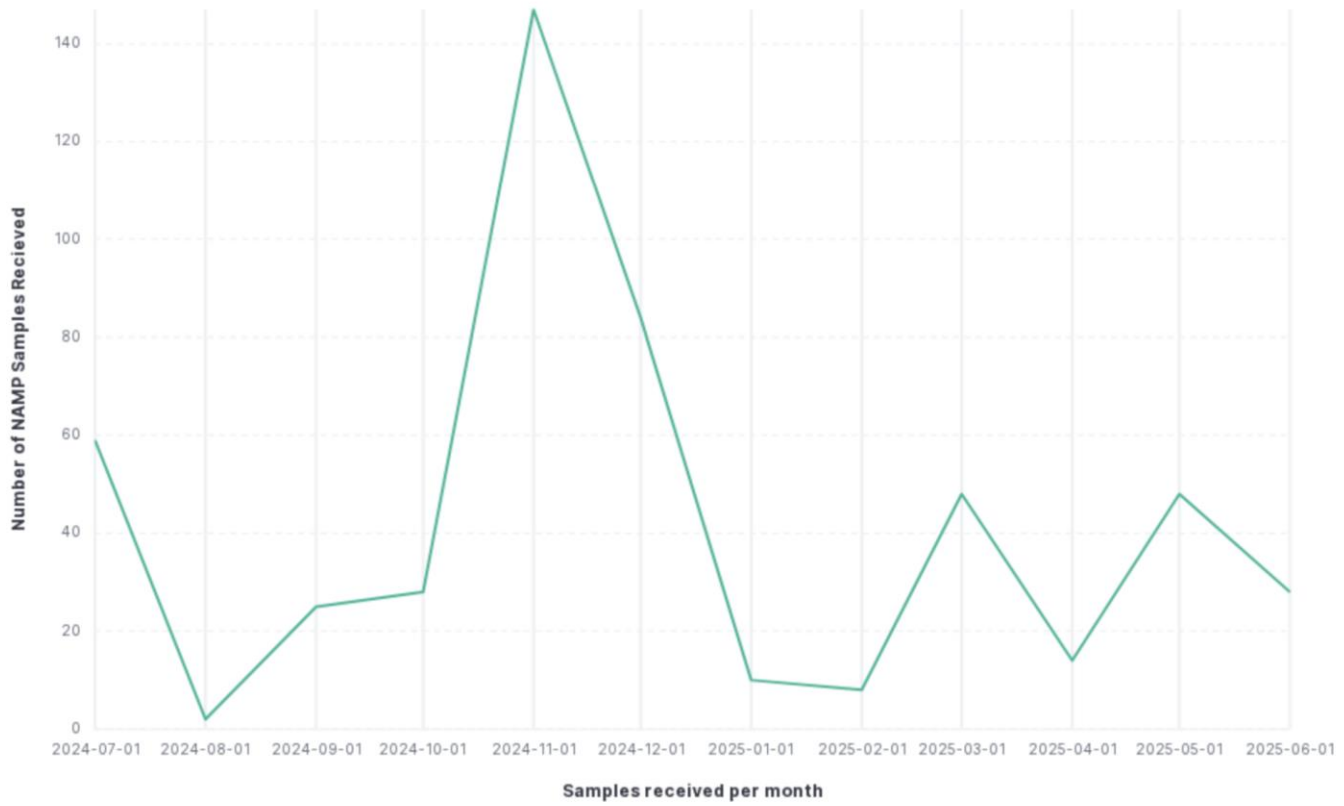


Figure 1. Bluetongue virus serotypes detected by sequencing from NAMP herds (FY2024-25).
Size of the pie charts are proportional to the total number of BTV TQM positive samples/isolates received. Note: Some locations are approximate. Includes characterisation of some NT isolates that originated from FY24 collections.

