FOR INFORMATION

NAMP Technical Committee Annual Meeting 2023
Agenda Item 4.9
Last updated: 21 August 2023
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ACDP VIROLOGY REPORT 2022-23

RECOMMENDATION

- That the NAMP Technical Committee NOTES
  - Testing performed by ACDP for WA and Qld in 2022-23
  - Detection of increased BTV-23 seroreactivity in QLD
  - Recent changes to the BTV Serology algorithm in use at ACDP, as previously communicated

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 FY2022/23

Appendix A: CSIRO ACDP – AAHL NAMP Activities Report FY2022/23

Contents

NAMP Testing Summary

Sentinel Herds

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

Western Australia:

- Kununurra
- Kalumburu Mission

Queensland:

- Capella
- Alligator Creek
- Hervey Range
- Cooktown
- East Palmerston
- Kalkie
- Gatton
- Charleville
- Chinchilla
- Etna Creek
- Peachester
- Springsure
- Allora
- Good Night
- Goondiwindi

Sentinel herd locations, and the serotypes detected by VP2 gene sequencing for submissions received in FY22-23 are shown in Figure 1. For comparison, data from FY2021-22, FY2020-21 and FY2019-20 are shown in Figures 2 & 3 & 4.

Figure 1. 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.

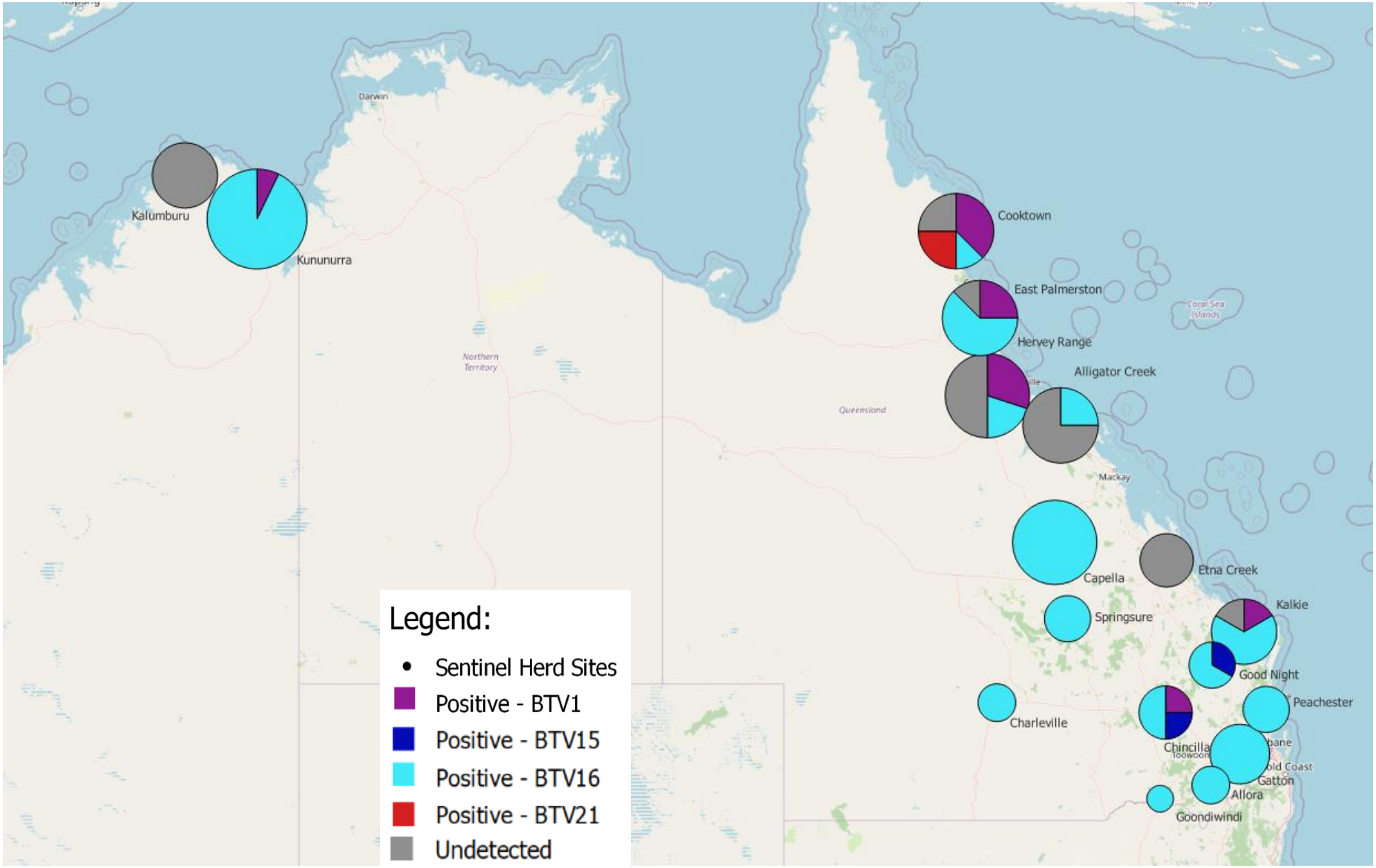


Figure 2. Bluetongue virus serotypes detected by sequencing from NAMP sentinel herds (FY2021-22).
Size of the pie charts are proportional to the total number of BTV TQM positive samples/isolates received. NB: Includes characterisation of some QLD isolates that originated from FY21 collections.

