Attachment A:

CSIRO ACDP – AAHL

NAMP Activities Report

FY2019/20

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:

- Broome
- Fossil Downs Station
- Halls Creek
- Kalumburu
- Carlton Hill Station

Northern Territory:

- Beatrice Hill
- Douglas Daly
- Katherine

Queensland:

- Seisia
- Merapah
- Cooktown
- Hervey Range
- Alligator Creek
- Marlborough
- Capella
- Springsure
- Eerwah Vale
- Bollon
- Moonie

Papua New Guinea:

- Trukai Farm

Timor Leste:

- Los Palos, Lautem District

Sentinel herd locations, and the serotypes detected by VP2 gene sequencing for submissions received in FY2019-20 are shown in Figure 1. For comparison, data from FY2018-19 is shown in Figure 2.

Figure 1. 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.

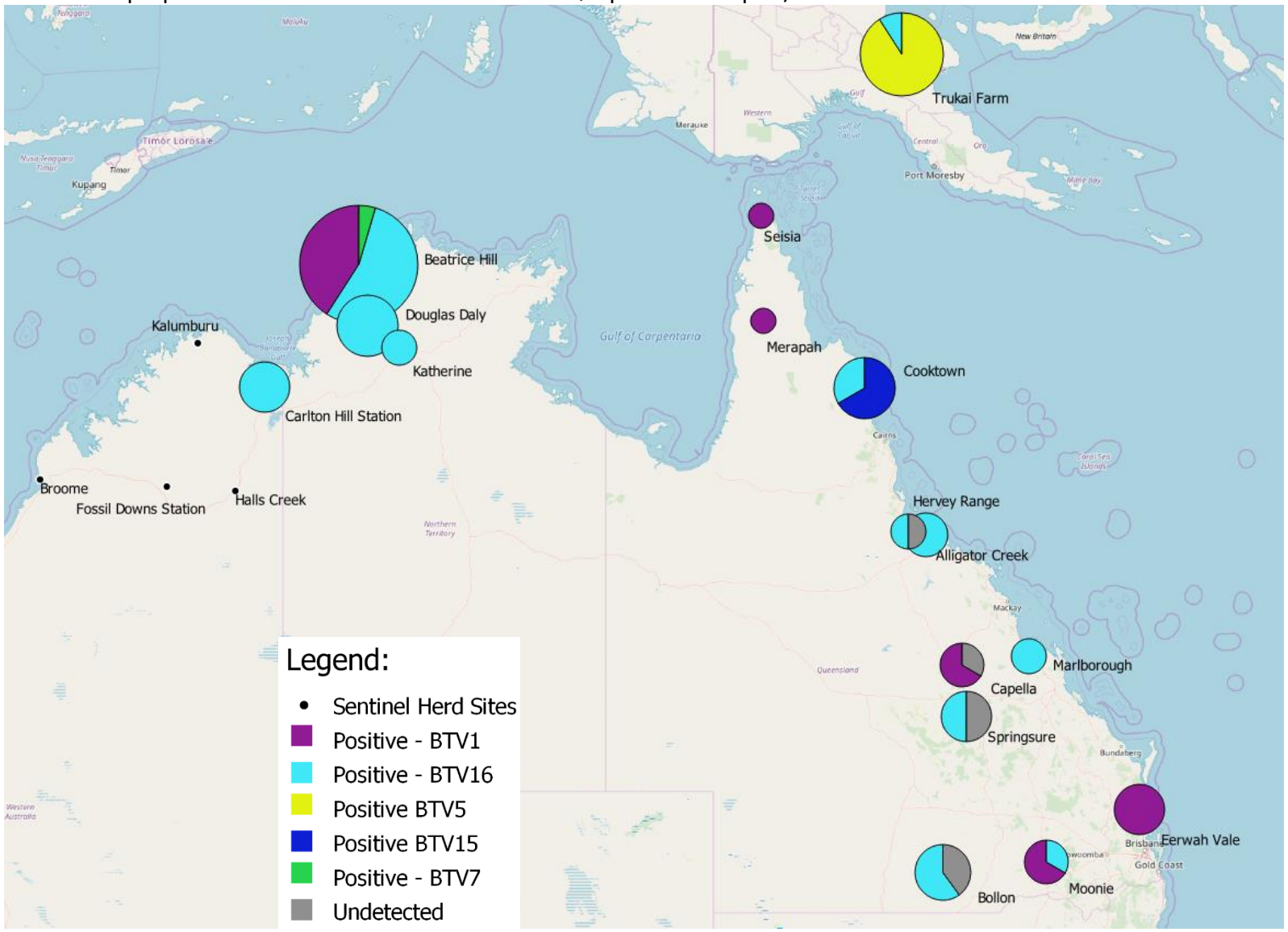
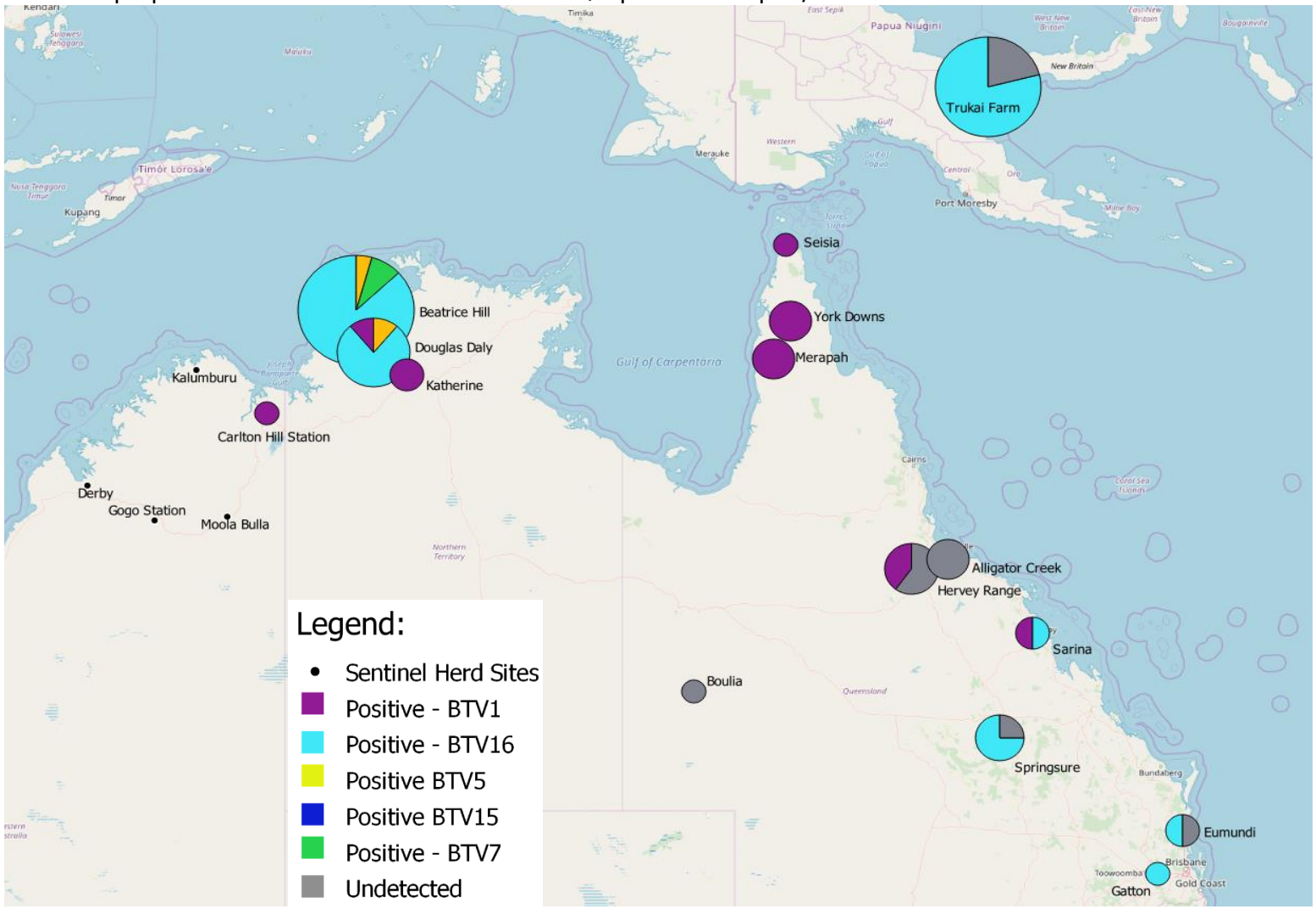


Figure 2. Bluetongue virus serotypes detected by sequencing from NAMP herds (FY2018-19).
Size of the pie charts are proportional to the total number of BTV TQM positive samples/isolates received.



