



FINAL REPORT

MURC PROJECT

DAQ.085

The vector competence of
Culicoides spp. for attenuated
Bluetongue virus vaccine strains
and studies on reversion to
virulence.

PROJECT SUMMARY

PROJECT TITLE

The vector competence of *Culicoides* spp. for attenuated Bluetongue virus vaccine strains and studies on reversion to virulence.

PROJECT NUMBER

DAQ.085

RESEARCH ORGANISATIONS AND LOCATIONS

Queensland Department of Primary Industries
Oonoonba Veterinary Laboratory
P.O. Box 1085
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Queensland

COMMENCEMENT

July 1992

COMPLETION

June 1994

PROJECT INVESTIGATORS

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SUPPLEMENTARY DESCRIPTION

The project aimed to provide information that would enable a more complete assessment of the risk associated with the use of live attenuated Bluetongue virus vaccines in Australia.

OBJECTIVES

- (i) By May 1993, to achieve an efficacy level of at least 80% for the detection of vector competence.
- (ii) To determine if attenuated Bluetongue vaccine viruses will appear in the semen of vaccinated rams.
- (iii) By August 1993, to evaluate the vector competence of *Culicoides brevitarsis* and *C. wadai* for Australian attenuated Bluetongue virus seed vaccine of serotype 23.
- (iv) By October 1994, to determine the vector competence of *Culicoides victoricae* for Australian Bluetongue virus serotypes 1 and 21.

METHODOLOGY

Culicoides brevitarsis and *C. wadai* were caught at Utchee Creek Research Station using the facilities established under Wool Research and Development Corporation (WRDC) project DAQ.4. Insects were transported to Oonoonba, held for 24 hours then induced to feed on viraemic sheep and cattle. Engorged females were held for 8 - 10 days then exposed on a susceptible animal to check for transmission. All insects were then killed, sorted into species and processed for virus isolation.

Rams were vaccinated with the attenuated seed stock vaccine developed under project CS. 129. They were bled and ejaculated on days 4, 7, 10, 13 and 16 post vaccination and blood and semen samples checked for presence of Bluetongue virus by PCR and inoculation into susceptible sheep. Recipient sheep were held for 35 days to check for seroconversion.

Attenuated vaccine viruses were passaged through insects either during vector competence trials or by direct inoculation of insects and through sheep or cattle. The virulence of progeny viruses was compared to that of the parental vaccine virus and wild unmodified virus by inoculation of susceptible sheep. Virulence was evaluated using the system developed at Oonoonba under WRDC project DAQ.4.

RESULTS

(i) Vector competence studies:

Three techniques, Polymerase chain reaction (PCR), immunospot test and virus isolation in cell culture were compared for their efficiency in detecting virus in infected vectors.

PCR detected virus with 85% efficacy whereas immunospot test and isolation in cell culture detected with efficacies of 42% and 10% respectively.

Wild caught *Culicoides brevitarsis* and *C. wadai* were fed on sheep vaccinated with the attenuated Bluetongue serotype 23 vaccine. Following a 10 day incubation period, virus was

detected and recovered from 0.9% of 761 *C. wadai* but from none of 501 *C. brevitarsis* that had fed on the sheep.

(ii) Excretion of virus in ram semen:

Two, 6 year old and 4, first season 3 year old rams were vaccinated with the attenuated seed stock vaccine to Bluetongue serotype 23. All rams were electroejacuated on days 4, 7, 10, 13, and 16 post inoculation.

Bluetongue virus appeared in the semen of the 2 older rams on 2 occasions each. One ram had virus in the semen on days 7 and 10 PI and the other on days 10 and 16 PI.

In the younger rams one had virus in the semen on day 4 PI, one on day 7 PI and a third was suspect on day 7 PI and positive on day 13 PI. The fourth young ram was negative for virus in semen on all occasions.

(iii) Reversion to virulence:

Attenuated Bluetongue virus serotype 23 was passaged through *C. brevitarsis* and cattle and *C. wadai* and sheep. The viruses recovered from the respective animals following the passages were inoculated into 20 susceptible merino sheep. The sheep were monitored for 20 days for evidence of clinical signs of Bluetongue which would indicate reversion to virulence.