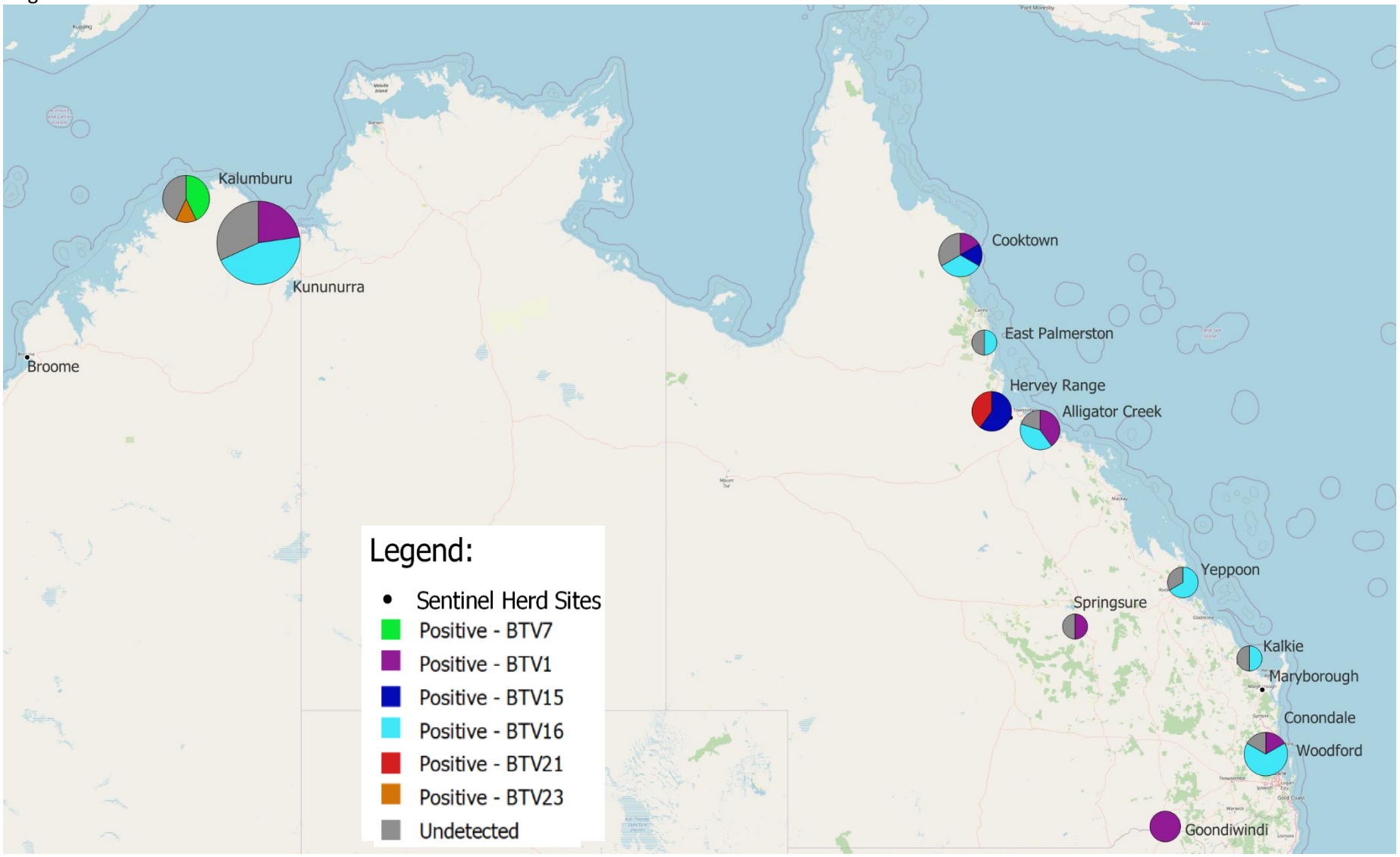


Figure 3. Bluetongue virus serotypes detected by sequencing from NAMP sentinel herds (FY2020-21).
Size of the pie charts are proportional to the total number of BTV TQM positive samples/isolates received.

