

FOR INFORMATION

NAMP Technical Committee Annual Meeting 2023

Agenda Item 5.9

Last updated: 13 August 2024

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ACDP VIROLOGY REPORT 2023-24**RECOMMENDATION**

1. That the NAMP Technical Committee NOTES:
 - a. **Testing Performed by ACDP for WA, QLD and NT in 2023-24**
 - b. **Notes on BTV-21 Activity**
 - c. **An update on Whole Genome Sequencing at ACDP**

BACKGROUND

- ACDP performed confirmatory molecular and serological testing of samples (whole blood, plasma/sera) from WA and Qld, as well as characterizing positive samples by neutralization assay, sequencing and/or virus isolation, as appropriate.

DISCUSSION

- See Appendix A for detailed results

FINANCIAL IMPLICATIONS

- N/A

CONSULTATION/COMMUNICATION

- N/A

NEXT STEPS/ACTIONS

- N/A

Attachment A: CSIRO ACDP – AAHL NAMP Activities Report FY2023/24



Appendix A: CSIRO ACDP – AAHL NAMP Activities Report FY2023/24

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NAMP Testing Summary

Sentinel Herds

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

Queensland

Alligator Creek
Allora
Bayrick
Bollon
Cairdbeign
Capella
Chinchilla
Cooktown
Corfield
Cunnamulla
Cuttaburra
East Palmerston
Gatton
Good Night
Goondiwindi
Hervey Range
Maleny
Mount MacArthur
Normanton
Quilpie

Western Australia

Halls Creek, SE Kimberley
Kalumburu Mission
East Kimberley
Gibb River, Kimberley
Central Kimberley

Northern Territory

Murray Downs Station
Stirling Station
Coomalie
Beatrice Hill Farm
Victoria River Research Station
Katherine Research Station
Douglas Daly Research Farm

Sentinel herd locations, and the serotypes detected by VP2 gene sequencing for submissions received in FY23-24 are shown in Figure 1. For comparison, data from FY2022-23, FY2021-22 & FY2020-21 are shown in Figures 2 & 3 & 4.

Figure 1. Bluetongue virus serotypes detected by sequencing from NAMP sentinel 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.