None of the sheep developed any clinical signs of bluetongue. A single sheep showed a transient febrile response on day 8 but all other clinical parameters remained within normal limits. All sheep developed strong antibody levels by day 14 post infection.

(iv) Vector competence of *C. victoriae*:

Culicoides victoriae and *C. multimaclatus* were collected in South Australia and Victoria, transported to Oonoonba and fed on sheep infected with Australian Bluetongue Serotype 1. A total of 445 *C. victoriae* and 500 *C. multimaclatus* which had fed and survived the incubation period were processed but no Bluetongue virus was recovered indicating that neither insect is a competent vector of bluetongue viruses.

CONCLUSIONS

- (i) Live attenuated Bluetongue serotype 23 vaccine was strongly antigenic and did not revert to virulence following passage in *Culicoides* spp. vectors and sheep or cattle.
- (ii) The Australian vector *C. wadai* was able to be infected with the vaccine virus after feeding on recently vaccinated sheep. There would be a high risk of the vaccine strain being transmitted in the field.
- (iii) The vaccine virus appeared commonly in the semen of vaccinated rams.
- (iv) *Culicoides victoriae* and *C. multimaclatus* are not competent vectors of Bluetongue virus and therefore, areas of Australia currently beyond the range of *C. brevitarsis* can be regarded as vector-free.

FINAL REPORT FOR PROJECT DAQ085 - PART 2

BACKGROUND AND INDUSTRY CONTEXT

Bluetongue virus was first identified from Australia in 1977, and since then 8 different serotypes have been recognised in this country at one time or another. So far, two of these serotypes (1 and 21) have moved from the Northern Territory to the east coast of the continent where they have come close to the commercial sheep raising areas, and continue to be more or less active in cattle from year to year.

Pathogenicity studies with Australian bluetongue virus serotypes have shown several serotypes capable of causing severe clinical disease and deaths in sheep. Recent vector competence and virus distribution studies have independently indicated that *Culicoides brevitarsis* and *C. wadai* are the principal vectors of bluetongue virus in eastern Australia. The former has a seasonal distribution that can extend well into sheep producing areas. Should virulent serotypes cross into eastern Australia then the potential exists for outbreaks of severe clinical disease in sheep.

An option for veterinary authorities to control an outbreak of disease is to vaccinate at-risk sheep and possibly in contact cattle. Live attenuated viruses were prepared and evaluated under project CS129 and although they appear efficacious they had undesirable side effects. A disadvantage already demonstrated under that project was that modified viruses were teratogenic. In ewes vaccinated in the first and second trimesters of pregnancy, up to 50% of foetuses were lost and up to 13% of surviving foetuses were born with mild to severe hydrancephaly and were non viable.

Preliminary results from Berrimah suggest that passage of attenuated virus in cattle may result in reversion to virulence. It was not known whether attenuated vaccine viruses would be taken up by vectors, transmitted to other livestock and revert to virulence in the process. Previous studies by CSIRO suggested that *C. brevitarsis* and *C. wadai* had higher infection rates with attenuated BTV20 than with unmodified virus.

Therefore 2 major risks may be associated with the use of live attenuated bluetongue vaccines if they are taken up by vectors. Firstly, the vaccine viruses may revert to virulence following replication in the insect or during passage in cattle and thereby provide a reservoir of virulent virus which could cause additional outbreaks of disease. If vaccination is undertaken in the face of an outbreak then vaccinated animals are likely to be exposed to increased numbers of competent vectors.

Secondly, even in the absence of a reversion to virulence the vaccine strains may persist in an insect-host cycle and result in subsequent infections of pregnant animals. The potential losses from foetal death and teratogenicity may be more costly to the local industry in the longer term and outweigh the benefits gained from the control of a limited outbreak. To fully assess the risks associated with the use of attenuated viruses it was necessary to know if the viruses were genetically stable and if existing vectors could be infected with, and transmit, the viruses.

The commercial production and future storage of attenuated bluetongue vaccines will involve industry and government in considerable expense. Industry must be aware of the risks before a decision is made to commit the substantial monies required to scale up to commercial vaccine production.