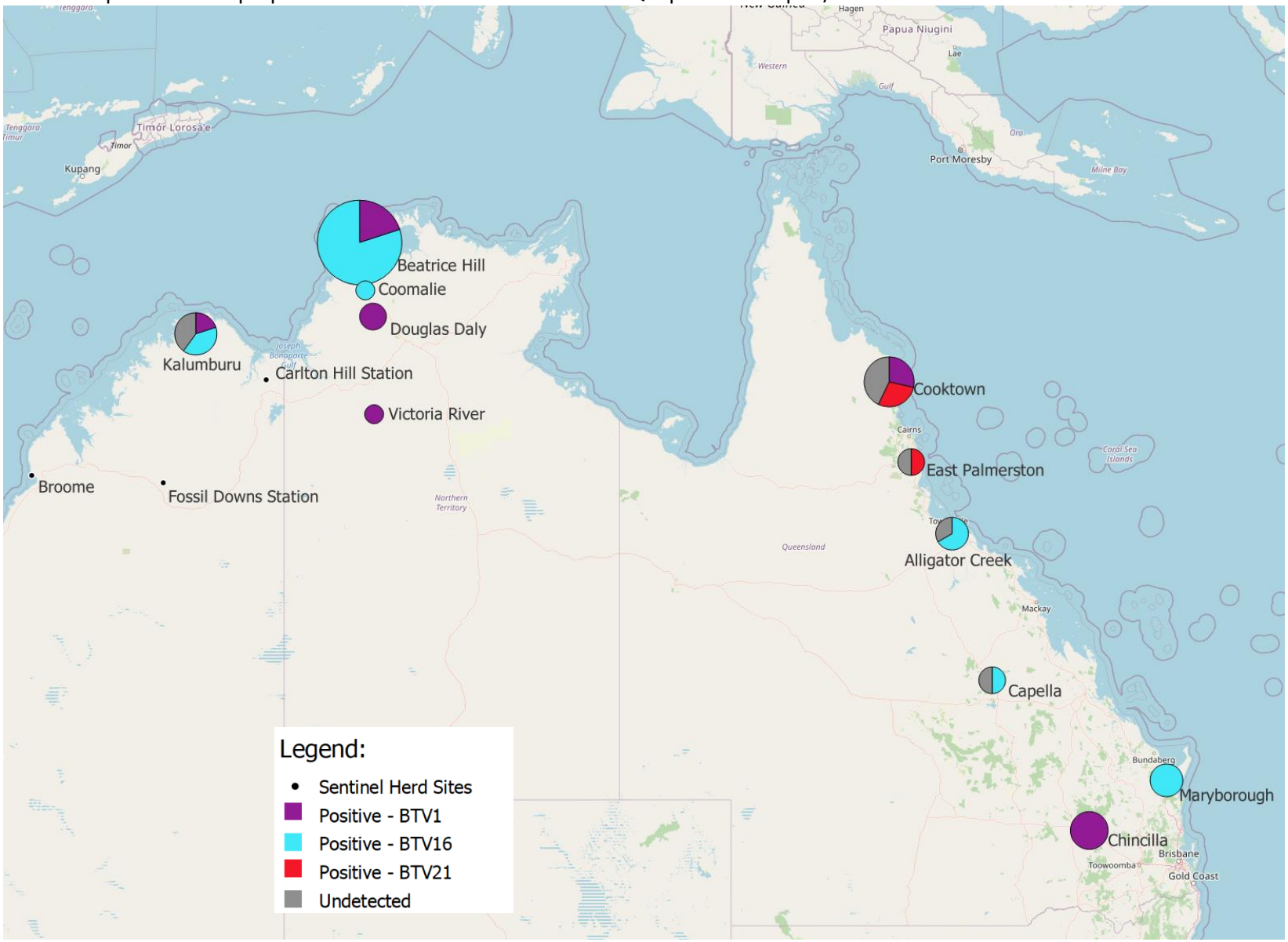
