FOR INFORMATION

NAMP Technical Committee Annual Meeting 2022
Agenda Item 3.9
Last updated: 12 August 2022
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ACDP VIROLOGY REPORT 2021-22

RECOMMENDATION

- That the NAMP Technical Committee NOTES
  - Testing performed by ACDP for WA, NT and Qld in 2021-22
  - Detection of BTV-23 in Australia (at Kalumburu, WA) for the first time since 1989. Whilst limited sequence data was obtained, the available sequence showed significant variability to BTV-23 previously seen in Australia.
  - Ongoing ACDP funded work on Whole Genome Sequencing and characterisation of genetic relationships between isolates

BACKGROUND

- ACDP performed confirmatory molecular and serological testing of samples (isolates, whole blood, plasma/sera) from WA, NT 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 FY2021/22

Appendix A: CSIRO ACDP – AAHL NAMP Activities Report FY2021/22

Contents

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:

Western Australia:

- Kununurra
- Broome
- Kalumburu Mission
- Halls Creek

Northern Territory:

- AZRI
- BHF
- Coomalie
- Douglas Daly
- Katherine Research Station
- VRRS

Queensland:

- Alligator Creek
- Bertiehaugh Station
- Conondale
- Cooktown
- East Palmerston
- Goondiwindi
- Hervey Range
- Kalkie
- Maryborough
- Springsure
- Woodford
- Yeppoon
- York Downs Station

Sentinel herd locations, and the serotypes detected by VP2 gene sequencing for submissions received in FY21-22 are shown in Figure 1. For comparison, data from FY2020-21 and FY2019-20 are shown in Figures 2 & 3.

Figure 1. 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.