Queensland

Samples Received:

Location	Sample Type		
	WHOLE BLOOD (EDTA/LITH HEP)	PLASMA/SERUM	VIRUS ISOLATES/ CELL CULTURE SUPERNATANTS
Alligator Creek	3	3	3
Bollon	5	5	-
Capella	3	3	-
Cooktown	4	4	6
Eerwah Vale	4	4	-
Hervey Range	2	2	-
Marlborough	-	-	2
Merapah	-	-	1
Moonie	3	3	1
Seisia	-	-	1
Springsure	4	4	-
Not advised	-	-	3
TOTAL	**28**	**28**	**17**

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
Alligator Creek	3	3	-	6	3 (+3#)	3 (+3#)	6	2 (+3#)
Bollon	5	5	-	5	5	5	5	-
Capella	3	3	-	3	3	3	3	-
Cooktown	4	3	1	10	6 (+4#)	6 (+4#)	10	2 (+4#)
Eerwah Vale	4	2	2	4	4	4	4	3
Hervey Range	2	1#	1#	2	2	2	-	-
Marlborough	-	-	-	2	2	2	2	2
Merapah	-	-	-	1	1	1	1	1
Moonie	3	3	-	4	3 (+1#)	3 (+1#)	4	1#
Seisia	-	-	-	1	1	1	1	1
Springsure	4	4	-	4	4	4	4	2
Not Advised	-	-	-	3	(+3#)	(+3#)	3	(+3#)
Grand Total	**28**	**24**	**4**	**45**	**34 (+11#)**	**34 (+11#)**	**43**	**13 (+11#)**

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

#Results pending

+ Isolates received may be passaged prior to VNT

Results by Location:

Agent detection

Location	Serotype (by sequencing)				Genotype (by sequencing)				Isolation	
	BTV1	BTV15	BTV16	Undetected	Australia A	Australia B2	Malaysia A	Undetected	Negative	Positive
Alligator Creek	-	-	3	-	3	-	-	-	3	-
Bollon	-	-	3	2	2	-	-	3	5	-
Capella	2	-	-	1	3	-	-	-	3	-
Cooktown	-	4	2	-	2	4	-	-	4	-
Eerwah Vale	4	-	-	-	4	-	-	-	1	3
Hervey Range	-	-	1	1	2	-	-	-	-	-
Marlborough	-	-	2	-	2	-	-	-	-	-
Merapah	1	-	-	-	-	-	1	-	-	-
Moonie	2	-	1	-	3	-	-	-	3	-
Seisia	1	-	-	-	-	-	1	-	-	-
Springsure	-	-	2	2	4	-	-	-	2	2
Grand Total	**10**	**4**	**14**	**6**	**25**	**4**	**2**	**3**	**21**	**5**

Serology

Location	cELISA			SNT
	Indeterminate	Negative	Positive	Dominant Serotypes detected
Alligator Creek	-	-	3	16, 4, 1, 15
Bollon	-	-	5	16
Capella	-	-	3	1
Cooktown	-	1	3	15*
Eerwah Vale	-	2	2	4*
Hervey Range	-	1	1	#
Moonie	-	-	3	16 (15^)
Springsure	1	-	3	16, 4, 21 (1^)
Grand Total	**1**	**4**	**23**	

Results pending

* Broad reactivity observed

^ Minor reactions

Western Australia

Samples Received:

Location	Sample Type		
	WHOLE BLOOD (EDTA/LITH HEP)	PLASMA/SERUM	BLOOD CLOT
Broome	-	3	-
Carlton Hill Station	11	11	-
Fossil Downs Station	5	6	1
Halls Creek	6	-	1
Kalumburu	65	65	-
TOTAL	**87**	**85**	**2**

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	3	3	-	-	-	-	-	-
Carlton Hill Station	11	4 (7#)	-	11	2	2	2	-
Fossil Downs Station	6	6	-	6	-	-	-	-
Halls Creek	-	-	-	7	-	-	-	-
Kalumburu	65	55	10	65	-	-	-	-
Grand Total	**85**	**68 (+7#)**	**10**	**89**	**4**	**4**	**2**	**0**