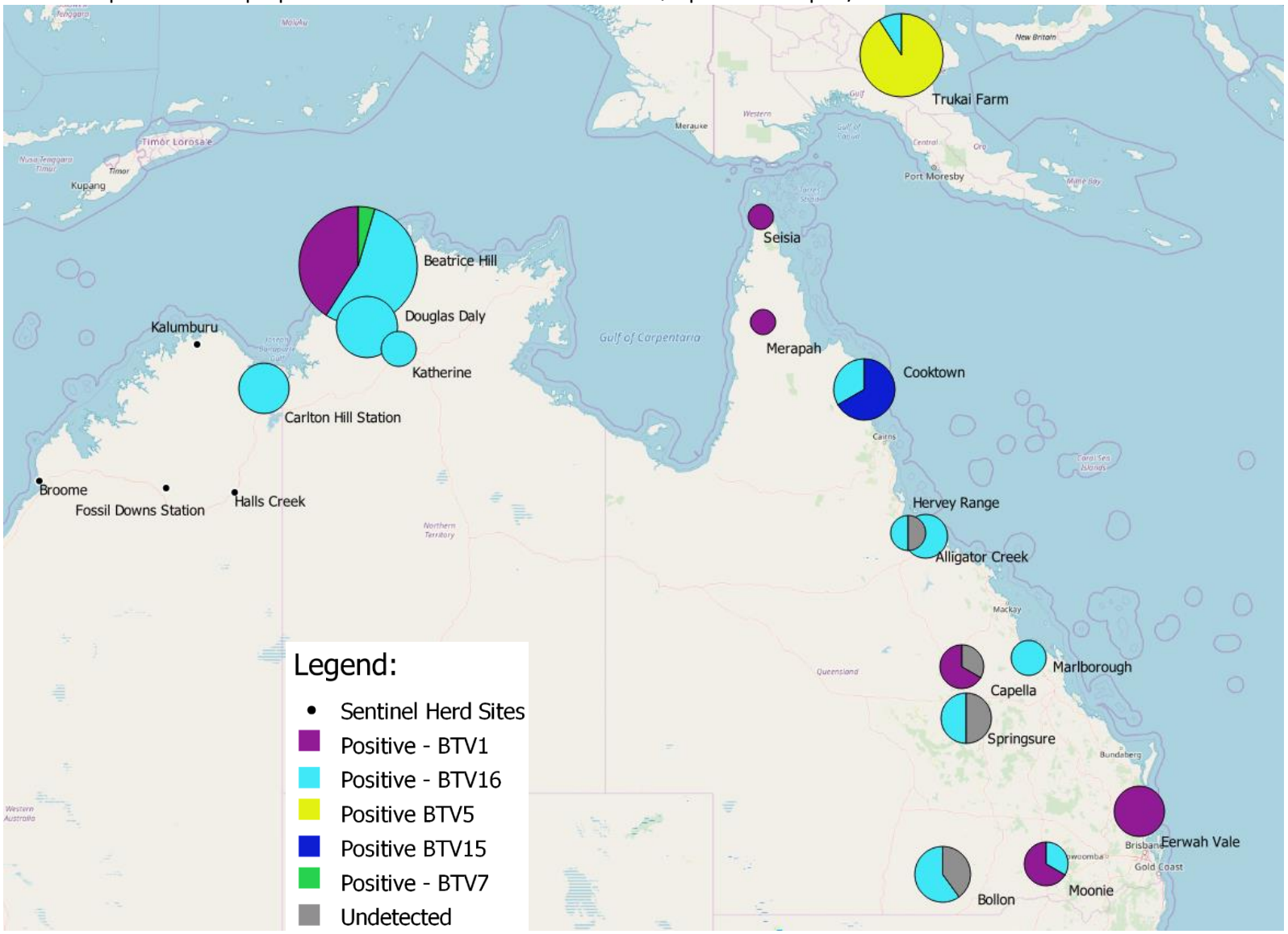


