

An overview of bluetongue viruses in Australia

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- History of bluetongue viruses in Australia;
- Epidemiology;
- Occurrence of disease and pathogenesis;
- Diagnosis and control measures;
- Parallels with Asia.
- Research directions



- Prior to 1788 no ruminants in Australia;
- Indigenous animals mostly marsupials (kangaroo, koala);
- Cattle originally imported from UK, sheep from UK and Spain;
- Approximately 25M cattle; 3M dairy cattle
- Currently 100M sheep;
- Small goat population 1M;
- Pigs, poultry, aquaculture



Livestock production in Australia

Cattle – raised throughout Australia:

- Beef production in all states;
- Tropical breeds in north based on Bos indicus;
- Bos taurus mainly in temperate areas;

Sheep production:

- Usually confined to the dryer, "cooler" regions;
- Few sheep in vector zones.









Bluetongue vectors

Distribution and abundance of the vector influenced by:

- Temperature limited by cold weather/frost
- Rainfall but not excessive
- Altitude influence of temperature and gradient
- Wind can be dispersed by strong winds



The biology of the vector determines:

- Distribution potential limits of virus
- Transmission patterns not always active annually
- Seasonality of infection (summer/autumn)
- Free areas for quarantine

Bluetongue virus – some history

- Prior to 1975, bovine ephemeral fever virus caused intermittent major epizootics of disease sweeping from the tropical north to the far south of Australia;
- The virus spread over several thousand kilometres in 1-2 months.
- A vector-borne virus was suspected;



Bluetongue virus investigations

- During the search for the vector of BEF, mosquitoes and biting midges (Culicoides spp) were caught and virus isolation attempted;
- In 1975, an unidentified virus found in the north.
- In 1977, unknown virus shown to be bluetongue.
- No evidence of disease;

(BTV also recognised in China in 1977 as a cause of disease in sheep)



- Systematic studies of the epidemiology of Bluetongue infection of cattle commenced in 1977, using sentinel cattle;
- Search for bluetongue took over from BEF;
- Many viruses isolated and characterised;
- Research undertaken to:
- Investigate epidemiology of BTVs in Australia;
- Study pathogenicity of viruses why no disease in the field?
- Develop improved diagnostic tests
- Study duration of viraemia, period of infectivity to insect



Tests available for virus detection:

- Virus isolation, Ag ELISA, PCR (esp qRT-PCR)
- Virus usually required for typing, molecular studies, vaccines
- Inoculation of ece, screen by Ag ELISA followed by passage in cell culture (C6/36 then mammalian cells)
- Identification: Group: Ag ELISA, IPX/IFAT, PCR
- Type specific: Neutralisation, qRT-PCR



Tests available for antibody detection:

- Group reactive assays: Sensitive, quick (once sample at lab) and economical;
- eg AGID, bELISA (cELISA) to test serum or plasma, detect any serotype, any animal species;
- Used to map BTV zones/free areas and facilitate virus isolation;
- Type specific serology VNT/PRNT research only
- Used to define distribution and movement of individual serotypes, risk of disease, incursions (cross reactivity an issue)

Biology of bluetongue infection

- Live virus can be detected from 3 to about 50 days (Zhang et al)
 [OIE 60 days]
- Levels of virus very low after 14 21 days
- Infective for insects for <28 days
- Vector competence requires 8-10 day incubation period
- Residual RNA (virus components) can be detected by qRT-PCR for many months and can complicate testing



- Virus and antibodies both detected after about 7 days
- Antibodies persist for several years without re-exposure
- Only indicate past exposure to virus not an indicator of risk



Considerations with tests for antibody detection:

- Tests must have very high sensitivity:
- Sensitivity:
 - the ability to detect truly infected animals;
 - detect antibodies from very early stages (day 6-7) but last for many years

(cannot predict when infection occurred);



Considerations with tests for antibody detection:

- Tests must have very high specificity;
- Specificity:
 - the ability to correctly classify an uninfected animal (negative result);
 - WITH THE BEST TEST specificity ranges from 98-99.5%; (that means 5-20 false positives/reactors per 1,000)
- As sensitivity increases, specificity decreases a balance (AGID less sensitive than bELISA)



Bluetongue viruses – the international situation

Globally:

- 29 recognised serotypes several others to be confirmed;
- Serotype determined by antigens that induce neutralising Ab;

In Australia:

- Currently 12 serotypes;
- Serotypes 1, 2, 3, 5, 7, 9, 12, 15, 16, 20, 21, 23.