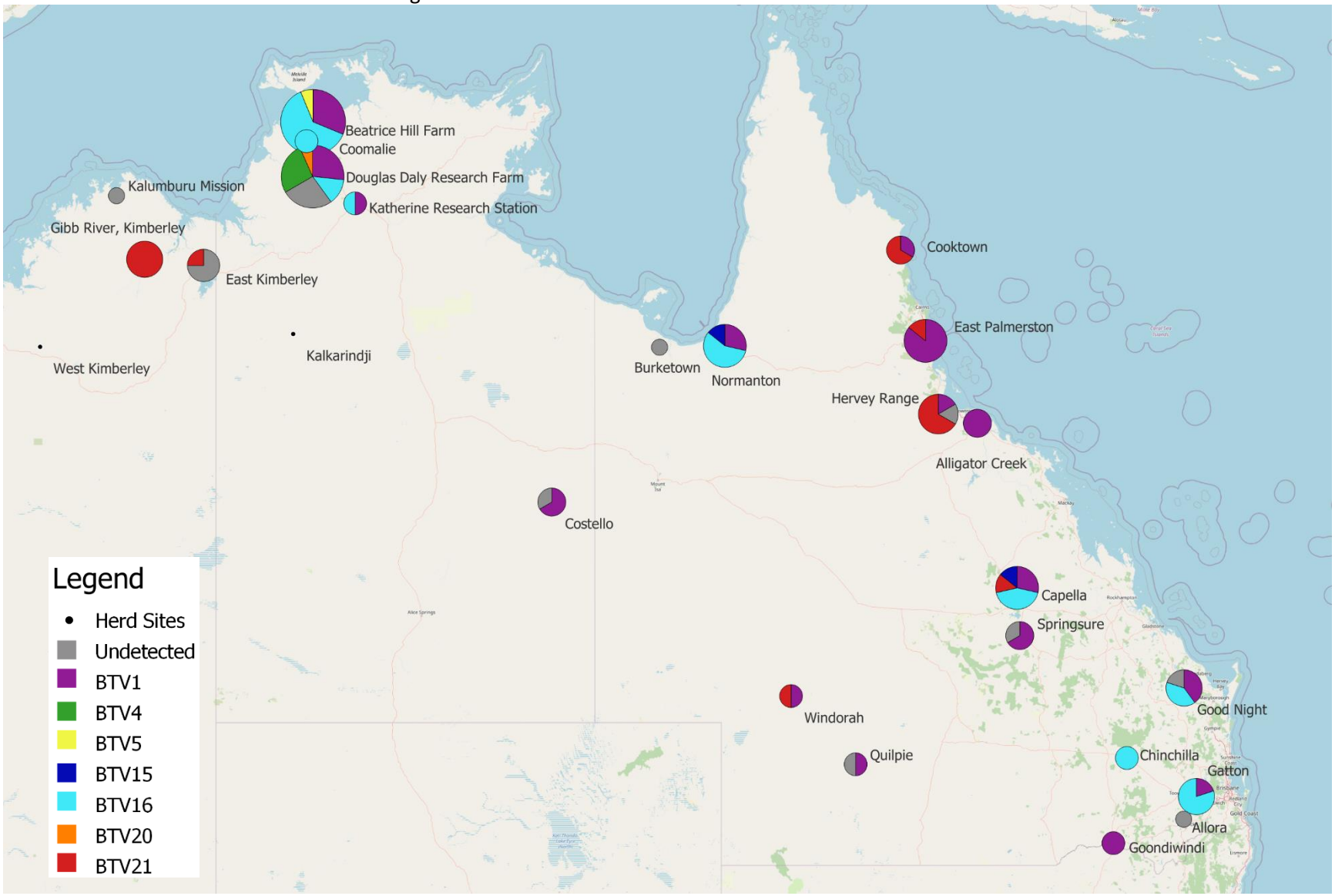


Figure 2. Bluetongue virus serotypes detected by sequencing from NAMP herds (FY2023-24).
Size of the pie charts are proportional to the total number of BTV TQM positive samples/isolates received. Note: Some locations are approximate. Includes characterisation of some NT isolates that originated from FY23 collections.