The recognised vectors in the east are *C. brevitarsis* and *C. wadai*. Both species are not usually found in the commercial sheep growing areas in numbers which might be expected to initiate and sustain a disease outbreak in sheep, nor are they known to extend into Victoria or South Australia. As yet there has been no serological evidence of bluetongue in that part of the continent. However there is concern that in seasons favourable for *C. brevitarsis* the insect could expand its distribution and abundance to the point where it could take bluetongue virus to areas where it has not occurred in the past. The virus may be picked up in those areas by biting midge species which feed on livestock and which have not been tested for bluetongue virus vector competence. One of the most abundant of these is *C. victoriae*, which is found in Victoria, South Australia, and also in Tasmania and the south-west of Western Australia. If this species can act as a vector of bluetongue it is possible that the virus could spread to these areas where sheep are farmed. *C. victoriae*, unlike the known vectors *C. brevitarsis* and *C. wadai*, does not breed in cow dung. If this species could act as a vector, it could take the virus into areas where sheep are raised without the presence of cattle. There would be potential disease problems and also difficulties with export of live animals from areas where bluetongue virus was known to occur. Determination of the vector competence of *C. victoriae* for bluetongue virus would enable assurances about the potential presence of bluetongue in these southern areas to be given to trading partners. Currently these trading partners can justifiably question the vector status of the biting midge species which they know are found in the areas which are considered free of bluetongue.

OBJECTIVES

The objectives of the project changed during the course of the project. The objectives of the original project which commenced on a 3 year cycle were related to the aim of the project as contained in the supplementary description.

“This project aims to provide information that will enable a more complete assessment of the risk associated with the use of live attenuated Bluetongue virus vaccines in Australia.”

The original objectives were as follows:

- (i) By November 1993, to evaluate the virulence of attenuated Bluetongue virus vaccine for BTV23 following passage in insects and cattle.
- (ii) By February 1995, to determine if attenuated Bluetongue vaccine viruses will appear in the semen of vaccinated rams.
- (iii) By August 1995, to evaluate the vector competence of *Culicoides brevitarsis* and *C. wadai* for Australian attenuated Bluetongue virus vaccine strains.
- (iv) By August 1995, to evaluate the virulence of attenuated Bluetongue virus vaccine for BTV21 and BTV1 following passage in insects and cattle.

By early 1993 preliminary results of the project supported by developments elsewhere in Bluetongue research indicated that live attenuated bluetongue virus vaccines were unlikely to be used in Australia. Therefore, the Corporation terminated the project under clause 27(a)(iii) of the agreement at the end of the first year.

The project was renegotiated to achieve dual aims. The first aim was to confirm the vector competence of known Australian bluetongue virus vectors for attenuated Bluetongue type 23 vaccine. The second aim was to provide virological support for AWRAPO project CT112 to establish the vector competence of *C. victoriae* for Australian bluetongue virus serotypes. The revised project was to operate for 1 year only.

The revised objectives were as follows:

- (i) By May 1993, to achieve an efficacy level of at least 80% for the detection of vector competence.
- (ii) By August 1993, to evaluate the vector competence of *Culicoides brevitarsis* and *C. wadi* for Australian attenuated Bluetongue virus (BTV) seed vaccine of serotype 23.
- (iii) By October 1994, to determine the vector competence of *Culicoides victoriae* for Australian bluetongue virus serotypes 1 and 21.

METHODOLOGY

(a) Vector competence of *Culicoides* spp. for attenuated BLU23 vaccine

C. wadai and *C. brevitarsis* were collected from wild populations at Utchee Ck Research Station between February and June 1993. Collections of dung reared *C. brevitarsis* were made from Burnside Stud (10km south of Ingham) in May and June 1994.

In 1993, insects were collected over penned cattle using the modified updraught light traps developed in the recently completed WRDC project DAQ4. Several sites were available for the collection of both vector species with the principal site being Utchee Creek Research Station near Innisfail, Queensland. Collected insects were transported daily to the laboratory in the high humidity cages developed under DAQ4. At the laboratory they were held for 24 hours, then exposed for feeding on viraemic sheep vaccinated with attenuated BLU23. The titre of virus in the blood of the animals was determined by plaque assay on BHK cells. Engorged females were held for 7 days, sorted to species then exposed to susceptible sheep to check for virus transmission. All surviving insects were killed and processed for virus isolation to determine vector infection rate. Insects were placed in liquid nitrogen until processing or processed immediately. Insects were individually homogenised in sterile water to which tissue culture medium was added. Part of each homogenate was then pooled into groups of 10. Each pool was inoculated onto BHK cells for up to 4 passages. Positive pools were defined by the presence of CPE.