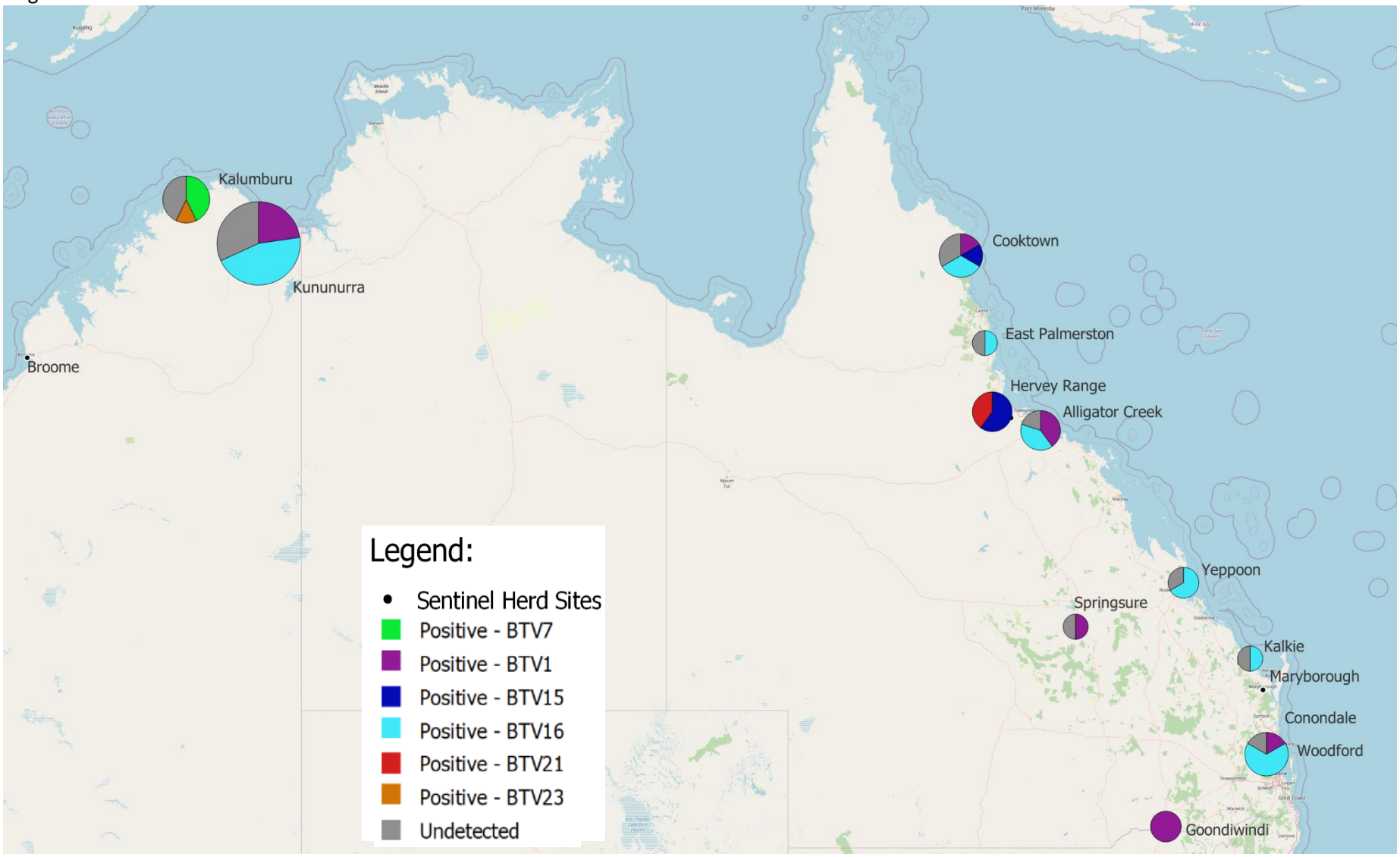


Figure 2. Bluetongue virus serotypes detected by sequencing from NAMP sentinel herds (FY2020-21).
Size of the pie charts are proportional to the total number of BTV TQM positive samples/isolates received.