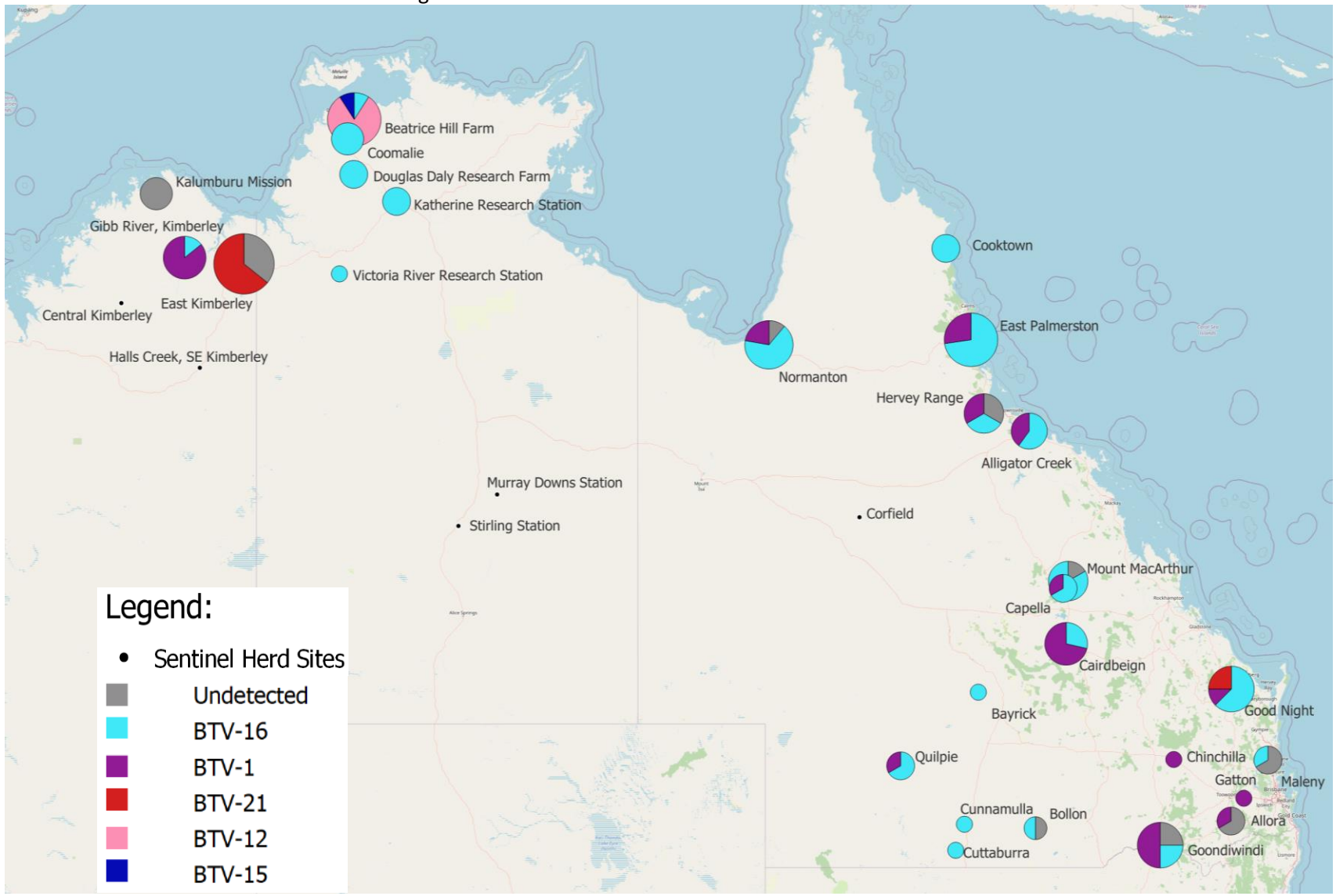


Figure 3. Bluetongue virus serotypes detected by sequencing from NAMP herds (FY2022-23).
Size of the pie charts are proportional to the total number of BTV TQM positive samples/isolates received. Note: Some locations are approximate.