In Asia:

- At least 14 serotypes;
- Serotypes 1, 2, 3, 4, 5, 9, 11, 12, 15, 16, 17, 20, 21, 23.



Bluetongue viruses – how many are pathogenic?

Globally:

- Virulence is independent of serotype;
- Can be a spectrum of virulence within a serotype eg serotype 1;
- Some serotypes may have few if any virulent strains.

In Australia:

- Strains within serotypes 2, 3, 16, 23 can be highly pathogenic.
- NSW BTV1 non-pathogenic; NT BTV1 mildly pathogenic



- Occurrence and severity (virulence) of disease is influenced by strain of virus;
- Sheep more susceptible than goats and cattle;
- Prior to 2007, disease not observed in cattle a unique feature of BTV8 (a lab adapted strain)
- Indigenous breeds tend to be less susceptible than introduced breeds.
- Significant disease induced experimentally in Australia with BTV 2, 3, 16, 23. BTV1 in eastern Australia non pathogenic – cf BTV1 (China)



Control measures for Bluetongue

AusVetPlan:

- No use of vaccine unless a continuing problem
- Live vaccines not an option have undesirable characteristics
 - foetal infection, excreted in semen, spread by insects;
- Vector control not practical on a large scale.

Inactivated vaccines:

- Have been used widely in India and Europe in last 10 years;
- Safe and effective but little cross protection between serotypes

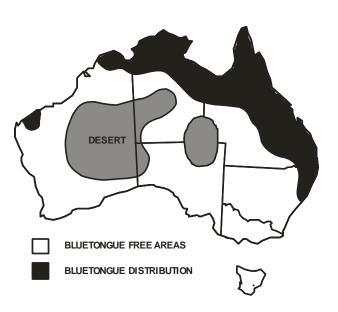
Distribution of Bluetongue viruses in Australia

- Natural distribution of viruses determined solely by distribution of insect vector;
- BTVs transmitted exclusively by biting midges Culicoides species;
- In eastern Australia (NSW & southern Qld), Culicoides brevitarsis
- In northern Australia, especially the 'Top End' of NT, C. brevitarsis and additional tropical vectors (C. actoni, fulvus, wadai).

Bluetongue virus epidemiology

 Geographical distribution of bluetongue virus in Australia may be similar to (but never exceed) the distribution of principal vector, Culicoides brevitarsis;





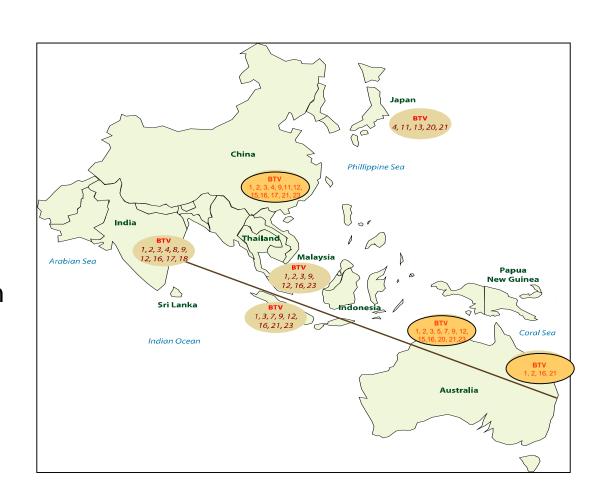
Trends in the Australasian region

- Considered to be an evolving episystem;
- Viruses moving from equatorial regions to the north and south



Trends in the Australasian region

- Recent incursions of BTV 2, 5, 7 &12 into Australia;
- Evidence of exchange of genotypes (within a serotype) between Australia and southern Asia;
- Will other serotypes move to Australia or the Asian region??





- Vector distribution and virus transmission patterns established;
- Research projects for virus-vector surveillance resulted in the development of the National Arbovirus Monitoring Program (NAMP)
- Vector-borne viruses have been systematically monitored for >30 years

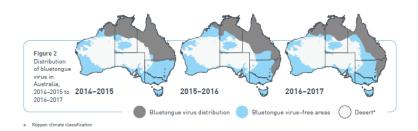




- NAMP is a nationally co-ordinated program for the monitoring of selected vector-borne viruses of importance to animal health in Australia
- The monitoring program is managed by a group representing state and federal governments and the major livestock industries and coordinated by Animal Health Australia (AHA)
- AHA is a company owned by the livestock industries in partnership with the state and federal governments
- Operationally NAMP is managed by state co-ordinators

What are the objectives?