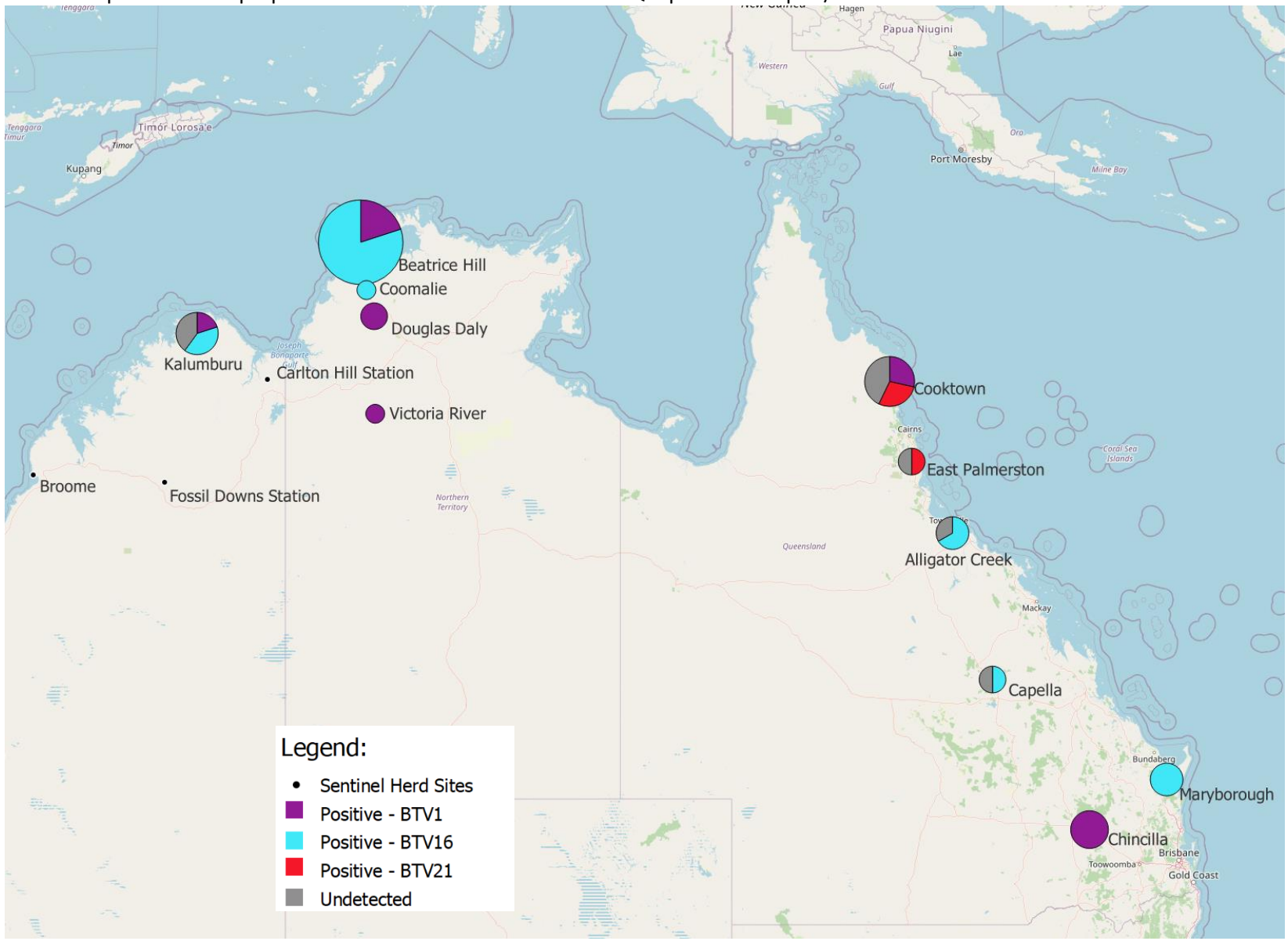
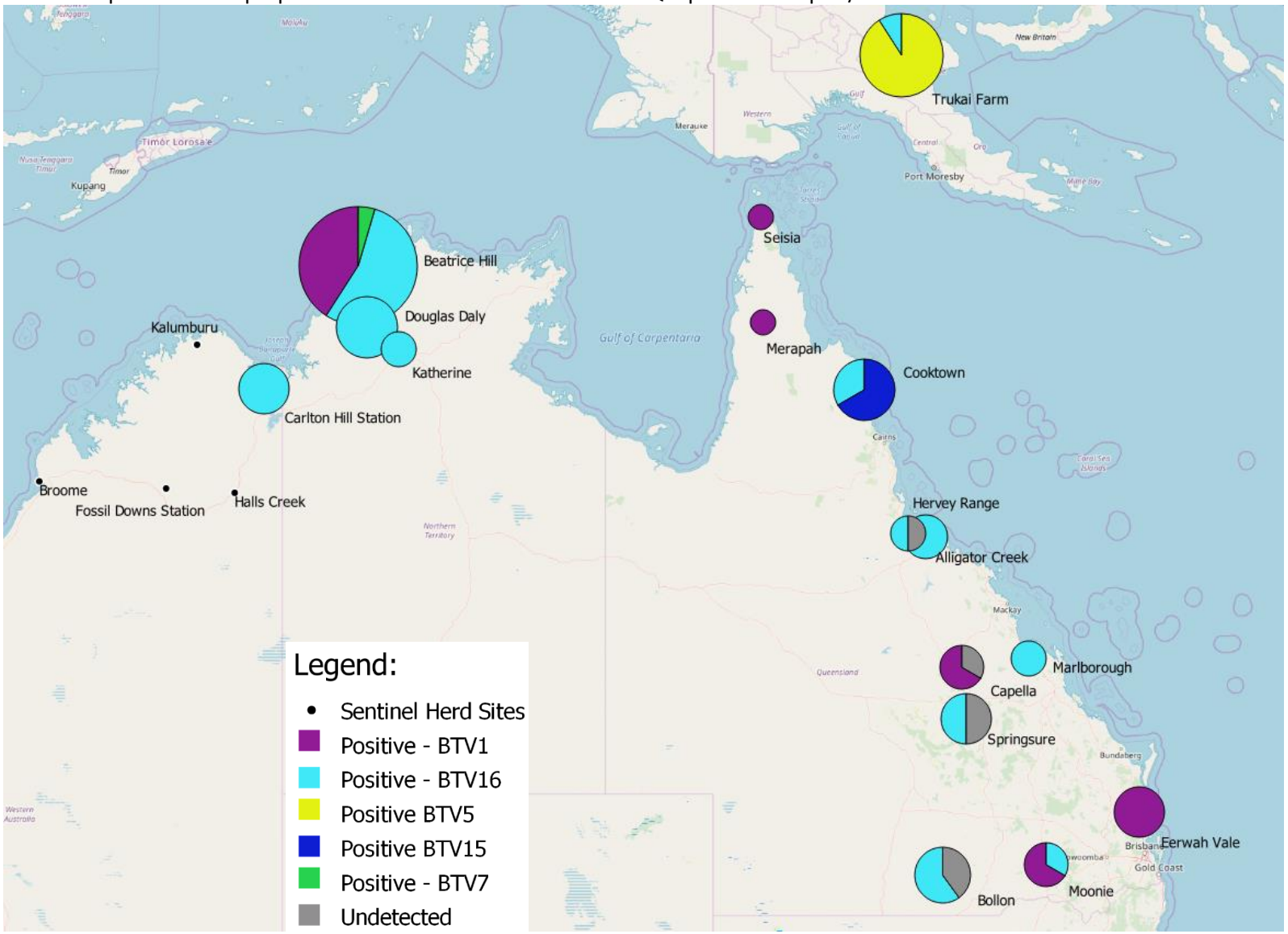