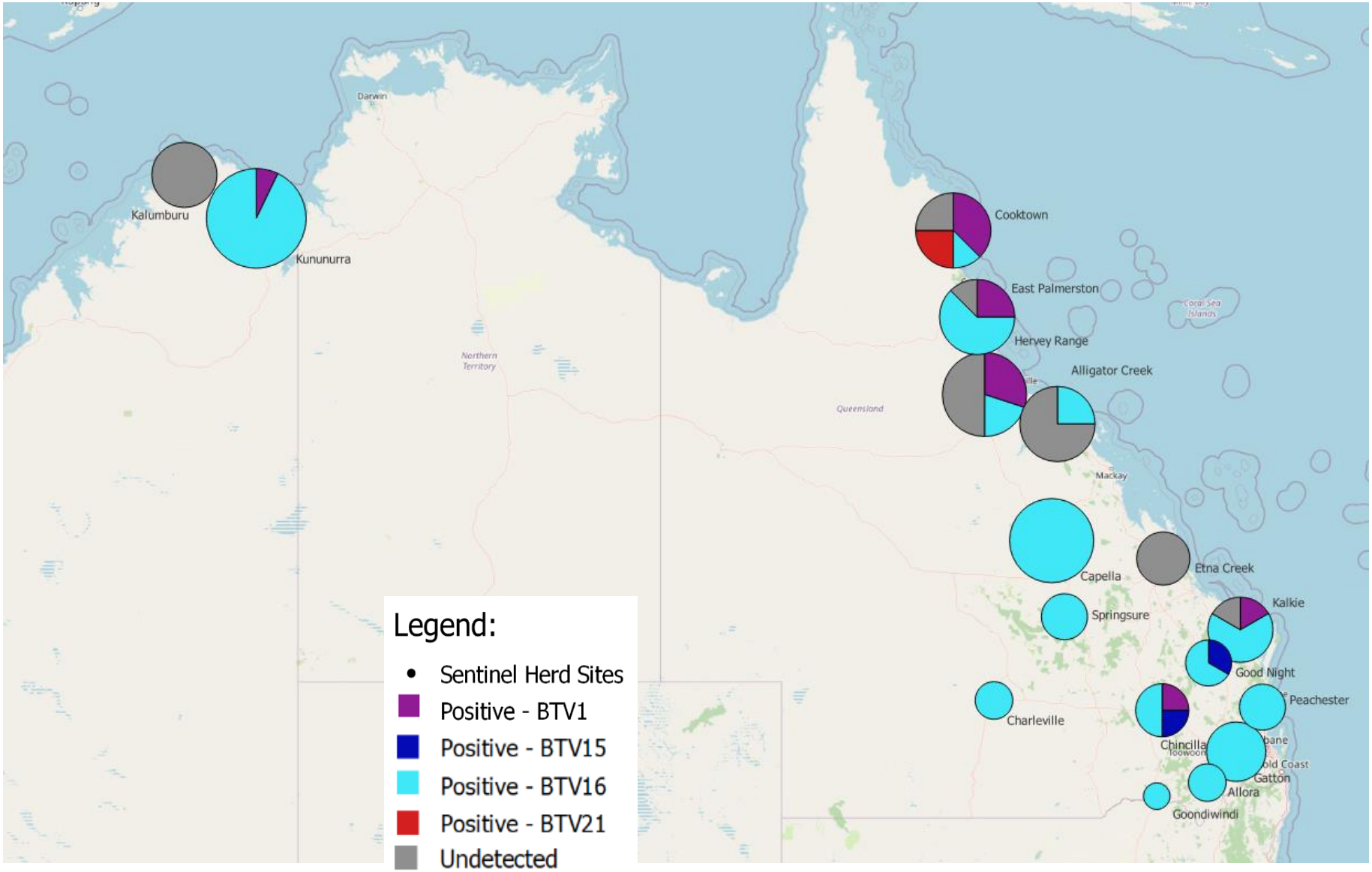