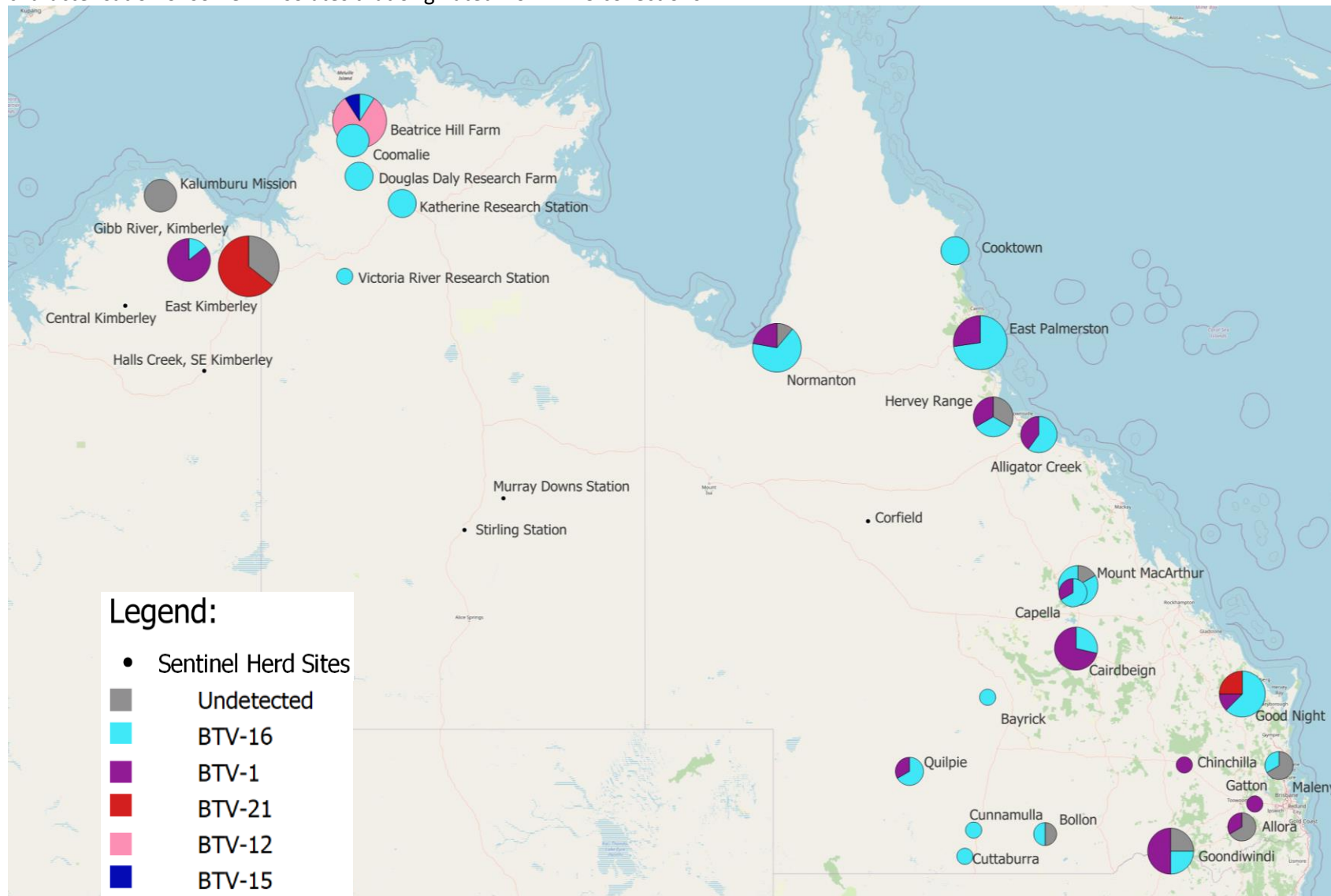


Figure 2. Bluetongue virus serotypes detected by sequencing from NAMP sentinel herds (FY2022-23).

Size of the pie charts are proportional to the total number of BTV TQM positive samples/isolates received. Note: Some locations are approximate.