Figure 3. Bluetongue virus serotypes detected by sequencing from NAMP sentinel herds (FY2019-20).
Size of the pie charts are proportional to the total number of BTV TQM positive samples/isolates received.



Queensland

Samples Tested:

Location	Sample Type		
	WHOLE BLOOD (EDTA/LITH HEP)	PLASMA/SERUM*	VIRUS ISOLATES/ CELL CULTURE SUPERNATANTS#
Alligator Creek	2	2	3
Bertiehaugh Station	-	21*	-
Conondale	-	-	1
Cooktown	6	6	1
East Palmerston	2	2	-
Goondiwindi	3	3	-
Hervey Range	2	2	4
Kalkie	2	2	-
Maryborough	-	-	2
Springsure	2	2	-
Woodford	6	6	-
Yeppoon	3	3	-
York Downs Station	-	50*	-
TOTAL	**28**	**99**	**11**

*Includes sera received from BVL for BTV ELISA only.

Includes previously characterised isolates that originated from FY21 collections.

Testing Performed:

Location	Serology			Molecular	Sequencing		BTV Isolation
	cELISA	BTV SNT PANEL (inc. 18 serotypes)	BTV SNT Serotype 15*	BTV TQM PCR	Genotype Sequencing (VP3)	Serotype Sequencing (VP2)	
Alligator Creek	2	2	-	5	5	5	1
Bertiehaugh Station	21[#]	-	-	-	-	-	-
Conondale	-	-	-	1	-	-	-
Cooktown	6	6	-	7	6	6	1
East Palmerston	2	1	1	2	2	2	-
Goondiwindi	3	3	-	3	3	3	-
Hervey Range	2	1	1	6	5	5	[+]
Kalkie	2	2	-	2	2	2	-
Maryborough	-	-	-	2	-	-	-
Springsure	2	-	2	2	2	2	-
Woodford	6	6	-	6	6	6	-
Yeppoon	3	3	-	3	3	3	-
York Downs Station	50[#]	-	-	-	-	-	-
Grand Total	**99**	**24**	**4**	**39**	**34**	**34**	**2**

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

[#]Samples received from BVL for BTV cELISA only, additional characterisation performed at BVL

[+] Additional results pending

Results by Location:

Agent detection

Location	Serotype (by sequencing)					Genotype (by sequencing)					Isolation
	BTV1	BTV15	BTV16	BTV21	Undetected	Australia A	Australia B	Malaysia A	Malaysia A2	Undetected	Negative
Alligator Creek	2	-	2	-	1	4	-	-	-	1	1
Cooktown	1	1	2	-	2	3	1	-	2	-	1
East Palmerston	-	-	1	-	1	1	-	-	1	-	-
Goondiwindi	3	-	-	-	-	3	-	-	-	-	-
Hervey Range	-	3	-	2	-	-	3	2	-	-	#
Kalkie	-	-	1	-	1	2	-	-	-	-	-
Springsure	1	-	-	-	1	2	-	-	-	-	-
Woodford	1	-	4	-	1	5	-	-	-	1	-
Yeppoon	-	-	2	-	1	3	-	-	-	-	-
Grand Total	**8**	**4**	**12**	**2**	**8**	**23**	**4**	**2**	**3**	**2**	**2[#]**