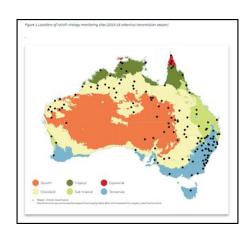
- Market access to facilitate the export of live cattle, sheep and goats, and ruminant genetic material, to countries with concerns about bluetongue, Akabane and bovine ephemeral fever (BEF) viruses
- Bluetongue early warning to detect incursions of exotic strains of bluetongue virus (BTV) and vectors (Culicoides species biting midges) into Australia by surveillance of the northern BTV epidemic area
- Risk management to detect changes in the seasonal distribution in Australia of endemic bluetongue, Akabane and BEF viruses and their vectors, to support livestock exporters



Bluetongue virus monitoring.

Bluetongue virus and vector surveillance in Australia:

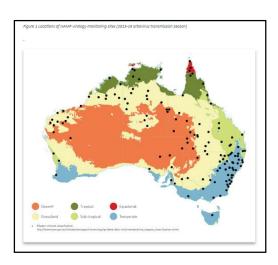
- Sentinel herds strategically located in BTV free and zone of possible transmission
- Vector collection at same locations
- Virology and entomology done in state laboratories
- Centralised data collection internet submission
- Virus submitted for centralised sequencing and molecular topotyping



How and where is monitoring conducted?

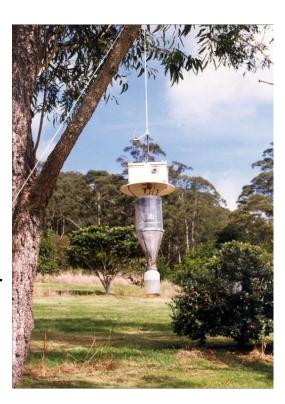


- Groups of young cattle (sentinel herds)
 are strategically located around
 Australia throughout the known range of
 the principal Culicoides species
- Serosurveillance herds
- Insects are collected in light traps



What testing is carried out?

- 10-15 animals (6-9 mths in spring)
- Blood samples collected regularly (monthly in coastal locations – weekly at CPRS, NT)
- Synchronised sampling between sites
- Tested for Akabane (Simbu serogroup),
 Bluetongue and Ephemeral Fever virus antibodies – ELISA and VNT
- BTV PCR (Pan); serotyping by PCR or VNT
- Bluetongue virus isolation ID of serotype and topotype
- Culiocoides midges sorted to species



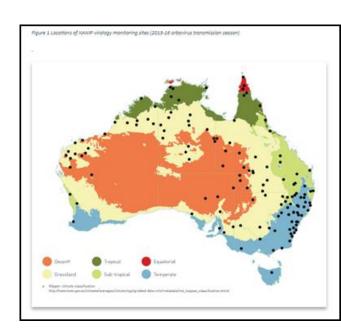


PATERSON - BLUETONGUE VIRUS - ELISA (2016/17)

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ANIMAL	EARTAG	ОСТ	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	END
1	1619	-		-	-	-	-	85+	90+	93+	93+	95+
2	1620	-		-	-	-	-	95+	94+	88+	92+	90+
3	1621	-			-	-	-	•	91+	92+	93+	97+
4	1622	96+		94+	95+	96+	92+	94+	93+	88+	95+	91+
5	1624	-		-		-	-	86+	88+	77+	91+	96+
6	1625	-			-	-	-	92+	92+	93+	83+	94+
7	1626	-		-	-	-	-	91+	93+	93+	91+	95+
8	1627	-		-	-	-	-	65?	92+	92+	90+	95+
9	1629	-		-	-	-	-	91+	93+	84+	90+	95+
10	1630	60?		-	-	-	-	92+	93+	91+	71+	96+
At Risk (A)		8		6	8	9	9	9	1	0	0	0
No S/C (S)		0		0	0	0	0	8	1	0	0	0
TOTAL (T)		10		8	9	10	10	10	10	10	10	10

PATERSON - BLUETONGUE VIRUS - PAN PCR (2016/17)