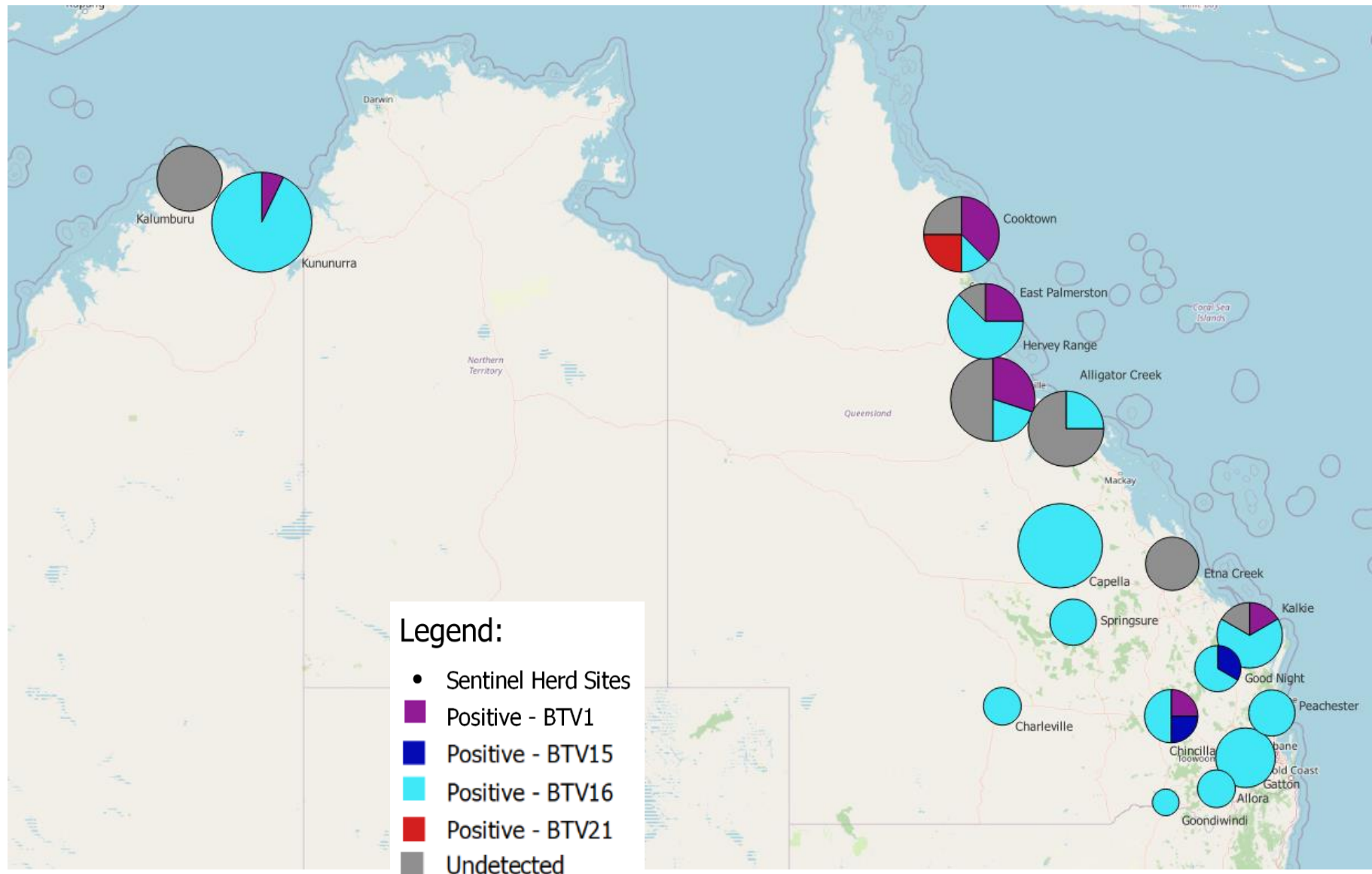


Figure 3. Bluetongue virus serotypes detected by sequencing from NAMP sentinel herds (FY2021-22).

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.