(b) Reversion to virulence studies

Infection of insects with attenuated BTV23 was attempted 3 ways; by feeding on the ears of sheep which had been inoculated 6-8 days previously, by intrathoracic inoculation, and by capillary feeding on concentrated virus suspension. Regardless of route of attempted infection, all insects were held at 27°C for 7 days after feeding/inoculation. Throughout their handling insects were fed only 10% sucrose. Following this incubation period the surviving insect were killed, grouped into appropriate sized pools and ground and aliquot's inoculated into susceptible sheep or cattle. Viruses recovered from the inoculated animals were subsequently inoculated into susceptible sheep to check for signs indicative of reversion to virulence.

(c) Presence of attenuated BLU23 in ram semen

Young and older rams were vaccinated with BLU23 vaccine and bled and electroejaculated on days 4, 7, 10, 13 and 16 post vaccination. On each occasion, half of each semen sample was inoculated into 2 susceptible sheep and the remainder was examined for presence of virus by PCR. Each blood sample taken at the time of ejaculation was inoculated into a susceptible sheep to confirm the presence of viraemia.

(d) Vector competence studies on *C. victoriae*

Collections of *C. victoriae* and the closely related *C. multimaculatus* were made in South Australia and Victoria and the live insects were transported to Townsville. At the laboratory they were fed on the ears of sheep infected with a field isolate of BLU1 or fed through artificial membranes on blood taken from sheep viraemic with the field BLU1. Insects that fed were held for 7 days at 25°C and then processed for virus isolation by serial passage in embryonated chicken egg, insect cells (C636) and repeatedly in mammalian cell line (BHK21).

ACHIEVEMENT OF OBJECTIVES

Original objectives

- (i) result achieved
- (ii) result achieved
- (iii) partial result achieved. Insufficient time to achieve complete result.
- (iv) not attempted due to early termination of project

Revised objectives

- (i) result achieved
- (ii) partial result obtained. Insufficient time to achieve complete result.
- (iii) partial result obtained. No result was achieved for serotype 21 because no field isolate of BLU21 was available.

RESULTS

(i) Vector competence of *C. brevitarsis* and *C. wadai* for attenuated BLU23 vaccine.

In 1993, a total of 846 *C. wadai* and 76 *C. brevitarsis* were harvested after the 7 day incubation period from a total of 2318 insects which fed on sheep. In 1994, a total of 461 *C. brevitarsis* were harvested from a total of 805 insects that fed.

C. wadai: 85 pools were initially tested. However 12 of these pools were contaminated by fungi and could not be judged as positive or negative. The contaminated pools were retested as pools of 5 insects to minimise the loss of data due to fungi. 17 of these smaller pools were infected resulting in a total loss of 85 insects reducing the total number of *C. wadai* reliably assessed to 761. These yielded 7 isolates. The isolates were identified as BLU23 by virus neutralisation assays. Assuming that each positive pool contained only 1 infected insect this would yield a minimum infection rate for *C. wadai* of 0.9%. By comparison, an infection rate of 1.4% was calculated for wild BLU23 in *C. wadai* collected from the same location in 1989.

C. brevitarsis: In 1993, 8 pools were initially tested. 1 pool was subsequently retested as 2 pools of 5 resulting in 5 insects being lost to fungal contamination. This reduced the total assessable number of *C. brevitarsis* to 71. No isolates were obtained from these insects. The pools were subsequently retested on a different line of BHK cells which appeared to be more sensitive to infection than the previous line. However no isolates were obtained. In 1994, 46 pools were tested. Once again, no isolates were obtained. Consequently these results estimate that the infection rate of *C. brevitarsis*, with the attenuated BLU23 is less than 0.19%. By comparison, an infection rate of 1.3% was obtained with wild BLU23 in insects collected from Utchee Ck Research Station in 1989. Other studies using different serotypes have found infection rates as low as 0.04% (Muller et al 1992). The results of this study cannot preclude the possibility of a similarly low infection rate for attenuated BLU23.