ANIMAL	EARTAG	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	END
1	1619	NT		NT	NT	NT	-	33.4	31.4	33.7	•	NT
2	1620	NT		NT	NT	NT	-	32.6	31.7	33.9	•	NT
3	1621	NT			NT	NT	-	32.2	30.3	32.2	32.8	NT
4	1622	NT		NT	NT	NT	-	31.1	29.9	28.8	30.4	NT
5	1624	NT		NT		NT	-	26.9	27.6	28.9	30.9	NT
6	1625	NT			NT	NT	-	33.4	33.8	34.3	•	NT
7	1626	NT		NT	NT	NT	-	32.6	31.5	31.9	33.4	NT
8	1627	NT		NT	NT	NT	-	28.6	28.4	29.4	32.6	NT
9	1629	NT		NT	NT	NT	-	34.9	30.7	29.9	32.0	NT
10	1630	NT		NT	NT	NT	-	31.5	29.4	32.1	33.0	NT
At Risk (A)		8		6	8	9	9	9	1	0	0	0
No S/C (S)		0		0	0	0	0	8	1	0	0	0
TOTAL (T)		10		8	9	10	10	10	10	10	10	10





Example of sentinel herd results

PATERSON - BTV1 PCR (2016/17)

ANIMAL	EARTAG	MAR	APR	MAY	JUN	JUL	END
1	1619	NT	35.8	38.1	-	NT	NT
2	1620	NT	32.9	38.1	-	NT	NT
3	1621	NT	32.8	37.7	-	•	NT
4	1622	NT	32.2	36.0	34.3	32.3	NT
5	1624	NT	-	39.0	-	•	NT
6	1625	NT	-	-	36.9	NT	NT
7	1626	NT	33.9	-	-	•	NT
8	1627	NT	-	34.4	33.8	34.8	NT
9	1629	NT	-	-	-	•	NT
10	1630	NT	33.0	35.8	-	•	NT
At Risk (A)		9	9	1	0	0	0
No S/C (S)		0	8	1	0	0	0
TOTAL (T)		10	10	10	10	10	10

PATERSON - BTV21 PCR (2016/17)

ANIMAL	EARTAG	MAR	APR	MAY	JUN	JUL	END
1	1619	NT	-	-	-	NT	NT
2	1620	NT	-	38.0	-	NT	NT
3	1621	NT	-	-	-	-	NT
4	1622	NT	36.7	-	36.1	-	NT
5	1624	NT	-	-	-	-	NT
6	1625	NT	-	-	34.6	NT	NT
7	1626	NT	-	33.1	30.7	-	NT
8	1627	NT	31.3	30.3	30.3	37.6	NT
9	1629	NT	35.6	31.6	32.5	31.9	NT
10	1630	NT	-	31.4	-	36.2	NT
At Risk (A)		9	9	1	0	0	0
No S/C (S)		0	8	1	0	0	0
TOTAL (T)		10	10	10	10	10	10

PATERSON - BTV16 PCR (2016/17)

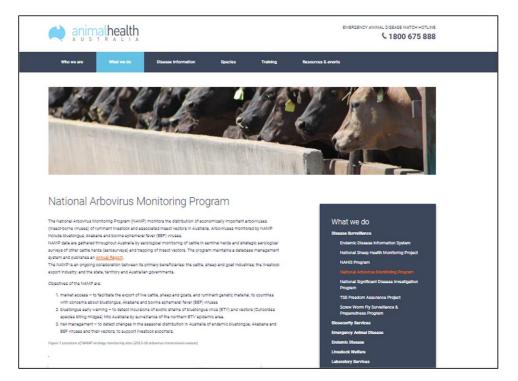
ANIMAL	EARTAG	MAR	APR	MAY	JUN	JUL	END
1	1619	NT	-	ı	ı	NT	NT
2	1620	NT	-	•	ı	NT	NT
3	1621	NT	-	1	ı	•	NT
4	1622	NT	-	1	ı	•	NT
5	1624	NT	29.0	30.3	31.9	31.9	NT
6	1625	NT	35.7	36.6	40	NT	NT
7	1626	NT	-	•	ı	-	NT
8	1627	NT	-	•	ı	-	NT
9	1629	NT	-	•	ı	-	NT
10	1630	NT	-	•	ı	-	NT
At Risk (A)		9	9	1	0	0	0
No S/C (S)		0	8	1	0	0	0
TOTAL (T)		10	10	10	10	10	10