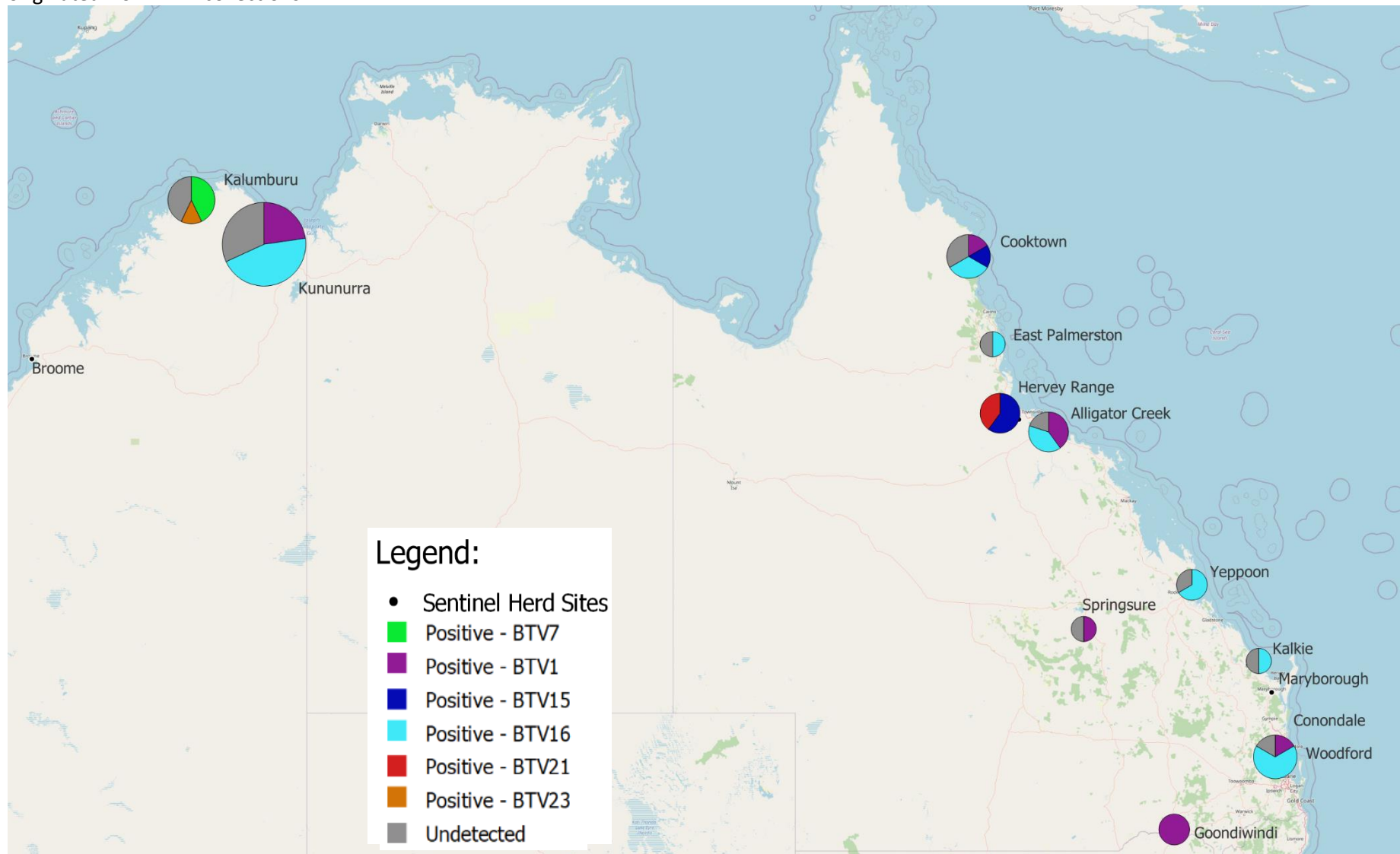
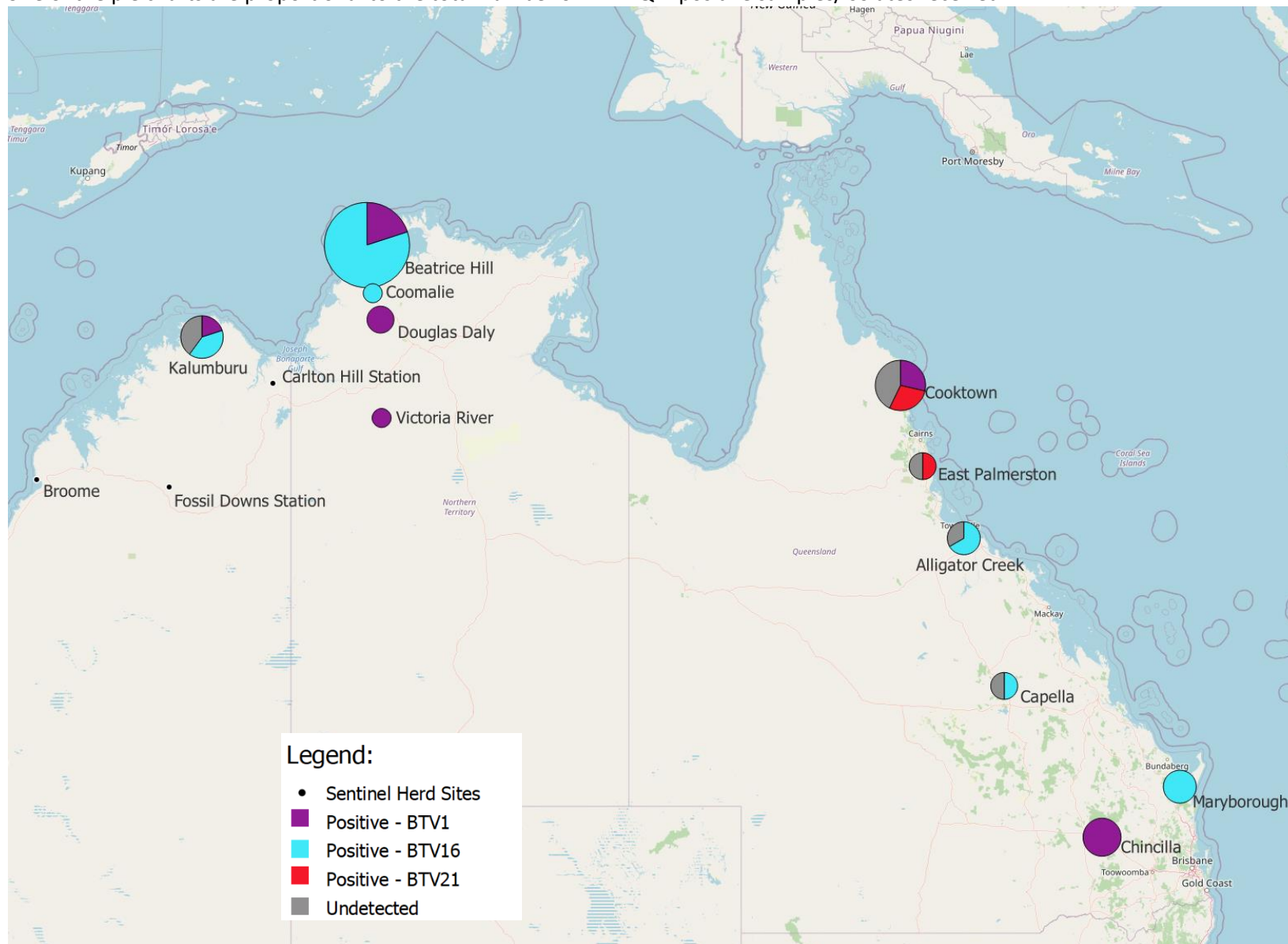