[#]Additional Results pending

Serology

Location	cELISA		SNT
	Negative	Positive	Dominant Serotypes detected
Alligator Creek	-	2	1, 15
Bertiehaugh Station[#]	7	14	NA
Cooktown	-	6	1, 4[^], 16, 21[^]
East Palmerston	1	1	16, 21
Goondiwindi	-	3	1, 21
Hervey Range	1	1	16, 21
Kalkie	-	2	1, 21
Springsure	2	-	NA
Woodford	-	6	1, 16, 21 (Broad reactivity)
Yeppoon	-	3	1, 16, 21
York Downs Station[#]	17	33	NA
Grand Total	**28**	**71**	

[*]Broad reactivity observed

[^] Minor reactions

[#]Samples received from BVL for BTV cELISA only, additional characterisation performed at BVL

Western Australia

Samples Tested:

Location	Sample Type	
	WHOLE BLOOD (EDTA/LITH HEP)	PLASMA/SERUM
Broome	1	1
Halls Creek	-	3
Kalumburu	103	64
Kununurra	30	30
TOTAL	**134**	**98**

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	Virus Isolate Typing Assay
Broome	1	1	-	1	-	-	-	-
Halls Creek	3	3	-	-	-	-	-	-
Kalumburu	64	32[#]	30	103	7	7	6	-
Kununurra	30	27[#]	-	30	22	22	6[#]	3
Grand Total	**98**	**63**	**30**	**134**	**29**	**29**	**12**	**3**

*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	BTV7	BTV16	BTV23	Undetected	Australia A	Malaysia A1	Malaysia A2	Malaysia B	Undetected	Negative	Positive
Kalumburu	-	3	-	1	3	-	1	3	-	3	6	-
Kununurra	5	-	10	-	7	16	-	-	3	3	3	3
Grand Total	**5**	**3**	**10**	**1**	**10**	**16**	**1**	**3**	**3**	**6**	**9**	**3**

Serology

Location	cELISA			SNT
	Indeterminate	Negative	Positive	Dominant Serotypes detected
Broome	-	-	1	21
Halls Creek	1	-	2	16, 21
Kalumburu	2	30	32	1, 4^, 16, 21, 23#
Kununurra	-	-	30	1, 16, 21#
Grand Total	**3**	**30**	**65**	

^ Minor reactions

#Additional Results pending

Northern Territory

Samples Tested:

Location	Sample Type*
	PLASMA/SERUM
Alice Springs	30
Beatrice Hill	46
Coomalie	10
Douglas Daly	15
Katherine	13
Victoria River	19
TOTAL	**133**

*An additional 57 historical isolates from various locations were received from BVL for research purposes.

Testing Performed:

Location	Serology
	cELISA
Alice Springs	30
Beatrice Hill	46
Coomalie	10
Douglas Daly	15
Katherine	13
Victoria River	19
Grand Total	**133**

Results by Location:

Agent detection

Location	Serotype (by sequencing)		
	Indeterminate	Negative	Positive
Alice Springs	-	30	-
Beatrice Hill	1	42	3
Coomalie	-	9	1
Douglas Daly	-	11	4
Katherine	-	8	5
Victoria River	1	14	4
Grand Total	**2**	**114**	**17**

Test numbers

Samples Tested:

Location	Sample Type		
	WHOLE BLOOD (EDTA/LITH HEP)	PLASMA/SERUM	VIRUS ISOLATES/ CELL CULTURE SUPERNATANTS
Northern Territory	-	133	-*
Queensland	28	99	11
Western Australia	134	98	-
TOTAL	**162**	**330**	**11**

*An additional 57 historical isolates from various locations were received from BVL for research purposes.