PCR techniques were developed for the diagnosis of BLU presence in insects. The pools reported above were tested in parallel in PCR. Approximately 90% of the pools were positive indicating a much higher infection rate for both *C. wadai* and *C. brevitarsis* than indicated by the isolations. While the PCR is likely to be more sensitive to infections in insects it is also possible that it detected remnants of viral RNA in the guts of uninfected insects and thus overestimated the infection rate.

Transmission of the attenuated BLU 23 by the bite of infected insects was attempted once. Transmission did not occur but was extremely unlikely as only 1 of the 100 *C. wadai* exposed to the sheep refed.

(ii) Reversion to virulence studies.

(a) Passage of vaccine virus in insects

(a.i) Capillary Feeding of Insects

45 insects were fed by glass capillary on a concentrated solution (7.7 logs TCID₅₀/ml) of the attenuated virus in tissue culture fluid to maximise the possibility of infection. Of these 2 *C. brevitarsis* and 12 *C. wadai* survived the 7 day incubation and were processed as 3 pools, one containing *C. brevitarsis* and the others containing 10 and 2 *C. wadai* respectively. An isolate of virus was obtained from the pool containing 10 *C. wadai*.

(a.ii) Intrathoracic Inoculation

Approximately 500 insects were inoculated with a concentrated virus solution (7.7 logs TCID₅₀) of which 32 *C. brevitarsis* and 59 *C. wadai* survived the 7 day incubation. These were mostly used in the development of the PCR and immunospot techniques. 7 *C. wadai* were tested for excretion of virus in the insects saliva by collecting saliva into a glass capillary of sucrose solution and testing both the insects and the sucrose/saliva with PCR.

Several inoculated *C. wadai* were titrated in cells and the 2 which showed the highest titre (calculated as 3.5 and 2.0 logs TCID₅₀ for the whole insect) were selected for the reversion to virulence trail.

(b) Virus passage in cattle and sheep

5 shorthorn cattle (approx 12 months old) were selected. The first was injected subcutaneously (SC) with 0.5ml from a total of 2.5ml of homogenate produced by crushing 10 untitrated inoculated *C. wadai*. A weak seroconversion was obtained but no virus could be isolated from any of the weekly blood collections made up to 35 days post-inoculation.

3 cattle each received 50µl SC of the homogenate (10 insects in 2.5ml) from a positive pool of sheep-fed *C. wadai*. Each received a different positive pool. However none seroconverted. Consequently each was inoculated intravenously with a further 150µl from the same pool as the first inoculation. Again, however, none seroconverted.

150µl from each of the same pools was inoculated intravenously into 3 sheep (1 pool per sheep). Once again, none seroconverted.

The fifth bovine was inoculated subcutaneously with 50µl of the homogenate (from a total of 250µl) from the inoculated *C. wadai* which had been titrated to 3.5 logs TCID₅₀. However the animal did not seroconvert. It was inoculated intravenously with a further 150µl of the same homogenate and subsequently seroconverted. Blood (300ml per collection) was collected weekly up to 35 days post-inoculation for testing in the pathogenicity trial against sheep. Virus was recovered from this blood.

A fourth sheep was inoculated intravenously with 150µl of the homogenate of the titrated inoculated *C. wadai* which contained 2.0 logs TCID₅₀. It subsequently seroconverted and

blood was collected weekly (300 ml per collection) for testing in the pathogenicity trial against sheep.

(c) Pathogenicity of passaged viruses for sheep

Each blood containing the viruses that had been passaged through insects-cattle and insect-sheep was inoculated in 10 susceptible merino sheep. The animals were clinically examined daily for signs of bluetongue infection. One sheep (1131) inoculated with the insect-cattle passaged virus showed a transient febrile response (Figure 1) and none inoculated with insect-sheep passaged virus showed a febrile response (Figure 2). No other clinical signs of bluetongue infection were seen in any of the sheep. All animals seroconverted confirming that they had been infected with bluetongue virus (Table 1).