Figure 4. Bluetongue virus serotypes detected by sequencing from NAMP sentinel herds (FY2020-21).

Size of the pie charts are proportional to the total number of BTV TQM positive samples/isolates received.



Queensland

Samples Tested:

Location	Sample Type	
	WHOLE BLOOD (LITH HEP)	PLASMA
Cairdbeign	17	17
Mount MacArthur	11	11
East Palmerston	10	10
Normanton	9	9
Goondiwindi	8	8
Hervey Range	6	6
Good Night	8	8
Alligator Creek	4	4
Allora	3	3
Capella	3	3
Cooktown	3	3
Maleny	3	3
Quilpie	3	3
Bollon	2	2
Cunnamulla	2	2
Bayrick	1	1
Chinchilla	1	1
Corfield	1	1
Cuttaburra	1	1
Gatton	1	1
TOTAL	97	97

Testing Performed:

Location	Serology		Molecular	Sequencing		BTV Isolation	Virus isolate typing assays
	cELISA	BTV SNT PANEL (8-18 serotypes [^])		Genotype Sequencing (VP3)	Serotype Sequencing (VP2)		
Alligator Creek	4	4	4	4	4	-	-
Allora	3	3	3	3	3	-	-
Bayrick	1	1	1	1	1	-	-
Bollon	2	2	2	2	2	-	-
Cairdbeign	17	17	17	7	7	3	-
Capella	3	3	3	3	3	2	1
Chinchilla	1	1	1	1	1	-	-
Cooktown	3	3	3	3	3	-	-
Corfield	1	1	1	-	-	-	-
Cunnamulla	2	2	2	1	1	-	-
Cuttaburra	1	1	1	1	1	-	-
East Palmerston	10	10	10	10	10	-	-
Gatton	1	1	1	1	1	-	-
Good Night	8	8	8	8	8	-	-
Goondiwindi	8	8	8	8	8	-	-
Hervey Range	6	6	6	6	6	1	-
Maleny	3	3	3	3	3	-	-
Mount MacArthur	11	11	11	6	6	1	-
Normanton	9	9	9	9	9	2	-
Quilpie	3	3	3	3	3	-	-
Grand Total	97	97	97	80	80	9	1