Figure 4. Bluetongue virus serotypes detected by sequencing from NAMP sentinel herds (FY2019-20).
Size of the pie charts are proportional to the total number of BTV TQM positive samples/isolates received.



Queensland

Samples Tested:

Location	Sample Type	
	WHOLE BLOOD (EDTA/LITH HEP)	PLASMA/SERUM
Capella	24	24
Alligator Creek	11	11
Hervey Range	11	11
Cooktown	7	7
East Palmerston	7	7
Kalkie	6	6
Gatton	5	5
Charleville	4	4
Chinchilla	4	4
Etna Creek	4	4
Peachester	3	3
Springsure	3	3
Allora	2	2
Good Night	2	2
Goondiwindi	1	1
TOTAL	**94**	**94**

Testing Performed:

Location	Serology			Molecular	Sequencing			BTV Isolation
	cELISA	BTV SNT PANEL (up to 18 serotypes^)	BTV SNT Serotype 15*	BTV TQM PCR	Genotype Sequencing (VP3)	Serotype Sequencing (VP2)	Next Generation Sequencing	
Capella	24	14	10	24	10	10	-	5
Alligator Creek	11	11	-	11	3	8	-	-
Hervey Range	11	10	1	11	10	10	-	-
Cooktown	7	7	-	7	7	7	-	-
East Palmerston	7	7	-	7	7	7	-	-+
Kalkie	6	6	-	6	6	6	-	2
Gatton	5	5	-	5	5	5	1	3+
Charleville	4	2+	-	4	2	2	-	-
Chinchilla	4	4	-	4	3	3	-	2
Etna Creek	4	4	-	4	4	4	-	-
Peachester	3	3	-	3	3	3	-	-+
Springsure	3	3	-	3	3	3	-	-
Allora	2	2	-	2	2	2	-	-
Good Night	2	2	-	2	2	2	-	1
Goondiwindi	1	1	-	1	1	1	-	-
Grand Total	**94**	**81**	**11**	**94**	**68**	**73**	**1**	**13**

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

^ACDP recently adopted a reduced initial SNT panel for all BTV cELISA positive serum samples. For further information, please see below.