(iii) Presence of attenuated bluetongue vaccine in ram semen

Two, 6 year old culled rams and 4, first season 3 year old rams were vaccinated with the attenuated seed stock vaccine to Bluetongue serotype 23. All rams were electroejaculated on days 4, 7, 10, 13 and 16 post inoculation (PI) and semen samples were examined for the presence of bluetongue virus by PCR and inoculation into susceptible sheep.

Bluetongue virus appeared in the semen of the 2 older rams on 2 occasions each. One ram had virus in the semen on days 7 and 10 PI and the other on days 10 and 16 PI.

In the younger rams one was positive on day 4 PI, one on day 7 PI and a third was suspect on day 7 PI and positive on day 13 PI. The fourth ram was negative for virus in semen on all occasions. Results are shown in Table 1. Virus was detected in semen only when rams were viraemic.

All semen samples were examined for the presence of blood and found to be free. The failure of some of the recipient sheep from the younger rams to seroconvert suggests very low level of virus in the semen.

Figure 1. Temperature responses of 10 sheep inoculated with attenuated BLU23 passaged in *Culicoides brevitarsis* and cattle.

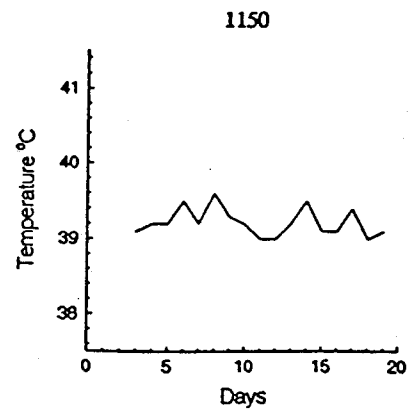
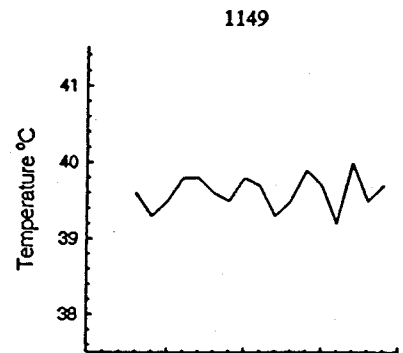
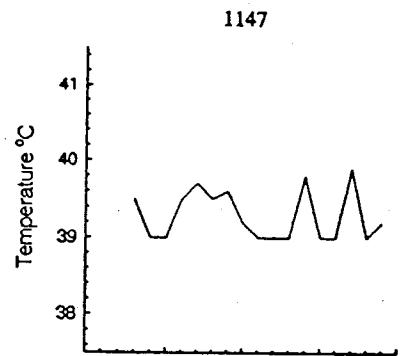
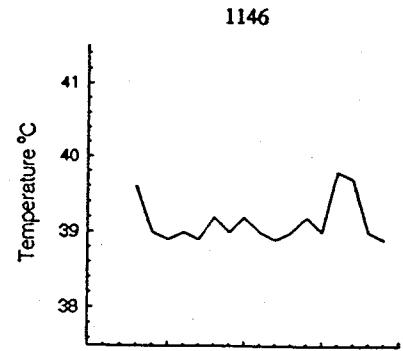
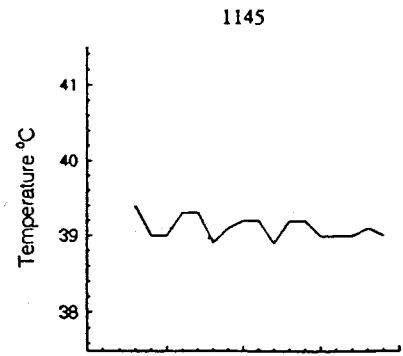
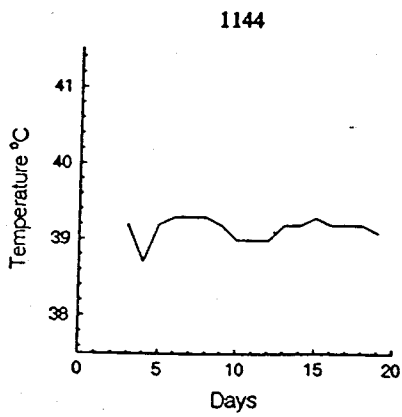
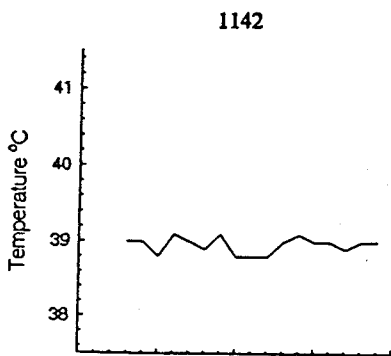
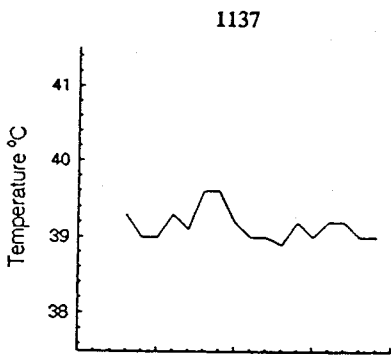
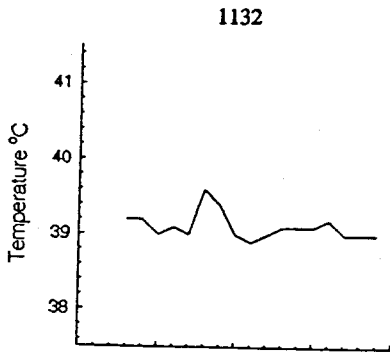
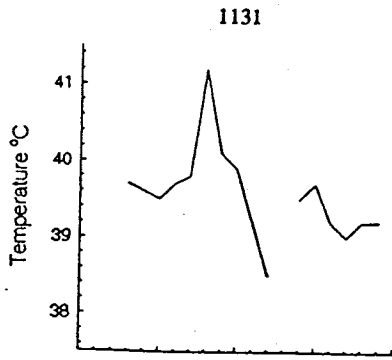
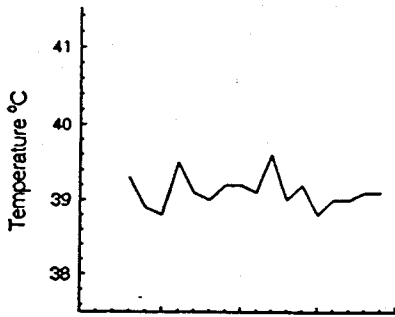
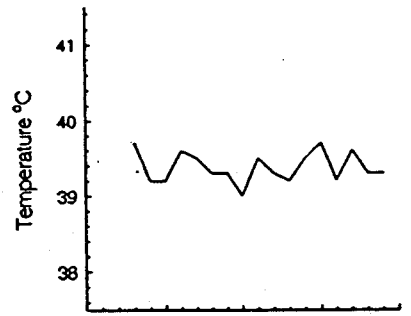