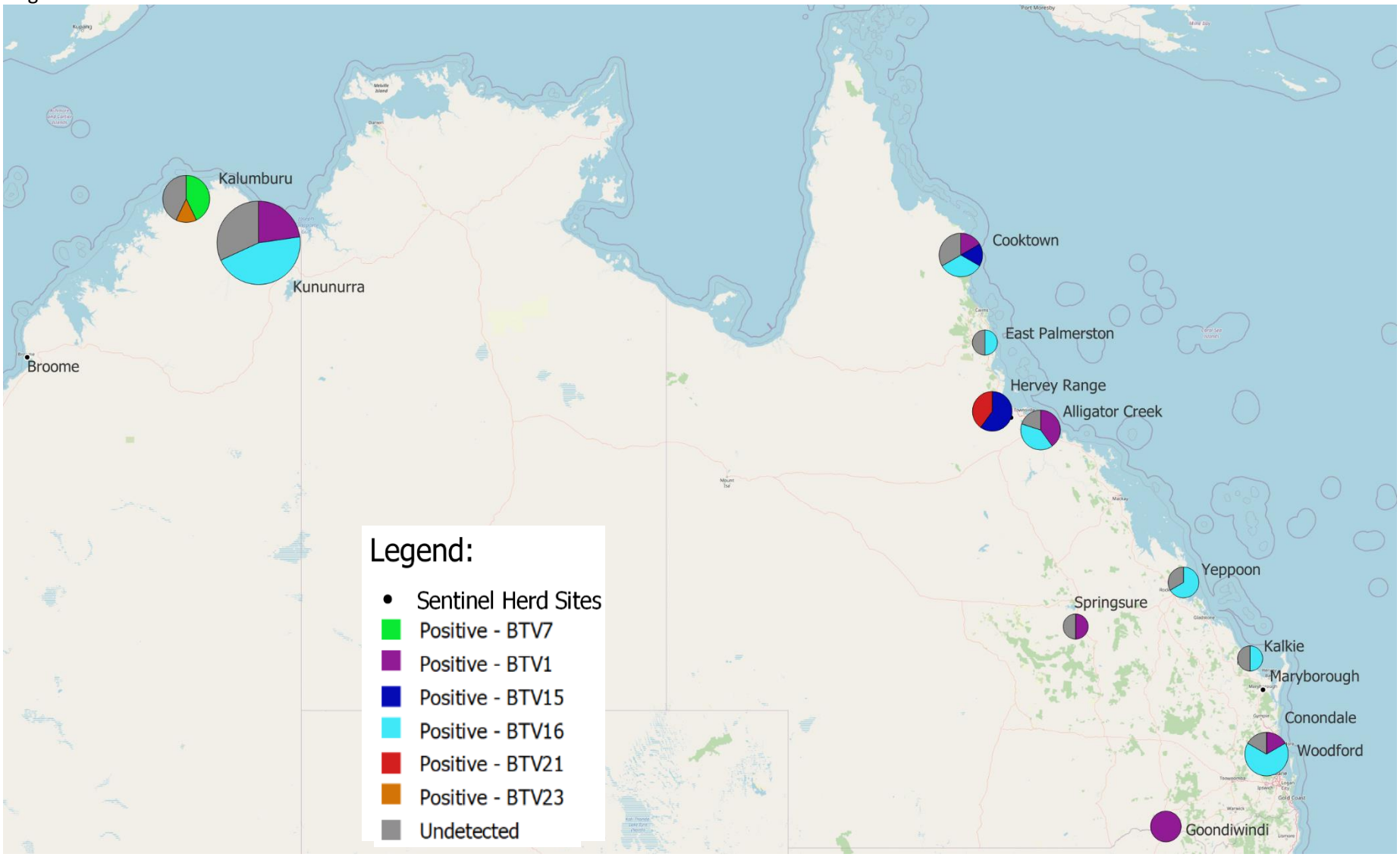