+ Additional results pending

Results by Location:

Agent detection

Location	Serotype (by sequencing)					Genotype (by sequencing)			Isolation	
	BTV1	BTV15	BTV16	BTV21	Undetected	Australia A	Malaysia A2	Undetected	Negative	Positive
Capella	-	-	10	-	-	10	-	-	5	-
Gatton	-	-	5	-	-	5	-	-	1+	2+
East Palmerston	2	-	5	-	1	4	2	1	-+	-+
Kalkie	1	-	4	-	1	6	-	-	2	-
Peachester	-	-	3	-	-	3	-	-	-+	-+
Springsure	-	-	3	-	-	3	-	-	-	-
Alligator Creek	-	-	2	-	6	1	-	2	-	-
Allora	-	-	2	-	-	2	-	-	-	-
Charleville	-	-	2	-	-	2	-	-	-	-
Hervey Range	3	-	2	-	5	6	-	4	-	-
Chinchilla	1	1	2	-	-	3	-	-	2	-
Good Night	-	1	2	-	-	2	-	-	1	-
Goondiwindi	-	-	1	-	-	1	-	-	-	-
Etna Creek	-	-	-	-	4	4	-	-	-	-
Cooktown	3	-	1	2	2	7	-	-	-	-
Grand Total	**10**	**2**	**44**	**2**	**19**	**59**	**2**	**7**	**11**	**2**

+ Additional results pending

Serology

Location	cELISA			SNT
	Negative	Positive	Inconclusive	Dominant Serotypes detected
Capella	10	13	1	1, 15, 16, 21 (23#)
Alligator Creek	-	11	-	1, 16, 21
Hervey Range	1	10	-	1, 15, 16, 21 (23#)
Cooktown	-	7	-	1, 16, 21 (23#)
East Palmerston	-	7	-	1, 15, 16, 21
Gatton	-	5	-	1, 15, 16, 21
Kalkie	-	5	1	15, 16, 21
Charleville	-	4	-	1, 21+
Chinchilla	-	4	-	1, 15, 16, 21 (23#)
Etna Creek	-	4	-	1, 16, 21
Peachester	-	3	-	16, 21
Springsure	-	3	-	16, 21 (23#)
Allora	-	2	-	1, 16, 21
Good Night	-	2	-	16, 21
Goondiwindi	-	1	-	15, 16, 21
Grand Total	**11**	**81**	**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

Western Australia

Samples Tested:

Location	Sample Type	
	WHOLE BLOOD (EDTA/LITH HEP)	PLASMA/SERUM
Kalumburu	50	52
Kununurra	23	32
TOTAL	**73**	**84**

Testing Performed:

Location	Serology			Molecular	Sequencing		Isolation and Typing
	cELISA	BTV SNT PANEL (inc. 18 serotypes)	BTV SNT Serotype 15*	BTV TQM PCR	Genotype Sequencing (VP3)	Serotype Sequencing (VP2)	Isolation
Kalumburu	30	13	17	50	6	6	4
Kununurra	32	31[#]	-	23	16	14	-[#]
Grand Total	**62**	**44**	**17**	**73**	**22**	**20**	**4[#]**

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

Results by Location:

Agent detection

Location	Serotype (by sequencing)			Genotype (by sequencing)					Isolation[#]	
	BTV1	BTV16	Undetected	Australia A	Malaysia A2	Malaysia B	Java C	Undetected	Negative	Positive
Kalumburu	-	-	6	-	2	1	2	1	4	-
Kununurra	1	13	-	11	-	2	-	3	-	-
Grand Total	**1**	**13**	**6**	**11**	**2**	**3**	**2**	**4**	**4**	**-**

[#]Additional Results pending

Serology

Location	cELISA			SNT
	Inconclusive	Negative	Positive	Dominant Serotypes detected
Kalumburu	-	17	13	1, 5, 16, 21 (7^)
Kununurra	2	-	30	1, 16, 21[#]
Grand Total	**2**	**17**	**43**	

^ Minor reactions

[#]Additional Results pending

Test numbers

Samples Tested:

Location	Sample Type	
	WHOLE BLOOD (EDTA/LITH HEP)	PLASMA/SERUM
Queensland	94	94
Western Australia	73	84
TOTAL	**167**	**178**

Testing Performed:

Location	Serology		Molecular	Sequencing		Isolation and Typing
	cELISA^	BTV SNT*†	BTV TQM PCR^	Genotype Sequencing (VP3)	Serotype Sequencing (VP2)**	Isolation†
Queensland	94	1,469	94	68	73	13
Western Australia	62	809	73	22	20	4
Grand Total	**156**	**2,278**	**167**	**90**	**93**	**17**

*Up to 18 SNTs/panel (15 Australian + 3 for cross reactions). ACDP recently adopted a reduced initial SNT panel for all BTV cELISA positive serum samples. For further information, please see below.

**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)

† Addition testing ongoing

BTV-23 Seroreactivity in QLD

Background:

- BTV-23 was detected by serotyping sequencing from a surveillance bleed in Kalumburu in October 2021. This was the first agent detection of BTV-23 in Australia since 1989.
- BTV-23 is known to share significant cross-reactivity with other BTV serotypes in the SNT panel. ACDP has consistently observed low levels of reactivity to BTV-23 in the SNT panel for many years.

Update:

- An apparent increase in the frequency and strength of BTV-23 reactivity was observed in some QLD samples in 22-23. In some cases, BTV-23 reactivity was observed as the dominant serotype in the SNT, with titres up to 160 with limited evidence of broad exposure to other serotypes. This pattern of results suggested possible prior exposure to BTV-23.

- Additional targeted investigations of suspect properties was performed in an attempt to detect BTV-23 by PCR and serotype sequencing, including adoption of an additional BTV-23 primer set. No BTV-23 was detected in any samples.

Conclusion:

- BTV-23 viruses have not been detected in QLD.
- There is some serological evidence that suggests possible low level circulation of BTV-23 in QLD. However, the possibility of cross-reactivity in the SNT cannot be excluded.
- ACDP has included BTV-23 as a screening serotype in the new targeted SNT panel, to continue to monitor for further exposure to this serotype.

New Serology Algorithm

ACDP has recently reviewed the diagnostic serology algorithm for Bluetongue virus. The purpose of this review was to identify opportunities for improved efficiencies, to facilitate more rapid reporting of results.

Following this review, a number of changes to the serological algorithm have been made.

The Bluetongue virus cELISA remains the primary screening test for the detection of BTV antibodies.

For routine NAMP and Exclusion submissions, ACDP has moved to a reduced initial SNT panel for all BTV cELISA positive serum samples.