- Monitoring of sentinel cattle demonstrates intermittent transmission of bluetongue viruses within range of *C. brevitarsis*
- Consistent north-south transmission pattern & southern limit of spread
- Virus isolation & qRT-PCR identify serotypes that are active
- Nucleic acid sequencing demonstrates genetic variation in strains within a serotype – confirms incursions and reassortment of BTV genes

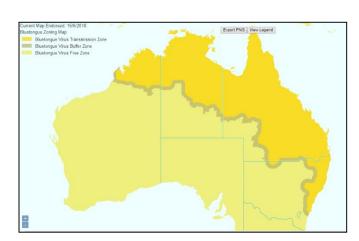


- AHA website
- Unique property identifiers
- Web-based data submission interactive error checking



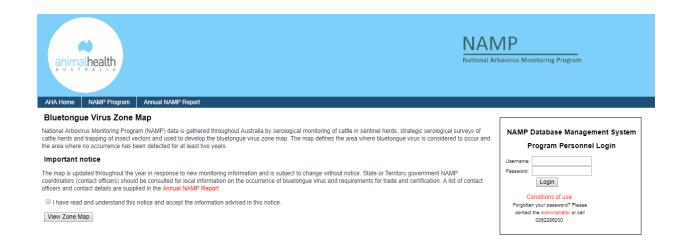


- BTV zone defined by composite distribution of viruses in previous 2 years
- A location must be BTV free continuously for 2 years to effect a change of status
- Detection of BTV in a free sentinel herd activates immediate notification, investigation and change of status if BTV confirmed;
- Zone map changed and notification by email to exporters or anyone registered to receive notification of changes;



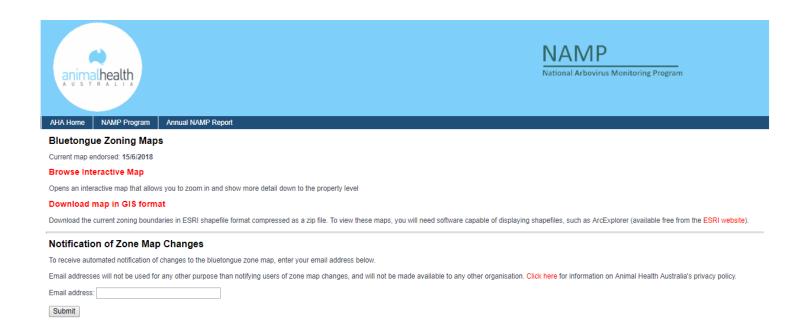
Outputs from NAMP

- BTV zone maps (interactive and GIS format)
- Zone maps dynamic, auto-notification system
- Synoptic annual reports