Figure 4. Bluetongue virus serotypes detected by sequencing from NAMP herds (FY2021-22).
Size of the pie charts are proportional to the total number of BTV TQM positive samples/isolates received. NB: Includes characterisation of some QLD isolates that originated from FY21 collections.



Queensland

Samples Tested:

Location	Sample Type	
	WHOLE BLOOD (LITH HEP)	PLASMA
Alligator Creek	10	18
Allora	1	1
Burketown	1	1
Capella	6	6
Chinchilla	2	2
Cooktown	3	3
East Palmerston	6	6
Gatton	4	4
Good Night	6	6
Goondiwindi	2	2
Hervey Range	18	42
Normanton	6	6
Quilpie	1	1
Springsure	5	5
Windorah	1	1
TOTAL	**72**	**104**

Testing Performed:

Location	Serology			Molecular	Sequencing		
	cELISA	sELISA[#]	BTV SNT PANEL (8-18 serotypes^)	BTV TQM PCR	Genotype Sequencing (VP3)	Serotype Sequencing (VP2)	Whole Genome Sequencing
Alligator Creek	18	-	5	10	-	-	3
Allora	1	-	1	1	-	-	1
Burketown	1	-	1	1	-	-	1
Capella	6	-	6	6	-	-	6
Chinchilla	2	-	2	2	-	-	2
Cooktown	3	-	3	3	2	2	3
East Palmerston	6	-	6	6	-	-	6
Gatton	4	-	4	4	-	-	4
Good Night	6	-	6	6	-	-	5
Goondiwindi	2	-	2	2	1	1	1
Hervey Range	42	3	42	18	-	-	6
Normanton	6	-	6	6	4	4	2
Quilpie	1	-	1	1	-	-	1
Springsure	5	-	5	5	-	-	3
Windorah	1	-	1	1	-	-	1
Grand Total	**104**	**3**	**91**	**72**	**7**	**7**	**45**

^ Only 1 test recorded/sample however 8-18 SNTs are performed per panel. ACDP recently adopted a reduced initial SNT panel for all BTV cELISA positive serum samples.
[#]Applied for additional investigation of serological reactors, where required.

Results by Location:

Agent detection

Location	Serotype (by sequencing)				
	BTV1	BTV16	BTV21	BTV15	Undetected
Alligator Creek	3	-	-	-	-
Allora	-	-	-	-	1
Burketown	-	-	-	-	1
Capella	2	3	1	1	-
Chinchilla	-	2	-	-	-
Cooktown	1	-	2	-	-
East Palmerston	6	-	1	-	-
Gatton	1	4	-	-	-
Good Night	2	2	-	-	1
Goondiwindi	2	-	-	-	-
Hervey Range	1	-	4	-	1
Normanton	2	4	-	1	-
Quilpie	1	-	-	-	1
Springsure	2	-	-	-	1
Windorah	1	-	1	-	-
Grand Total	**24**	**15**	**9**	**2**	**6**

Western Australia

Samples Tested:

Location	Sample Type	
	WHOLE BLOOD (EDTA/CLOTTED)	SERUM
Kalumburu Mission	61	68
East Kimberley	20	31
Gibb River, Kimberley	20	23
West Kimberley	13	16
TOTAL	**114**	**138**

Testing Performed:

Location	Serology				Molecular	Sequencing		
	cELISA	sELISA[#]	BTV SNT PANEL (8-18 serotypes^)	BTV SNT Serotype 15*	BTV TQM PCR	Genotype Sequencing (VP3)	Serotype Sequencing (VP2)	Whole Genome Sequencing
Kalumburu Mission	38	-	28	10	61	-	-	1
East Kimberley	31	16	31	-	20	4	4	2
Gibb River, Kimberley	23	-	20	1	20	5	5	4
West Kimberley	16	-	15	1	13	-	-	-
Grand Total	**108**	**16**	**94**	**12**	**114**	**9**	**9**	**7**

*BTV-15 SNT is performed on cELISA negative samples, as the cELISA is known to have reduced sensitivity to antibodies to BTV-15.

^ Only 1 test recorded/sample however 8-18 SNTs are performed per panel. ACDP recently adopted a reduced initial SNT panel for all BTV cELISA positive serum samples.