[^] 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)				Genotype (by sequencing)			Isolation	
	BTV1	BTV16	BTV21	Undetected	Australia A	Malaysia A2	Undetected/Indeterminate	Negative	Positive
Alligator Creek	2	3	-	-	3	-	1	-	-
Allora	1	-	-	2	3	-	-	-	-
Bayrick	-	1	-	-	1	-	-	-	-
Bollon	-	1	-	1	2	-	-	-	-
Cairdbeign	5	2	-	-	7	-	-	2	1
Capella	1	2	-	-	3	-	-	1	1
Chinchilla	1		-	-	1	-	-	-	-
Cooktown	-	3	-	-	2	-	1	1	-
Corfield	-		-	-	-	-	-	-	-
Cunnamulla	-	1	-	-	1	-	-	-	-
Cuttaburra	-	1	-	-	1	-	-	-	-
East Palmerston	3	10	-	-	7	2	1	-	-
Gatton	1	-	-	-	1	-	-	-	-
Good Night	1	5	2	-	8	-	-	-	-
Goondiwindi	4	2	-	2	7	-	1	-	-
Hervey Range	3	2	-	2	5	-	-	-	-
Maleny	-	1	-	2	2	-	1	-	-
Mount MacArthur	-	5	-	1	6	-	-	1	-
Normanton	2	6	-	1	9	-	-	2	-
Quilpie	1	2	-	-	3	-	-	-	-
Grand Total	22	42	2	11	72	2	5	7	2

Serology

Location	cELISA		SNT
	Positive	Inconclusive	Dominant Serotypes detected
Alligator Creek	4	-	1, 16, 21
Allora	3	-	1, 16, 21
Bayrick	1	-	16
Bollon	1	1	16, 21
Cairdbeign	17	-	1, 16, 21
Capella	3	-	1, 16, 21 (23 [#])
Chinchilla	1	-	1 (21 [^])
Cooktown	3	-	1, 16, 21 (3 ^{*^})
Corfield	1	-	1
Cunnamulla	2	-	16
Cuttaburra	1	-	16 (21 [^])
East Palmerston	9	1	1, 16, 21
Gatton	1	-	1, 16, 21
Good Night	8	-	1, 15, 16, 21
Goondiwindi	8	-	1, 16, 21 ⁺
Hervey Range	6	-	1, 16
Maleny	3	-	16
Mount MacArthur	11	-	1, 16, 21
Normanton	9	-	1, 16, 21
Quilpie	3	-	1, 16, 21
Grand Total	95	2	

#Whilst low titre reactions to BTV-23 are frequently observed as cross reactions, the level of reactivity observed is potentially significant and may indicate exposure to this serotype.

* Additional results pending

[^] Minor reactions

*Previous exposure to BTV-16 can result in VN reactivity to the serologically related BTV-3.

Western Australia

Samples Tested:

Location	Sample Type	
	WHOLE BLOOD (EDTA)	SERUM
Central Kimberley	-	12
East Kimberley	26	21
Gibb River, Kimberley	16	16
Halls Creek, SE Kimberley	-	48
Kalumburu	95	84
TOTAL	181	137

Testing Performed:

Location	Serology			Molecular	Sequencing		Isolation and Typing	
	cELISA	BTV SNT PANEL (8-18 serotypes [^])	BTV SNT Serotype 15*	BTV TQM PCR	Genotype Sequencing (VP3)	Serotype Sequencing (VP2)	Isolation	Virus isolate typing assays
Central Kimberley	12	12	-	-	-	-	-	-
East Kimberley	21	18	4	26	14	14	-	-
Gibb River, Kimberley	16	16		16	7	7	3	1
Halls Creek, SE Kimberley	48	48	-	-	-	-	-	-
Kalumburu	60	47	13	95	4	4	2	-
Grand Total	157	141	17	137	25	25	5	1

*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.

Results by Location:**Agent detection**

Location	Serotype (by sequencing)				Genotype (by sequencing)		Isolation [#]	
	BTV1	BTV16	BTV21	Undetected	Australia A	Undetected	Negative	Positive
East Kimberley	-	-	9	5	13	1	-	-
Gibb River, Kimberley	6	1	-	-	7	-	2	1
Kalumburu	-	-	-	4	3	1	2	-
Grand Total	6	1	9	9	23	2	4	1

[#]Additional Results pending**Serology**

Location	cELISA		SNT
	Negative	Positive	Dominant Serotypes detected
Central Kimberley	-	12	1, 16, 21
East Kimberley	7	14	21, 16 [#]
Gibb River, Kimberley	-	16	1, 16, 21
Halls Creek, SE Kimberley	-	48	1, 16, 21, 23
Kalumburu	13	47	1, 16, 21 (5 [^]) [#]
Grand Total	20	137	