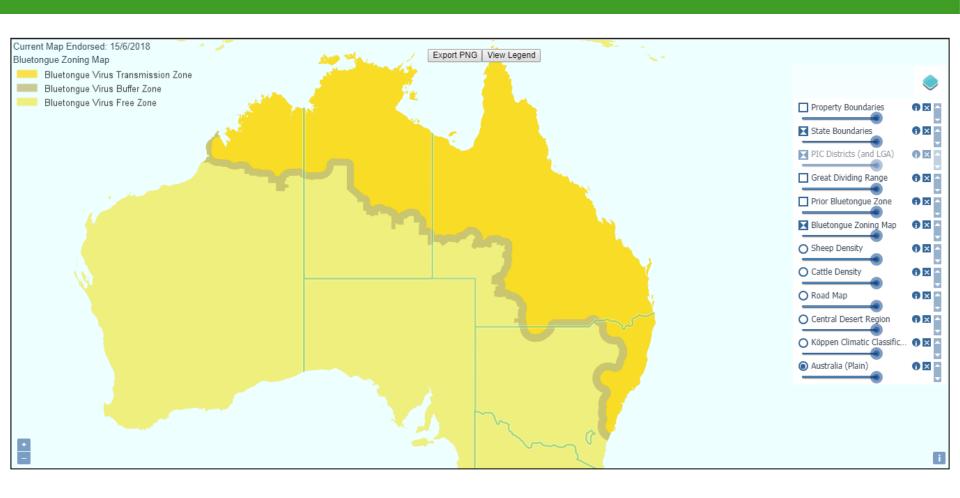


Availability of NAMP data

BTV zone maps and reports available on line

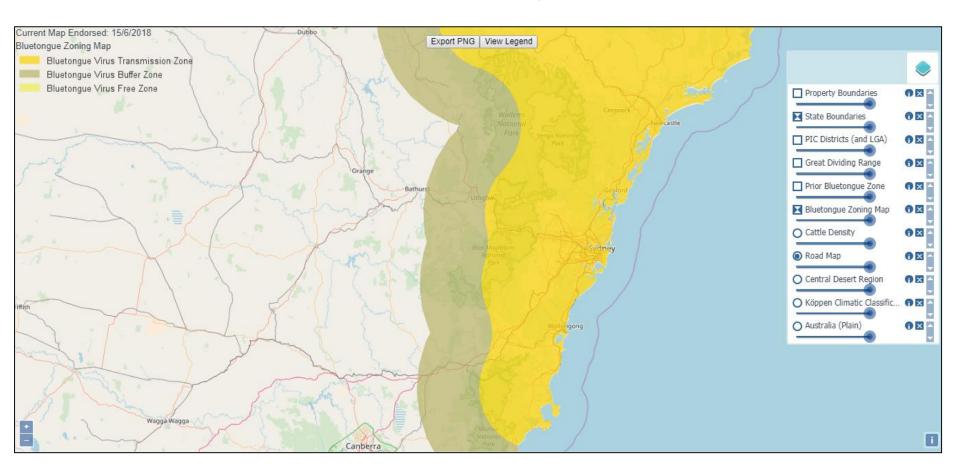


BTV Zone – Interactive Map



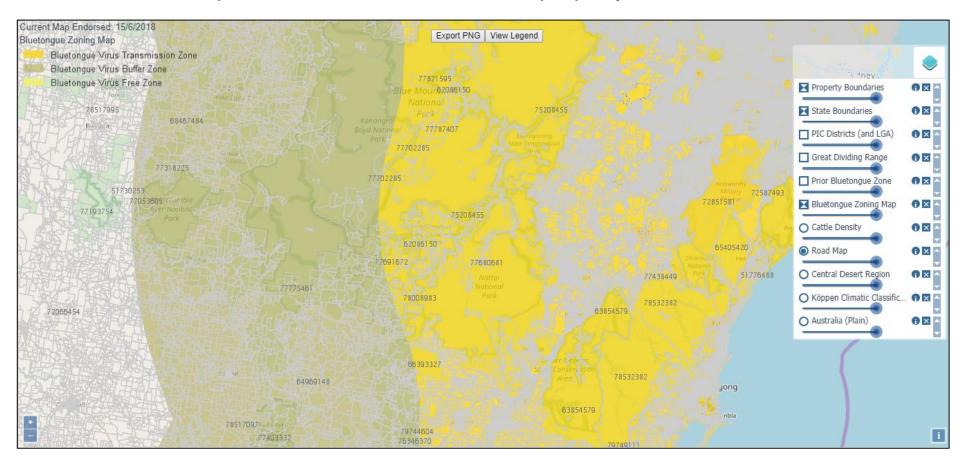
BTV free zones – interactive

NAMP data can be used to identify BTV boundaries



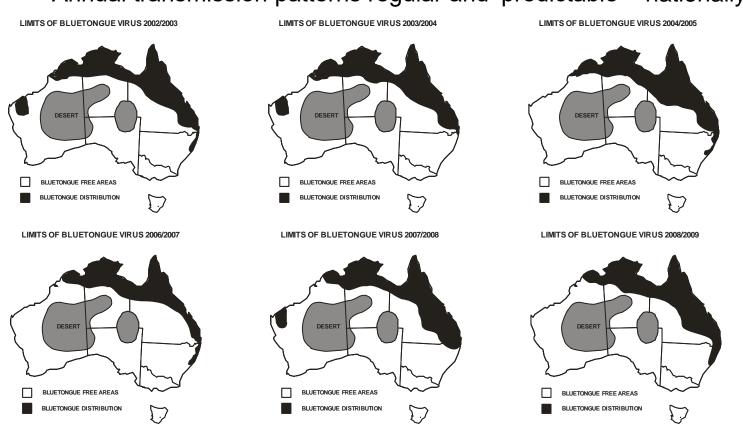
BTV free zones - interactive

Interactive map can be used to determine if property in or out of BTV free zone



Bluetongue virus epidemiology

Annual transmission patterns regular and 'predictable' - nationally





- Arboviruses insect borne
- Vectors only a few competent Culicoides species
- No vector no spread
- No direct spread to animals by close contact