[#]Applied for additional investigation of serological reactors, where required.

Results by Location:

Agent detection

Location	Serotype (by sequencing)	
	BTV21	Undetected
Kalumburu Mission	-	1
East Kimberley	2	3
Gibb River, Kimberley	5	-
Grand Total	**7**	**4**

Northern Territory

Samples Received:

Location	Sample Type		
	VIRUS ISOLATES	SERUM	WHOLE BLOOD
Costello	-	3	3
Coomalie	2	2	2
Douglas Daly Research Farm	5	10	10
Beatrice Hill Farm	17	-	-
Katherine Research Station	3	-	-
Gunbalanya	-	1	1
Victoria River Research Station	-	1	1
Kalkarindji	-	1	-
Not defined	1	4	5
TOTAL	**28**	**22**	**22**

Testing Performed:

Location	Serology		Molecular	Sequencing		
	cELISA	BTV SNT PANEL (8-18 serotypes^)	BTV TQM PCR	Genotype Sequencing (VP3)	Serotype Sequencing (VP2)	Whole genome sequencing
Costello	3	1	3	-	-	3
Coomalie	2	2	4	-	-	2
Douglas Daly Research Farm	10	10	15	-	-	14
Beatrice Hill Farm	-	-	17	-	-	16
Katherine Research Station	-	-	3	-	-	2
Gunbalanya	1	1	1	-	-	-
Victoria River Research Station	1	1	1	-	-	-
Kalkarindji	1	1	-	-	-	-
Not defined	4	4	6	5	5	5
Grand Total	**22**	**20**	**50**	**5**	**5**	**42**

^ Only 1 test recorded/sample however 8-18 SNTs are performed per panel. ACDP recently adopted a reduced initial SNT panel for all BTV cELISA positive serum samples.

Results by Location:

Agent detection

Location	Serotype (by sequencing)					
	BTV1	BTV4	BTV5	BTV16	BTV20	Undetected
Costello	2	-	-	-	-	1
Coomalie	-	-	-	2	-	-
Douglas Daly Research Farm	4	4	-	2	1	4
Beatrice Hill Farm	5	-	1	10	-	-
Katherine Research Station	1	-	-	1	-	-
Not defined	1	-	-	-	-	-
Grand Total	**13**	**4**	**1**	**15**	**1**	**5**

Victoria

A single submission of a single sample was received from Victoria, from a potential ELISA reactor at Goon Nure. The sample tested negative on cELISA and BTV-15 SNT at ADCP.

Test numbers

Samples Tested:

Location	Sample Type		
	WHOLE BLOOD (EDTA/LITH HEP/CLOTTED)	PLASMA/SERUM	VIRUS ISOLATES
Queensland	72	104	-
Western Australia	114	138	-
Northern Territory	22	22	28
Victoria	-	1	-
TOTAL	**208**	**265**	**28**

Testing Performed:

Location	Serology			Molecular	Sequencing		
	cELISA^	sELISA#	BTV SNT panels*†	BTV TQM PCR^	Genotype Sequencing (VP3)	Serotype Sequencing (VP2)**	Whole Genome Sequencing
Queensland	104	3	91	72	7	7	45
Western Australia	108	16	94	114	9	9	7
Northern Territory	22	-	20	50	5	5	42
Victoria	1	-	-	-	-	-	-
Grand Total	**235**	**19**	**205**	**236**	**21**	**21**	**94**

* Only 1 test recorded/sample however 8-18 SNTs are performed per panel. ACDP recently adopted a reduced initial SNT panel for all BTV cELISA positive serum samples.
**Only 1 test recorded/sample (regardless of # primer sets used)
^ Generally repeated testing to confirm result prior to follow-up testing (and to determine OD/Ct)
† Additional testing ongoing
# Applied for additional investigation of serological reactors, where required.

WGS TRANSITION

As previously notified, ACDP has completed a transition from capillary sequencing to whole genome sequencing (WGS) for NAMP submissions. WGS results will be reported with a serotype designation. ACDP is reviewing the genomic characterisation of BT viruses and as such, is no longer reporting the VP3 genotype of detected viruses. For more information on ACDP's planned approach to WGS and genomic analysis, please see our accompanying meeting paper.