Testing Performed:

Location	Serology		Molecular	Sequencing		Isolation and Typing	
	cELISA^	BTV SNT*	BTV TQM PCR^	Genotype Sequencing (VP3)	Serotype Sequencing (VP2)**	Isolation+	Virus Isolate Typing Assay##
Northern Territory	133	-	-	-	-	-	-
Queensland	99	436†	39	34	34	2†	-
Western Australia	98	1,164†	134	29	29	12†	39
Grand Total	**330**	**1,600†**	**173**	**63**	**63**	**14†**	**39**

*18 SNTs/panel (15 Australian + 3 for cross reactions)
**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)
VNT performed on both isolates received and isolates grown at ACDP. 13 VNTs/isolate
+ Isolates received may be passaged prior to VNT
† Addition testing ongoing

Significant Results summary

BTV-23 at Kalumburu

BTV23 was detected by VP2 Sequencing in a single sample from Kalumburu, collected in October 2021 (ACDP SAN 21-04192). This was the first agent detection of BTV23 by ACDP-AAHL since 1989. Whilst limited sequence data was obtained, the available sequence showed significant variability to BTV-23 previously seen in Australia. This is suggestive of a novel incursion.

In addition, SNT results from both this submission (Oct 2021) and a subsequent submission (ACDP SAN 22-02546, May 2022) provided further serological evidence of BTV23 exposure in this herd.

Whilst no isolate was obtained from this sample, additional whole genome sequencing attempts are currently underway using recently developed probe-based next generation sequencing technologies (myBaits, see below).

BTV-7 at Kalumburu

BTV-7 was detected by VP2 Sequencing in a number of samples from Kalumburu, collected in May 2022 (ACDP SAN 22-02546).

Interestingly, there was no concurrent serological evidence of seroconversion to BTV-7 in the herd in the SNT. Additional SNT testing is ongoing to further investigate this result.

ACDP's most recent detection of BTV7 by sequencing was in 2019, in two isolates obtained from BVL. Prior to this, the last detection was in 2014. In addition, an increase in low-level BTV7 seroreactivity had been identified at the Kalumburu herd in 2018 (as presented at the 2019 NAMP Technical Committee meeting).

Update on Whole Genome Sequencing Activities and Nextstrain

Through 2021-22, ACDP has undertaken a project to develop a novel method of characterisation of the genetic relationships between BTV isolates, at the whole genome level. This project aimed to overcome the limitations of the current BTV characterisation methods, that rely heavily on serotyping alone and as such are poorly sensitive to detecting potentially significant changes in the molecular epidemiology of BTV. Rapidly analysing viruses at the whole genome level has the potential to greatly improve our understanding of the molecular epidemiology of BTV.

Through this project, we have developed a Machine Learning model (Support Vector Machine, SVM) that assigns isolates into clusters using kmer-based investigations of nucleotide sequence variability in three series of concatenated genome sequences. During testing, the model has shown to produce predictable cluster designations consistent with the geographical origin of the isolates.

Further intra-cluster phylogenetic analysis can then be performed in a newly developed Bluetongue virus Nextstrain platform, to identify specific within-cluster clade designations.

Based on the model outputs and further analysis, a novel nomenclature has been proposed for the classification of BTV isolates that incorporates geographical origin, date, topotype, serotype and machine learning-determined cluster designations.

Newly obtained whole genome sequence data (i.e. from new diagnostic submissions) can be rapidly assessed in the model to identify genetic relationships to historical isolates. These relationships can then be visualised in the Nextstrain platform. Whilst validation of this methodology is ongoing, it is hoped that this system will greatly assist in understanding the patterns of BTV transmission at a whole genome level.

Additional applications of the new Nextstrain platform may include identification of:

- changes in patterns of virus transmission
- novel virus (or segment) incursions, and
- significant viral recombination events.

Full details of the methodology utilised in the development and testing of this model, as well as the proposed nomenclature for whole genome characterisation, will appear in a future manuscript. This manuscript will be circulated to NAMP for comment prior to submission for publication. Subject to peer review and further testing, ACDP aims to utilise this model to report on whole genome characterisation of NAMP isolates in the future. We envisage that once complete, NAMP stakeholders will be able to log into the system to access this enhanced, interactive genomic surveillance tool for their own purposes.

One of the limitations of this approach is that whole genome sequencing currently requires high-titre isolates and cannot be performed on field samples. To overcome this, ACDP is currently investigating

the use of myBaits target capture kits for probe-enriched next-generation sequencing of BTV samples. It is hoped that this technology may allow whole genome sequencing of PCR-positive field samples. Further updates will be provided once this technology has been fully assessed.

ACDP would like to thank all NAMP submitters who have assisted in providing isolates and PCR positive samples for use in these activities.