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

#Results pending

Results by Location:

Agent detection

Location	Serotype (by sequencing)	Genotype (by sequencing)	Isolation	
	BTV16	Australia A	Negative	Positive
Carlton Hill Station	2	2	2	0
Grand Total	**2**	**2**	**2**	**0**

Serology

Location	cELISA			SNT
	Indeterminate	Negative	Positive	Dominant Serotypes detected
Broome	-	-	3	16 (4^, 21^)
Carlton Hill Station	-	-	11	16, (21^, 23^)#
Fossil Downs Station	1	-	5	4, 16 (1^)
Kalumburu	1	10	54	16, 4, 21, (1^)
Grand Total	**2**	**10**	**73**	

^ Minor reactions

Further results pending

Northern Territory

Samples Received:

Location	Sample Type
	VIRUS ISOLATES
Beatrice Hill	22
Douglas Daly	6
Katherine	2
TOTAL	**30**

Testing Performed:

Location	Molecular	Sequencing		Isolation and Typing	
	BTV TQM PCR	Genotype Sequencing (VP3)	Serotype Sequencing (VP2)	Isolation+	Virus Isolate Typing Assay
Beatrice Hill	22	22	22	5 (+17#)	5 (+17#)
Douglas Daly	6	6	6	6	6
Katherine	2	2	2	2	2
Grand Total	**30**	**30**	**30**	**13 (+17#)**	**13 (+17#)**

#Results pending

+ Isolates received may be passaged prior to VNT

Results by Location:

Agent detection

Location	Serotype (by sequencing)			Genotype (by sequencing)			Virus Isolate Typing Assay
	BTV16	BTV1	BTV7	Australia A	Malaysia A	Untyped	Serotype 16
Beatrice Hill	12	9	1	9	12	1	5#
Douglas Daly	6	-	-	-	6	-	6
Katherine	2	-	-	2	-	-	2
Grand Total	**20**	**9**	**1**	**11**	**18**	**1**	**13**

Further results pending

Papua New Guinea

Samples Received:

Location	Sample Type
	Blood Clots
Erap, Morobe Province (Trukai Farm)	11
TOTAL	**11**

Testing Performed:

Location	Molecular	Sequencing		Isolation and Typing	
	BTV TQM PCR	Genotype Sequencing (VP3)	Serotype Sequencing (VP2)	Isolation	Virus Isolate Typing Assay
Erap, Morobe Province (Trukai Farm)	11	11	11	11	2
Grand Total	**11**	**11**	**11**	**11**	**2**

Results by Location:

Agent detection

Location	Serotype (by sequencing)		Genotype (by sequencing)	Virus Isolate Typing Assay	
	BTV16	BTV5	Malaysia A	Serotype 16	Serotype 5
Erap, Morobe Province (Trukai Farm)	1	10	11	1	1
Grand Total	**1**	**10**	**11**	**1**	**1**

Timor Leste (NAQS)

Samples Received:

Location	Sample Type
	Blood Clots
Los Palos, Lautem District	2
TOTAL	**2**

Testing Performed:

Location	Molecular	Sequencing		Isolation and Typing	
	BTV TQM PCR	Genotype Sequencing (VP3)	Serotype Sequencing (VP2)	Isolation	Virus Isolate Typing Assay
Los Palos, Lautem District	2	2	2	2	2
Grand Total	**2**	**2**	**2**	**2**	**2**

Results by Location:

Agent detection

Location	Serotype (by sequencing)	Genotype (by sequencing)	Virus Isolate Typing Assay
	BTV1	Malaysia A	Serotype 1
Los Palos, Lautem District	2	2	2
Grand Total	**2**	**2**	**2**

Test numbers

Samples Received:

Location	Sample Type			
	WHOLE BLOOD (EDTA/LITH HEP)	PLASMA/SERUM	VIRUS ISOLATES/ CELL CULTURE SUPERNATANTS	BLOOD CLOTS
Queensland	28	28	17	0
Western Australia	87	85	-	2
Northern Territory	-	-	30	-
Papua New Guinea	-	-	-	11
Timor Leste	-	-	-	2
TOTAL	**115**	**113**	**47**	**15**