SAMPLE VOLUMES

To allow for all characterization testing requirements, ACDP kindly requests jurisdictions to submit a minimum of:

- 3mL of uncoagulated whole blood, to allow for all sequencing and virus isolation assays to be performed (if required), and
- 1mL of serum/plasma for serological testing.

BTV-5 & BTV-9 REACTIVITY IN QLD

ACDP detected significant serological titers to BTV-5 and BTV-9 in a single animal from Hervey Range, QLD, from a January 2025 serosurvey bleed. BTV-5 and BTV-9 share strong cross-reactivity in serological assays. No BTV-5 or BTV-9 virus was detected by molecular methods.

Following this result, and in consultation with QLD, ACDP subsequently received four submissions of additional samples from this herd for further investigation, including:

- BTV PCR positive samples from earlier timepoints (December 2024)
- Additional ELISA-positive samples from the same herd bleed (January 2025)
- Additional PCR and ELISA positive samples from subsequent herd bleeds (March-April 2025).

Whilst these investigations are ongoing, initial testing of these additional samples has not demonstrated sufficient evidence to confirm BTV-5 and/or BTV-9 exposure in this herd. Furthermore, there is no supporting evidence of BTV-5 and/or BTV-9 exposure in other QLD surveillance herds. Ongoing surveillance for BTV-5 and/or BTV-9 transmission in the eastern episystem is recommended.

BVL BTV-20 INVESTIGATION

ACDP assisted Berrimah Veterinary Laboratory with an investigation into an untyped sample from Douglas Daly. This sample was not initially typed by serotype-specific PCRs and was submitted to ACDP for characterisation by sequencing. Both segment 2 capillary sequencing and whole genome sequencing results demonstrated the sample was a BTV-20. With the permission of the NT CVO, ACDP has shared BTV-20 sequence data with EMAI to support further refinement of the serotype specific PCRs.

BTV-7 SNT

ACDP has engaged collaboratively with Berrimah Veterinary Laboratory to review results of a longitudinal study into BTV infections in the Douglas Daly herd. Through this collaboration, it was identified that BTV-7 exposures detected by serotype-specific PCR were not reflected in ACDP's serological SNT panel. ACDP has reviewed serology results from earlier BTV-7 positive samples, and has recently received additional BTV-7 reference samples from BVL to assist in this investigation. Initial findings indicate that there has not been a significant change in BTV-7 antigenicity, however it has been identified that BTV-7 does not appear to trigger a strong neutralising antibody response. As such, ACDP is altering the BTV-7 SNT methodology to begin at a dilution of 1:5 (as opposed to 1:10), in an attempt to increase the sensitivity of this assay for the detection of BTV-7 specific neutralising antibodies.

WA ELISA REACTIVITY INVESTIGATION

ACDP has assisted WA to investigate BTV ELISA reactivity in a surveillance herd in the East Kimberley. A number of animals tested positive by screening BTV cELISA, however only minor titres were observed to BTV-3 on the SNT panel. No corresponding EDTA blood samples were positive by BTV TQM PCR. Further investigation of these results included testing by BTV sELISA and screening for exposure to other orbiviruses that may cause cross-reactivity in BTV serological assays (including EDHV) by both serological and molecular methods. BTV-13 SNT was performed to assess for exposure to this exotic serotype, due to known cross-reactivity with BTV-3 and BTV-16, with negative results. Based on the additional investigation performed it was deemed likely that the BTV serological reactivity observed in this submission was caused by exposure of the herd to a related non-BTV orbivirus, the identity of which remains unknown.

BTV ISOLATION

ACDP is once again investigating alternatives to BTV isolation in embryonated chicken eggs (ECEs). Virus isolations are currently being conducted in KC cells and ECEs in parallel to generate additional comparative data to support a transition away from ECEs. KC cell lines are currently used successfully overseas as the primary method of BTV isolation.