[^] Minor reactions[#]Additional Results pending

Northern Territory

Samples Tested:

Location	Sample Type	
	VIRUS ISOLATES	SERUM
Murray Downs Station	-	2
Stirling Station	-	1
Coomalie	4	-
Beatrice Hill Farm	12	-
Victoria River Research Station	1	-
Katherine Research Station	3	-
Douglas Daly Research Farm	3	-
TOTAL	23	3

Testing Performed:

Location	Serology		Molecular	Sequencing		Virus Isolate Typing Assay
	cELISA	BTV SNT PANEL (8-18 serotypes^)		Genotype Sequencing (VP3)	Serotype Sequencing (VP2)	
Murray Downs Station	2	2	-	-	-	-
Stirling Station	1	1	-	-	-	-
Coomalie	-	-	4	4	4	1
Beatrice Hill Farm	-	-	12	11	11	8
Victoria River Research Station	-	-	1	1	1	-
Katherine Research Station	-	-	3	3	3	-
Douglas Daly Research Farm	-	-	3	3	3	-
Grand Total	2	2	23	22	22	9

Results by Location:**Agent detection**

Location	Serotype (by sequencing)			Genotype (by sequencing)			Virus Isolate Typing Assay	
	BTV12	BTV15	BTV16	Australia A	Australia B	Malaysia A2	Serotype 12	Serotype 16
Coomalie	-	-	4	4	-	-	-	1
Beatrice Hill Farm	9	1	1	1	1	9	8	-
Victoria River Research Station	-	-	1	1	-	-	-	-
Katherine Research Station	-	-	3	3	-	-	-	-
Douglas Daly Research Farm	-	-	3	3	-	-	-	-
Grand Total	9	1	12	12	1	9	8	1

Serology

Location	cELISA	SNT
	Positive	Dominant Serotypes detected
Stirling Station	1	16
Murray Downs Station	2	16
Grand Total	3	

Test numbers

Samples Tested:

Location	Sample Type		
	WHOLE BLOOD (EDTA/LITH HEP)	PLASMA/SERUM	VIRUS ISOLATES
Queensland	97	97	-
Western Australia	181	137	-
Northern Territory	-	3	23
TOTAL	278	237	23

Testing Performed:

Location	Serology		Molecular	Sequencing		Isolation and Typing	
	cELISA [^]	BTV SNT panels ^{*†}	BTV TQM PCR [^]	Genotype Sequencing (VP3)	Serotype Sequencing (VP2)**	Isolation [†]	Typing Assays
Queensland	97	97	97	80	80	9	1
Western Australia	157	141 (+17 BTV-15 only [#])	137	25	25	5	1
Northern Territory	2	2	23	22	22	-	9
Grand Total	256	240 (+17)	257	127	127	14	11

* 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

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

BTV-21 Activity

Whilst BTV-1 and BTV-16 continue to be the predominate serotypes detected across the country, ACDP has observed, and is continuing to investigate, a potentially significant change in the Australian BTV-21 population.

Very high serological titres (>1280) to BTV-21 have been observed in a number of samples in both QLD and WA in the reporting period. Whilst the significance of these results remains unclear, ACDP is conducting further whole genome sequencing on a number of BTV-21 positive blood samples to establish if this serological behaviour can be attributed to a change in the molecular epidemiology of this virus.

Whole Genome Sequencing @ ACDP

ACDP has made significant progress in developing the diagnostic pipelines to allow whole genome sequencing of Bluetongue viruses directly from blood samples. This technology utilises the MyBaits probe system, allowing generation of whole genome sequence data from BTV TQM positive samples. Whilst validation is ongoing, the sensitivity of this approach appears to be similar to that of current capillary sequencing technology, with whole genome data obtained from samples with BTV TQM Cts of approximately <28-30.

Once validation is complete, it is likely that ACDP will transition our routine diagnostic sequencing approach for NAMP submissions from VP3 and VP2 sequencing to WGS. This approach, with utilisation of the Nextstrain platform and ACDP's novel machine learning model for whole genome characterisation, will facilitate greater insights into the molecular epidemiology of BTV at the national level.

ACDP will advise the NAMP TC prior to any change being implemented.