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##
Queensland	28	436	45	34 (+11#)	34 (+11#)	43	169 (+143#)
Western Australia	85	1,234 (+126#)	89	4	4	2	0
Northern Territory	-	-	30	30	30	30	169 (+221#)
Papua New Guinea	-	-	11	11	11	11	26
Timor Leste	-	-	2	2	2	2	26
Grand Total	**113**	**1,670** (+126#)	**177**	**81**	**81**	**88**	**390** (+364#)

*18 SNTs/panel (15 Australian + 3 for cross reactions)

**Only 1 test recorded/sample (regardless of # primer sets used)

Results pending

^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 AAHL. 13 VNTs/isolate

+ Isolates received may be passaged prior to VNT

Appendix 1 - BTV Whole Genome Sequencing

Initiatives

ACDP is in the process of collating all currently available Whole Genome Sequencing (WGS) data for Australian BTV isolates and associated metadata into a Nextstrain[1] data bank. The aim of this project is to utilise the Nextstrain platform to analyse various aspects of virus isolation and virus and individual gene segment movement within the Australian BTV episystem.

In 2019 we also selected approximately 30 BTV isolates selected from those provided by Berrimah, EMAI and isolations made at AAHL from field samples from Queensland. These were specifically chosen to augment 'gaps' in our existing bank of WGS BTV data. Particularly, our collection of Australian BTV-16 isolates was expanded from the single prototype isolate to 12 (including a recent PNG isolate) and included isolates from WA, NT, Qld and NSW.

Earlier sequencing investigations revealed many of existing BTV-20 isolates were in fact BTV-4 (as previously advised to NAMP). To further investigate this, six BTV-4 isolates from the NT (1996 to 2014) were also analysed by WGS. These collective data (approximately 140 isolates) are currently being analysed using Nextstrain to determine the extent of changes in the resident gene segment constellation of each of the regional BTV episystems in Australia (Northern, Eastern and South Eastern) over the last 40 years.

Additionally, two separate isolates of BTV-16 from NSW (2017 and 2019) have been identified as having quite separate genetic lineages. The 2017 isolate displays a unique genome composition with many segments being alien to the South-Eastern regional pool. The genome of the 2019 isolate also displays some significant regional departures and interestingly has a genetic profile that is very similar to a likely new incursion into WA in 2017. More 'fine-grained' analysis of the total data is currently being undertaken.

Future Directions

The collective database was intended to be augmented by a another large batch of selected BTV isolates (including disease-associated isolates form Qld from this year, an increased number of BTV-5 isolates including a PNG isolate and more recent isolates of BTV-2 and BTV-7), however this work has been suspended due to the COVID-19 crisis.

We intend to continue to incorporate data from newly detected isolates into our Nextstrain database. Ultimately, we intend to explore opportunities to utilise the Nextstrain platform to generate regular reports for the NAMP technical committee, thereby providing more fine-tuned analyses of gene segment movements in addition to movements of individual serotypes between the regional episystems. Surveillance at this level will potentially provide for early warning of the presence of particular genomic combinations of disease causing potential existing within the Australian BTV episystem.

[1]Hadfield et al., Nextstrain: real-time tracking of pathogen evolution, Bioinformatics (2018).

Appendix 2 - BTV sELISA assay development

The current cELISA method utilised at ACDP - AAHL for screening BTV serology is known to have reduced sensitivity to BTV-15 specific antibodies. As such, serum samples testing negative in the BTV cELISA are subsequently tested in the BTV-15 serum neutralisation test.
In order to overcome this limitation, ACDP – AAHL has been developing and evaluating a new BTV sELISA method. The new assay is showing promising signs, including:

1. The sELISA is more sensitive and seems to be (at least) as specific as the cELISA when testing bovine sera;
2. The sensitivity of the sELISA for the detection of antibodies to BTV does not appear to vary by serotype, whereas the cELISA poorly detects or does not detect antibodies to BTV-15;
3. The sELISA for ovine sera appears to be less sensitive then the cELISA at early stage of infection, but it does detect antibodies to BTV-15 as well as other serotypes;
4. The BTV sELISA for ovine sera seems to be more specific then the cELISA;

The validation of this assay is ongoing, with repeatability studies still to be completed.