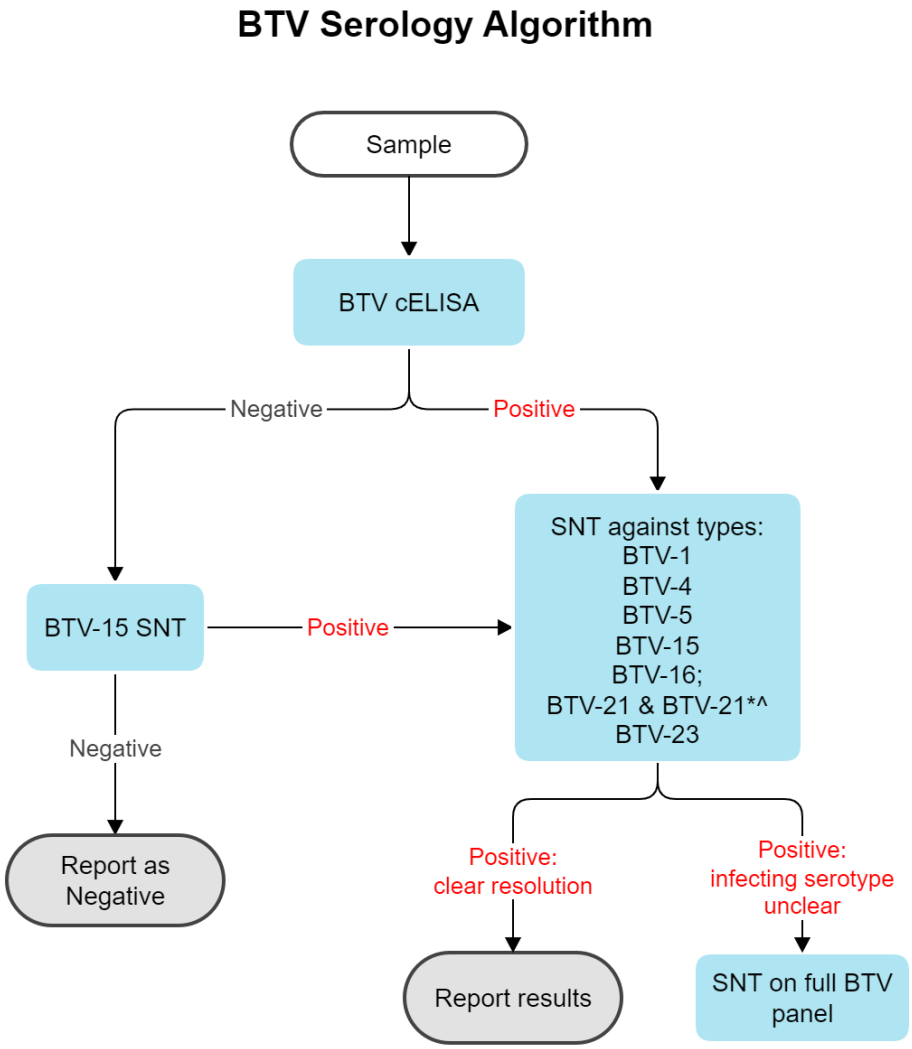
- The serotypes tested are BTV-1, BTV-4, BTV-5, BTV-15, BTV-16, BTV-21 and BTV-23.
- If this panel provides clear resolution of the infecting serotypes, these results will be reported.
- If there is no clear resolution of the infecting serotypes, samples will be progressed to the full SNT panel currently in use. The full SNT panel tests for Australian serotypes of BTV (1, 2, 3, 4, 5, 7, 9, 12, 15, 16, 20, 21 and 23), South African BTV 4 and Indonesian BTV 3 and 21.

Please Note:

- The reduced BTV SNT initial panel will be reviewed on a regular basis, as well as in response to changes in circulating serotypes.
- For high value strategic sampling sites, such as Kalumburu Mission, the full BTV SNT panel will continue to be used. This reflects the higher probability of encountering exotic serotypes in such locations.
- ACDP has completed a review of performance of two non-prototype viruses (BTV-4$^{\#}$ and BTV-21*) that have been routinely used in the SNT panel in recent years. These viruses were introduced into the panel to assess for the possibility of circulation of BTV-4 and BTV-21 viruses that were antigenically distinct from the prototype viruses previously used. The outcome of this review is that the BTV-4$^{\#}$ virus will be removed from the reduced panel, whilst the BTV-21* will remain in use. As such, results will be reported for testing against the prototype BTV-4 (only) but both the prototype and non-prototype BTV-21.
- Samples testing negative in the BTV cELISA will still be routinely tested in the BTV-15 SNT, as the BTV cELISA is known to have reduced sensitivity for the detection of BTV-15 antibodies.
- For other submission categories, such as characterisation of export reactors, ACDP will review the diagnostic approach on a case-by-case basis in collaboration with the submitter and with consideration of the relevant clinical and epidemiological information available.

This new approach has been developed to balance the resource requirements and reporting timelines commensurate with the level of risk of failing to detect circulation of novel serotypes.

A schematic diagram of the new serology algorithm is shown below:



^BTV-21* is a 2017 isolate of BTV-21 from the Northern Territory. This virus is included in the panel to detect circulation of BTV-21 viruses that are antigenically divergent from the prototype virus.