Figure 2. Temperature responses of 10 sheep inoculated with attenuated BLU23 passaged in *Culicoides wadai* and sheep.

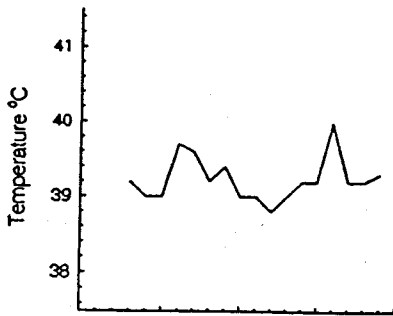
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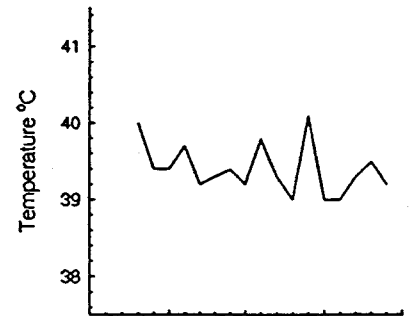
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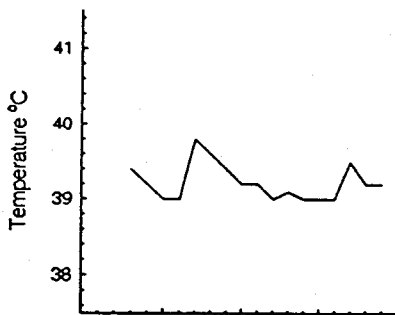
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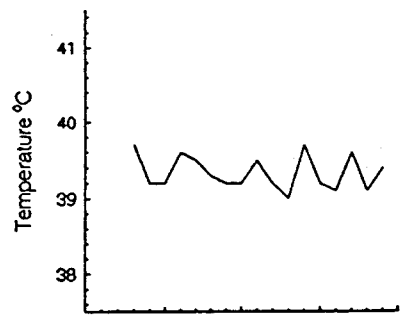
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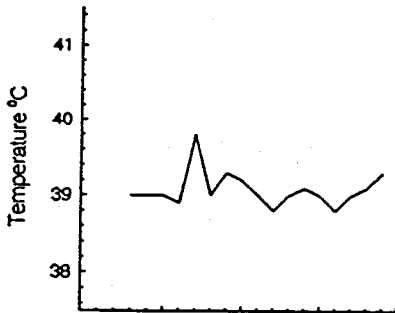
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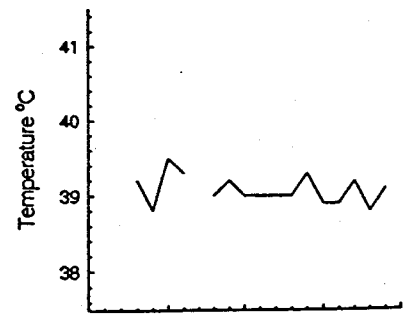
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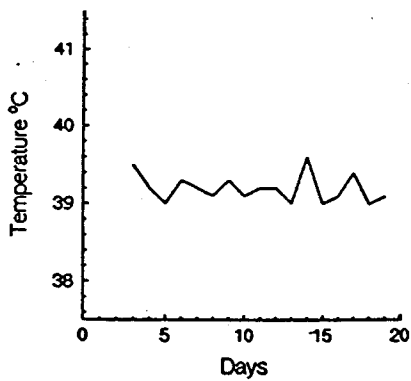
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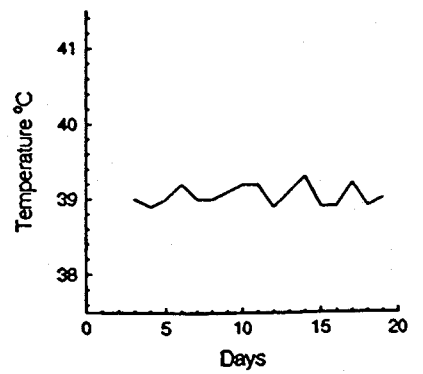


Table 1. Group antibody levels of sheep inoculated with attenuated BLU23 that had been passaged in insects and cattle or sheep.

Sheep		Elisa	Sheep		Elisa
		<u>Titre</u>			<u>Titre</u>
1131	Positive	99	1141	Positive	88
1132	Positive	63	1142	Positive	102
1133	Positive	95	1143	Positive	93
1134	Positive	96	1144	Positive	97
1135	Positive	75	1145	Positive	84
1136	Positive	61	1146	Positive	93
1137	Positive	72	1147	Positive	93
1138	Positive	91	1148	Positive	87
1139	Positive	84	1149	Positive	83
1140	Positive	92	1150	Positive	112

Table 2

Appearance of attenuated BLU23 vaccine in the semen of rams following vaccination

Ram	768			980			1001			1002			1003			1004		
	Semen		Blood	Semen		Blood	Semen		Blood	Semen		Blood	Semen		Blood	Semen		Blood
	Sheep	PCR		Sheep	PCR		Sheep	PCR		Sheep	PCR		Sheep	PCR		Sheep	PCR	
Day PI																		
4	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	+	+
7	+	+	+	-	-	+	-	+	+	-	S	+	-	-	+	-	-	+
10	+	+	+	+	+	+	-	-	+	-	-	+	-	-	+	-	-	+
13	-	-	+	-	-	+	-	-	+	+	-	+	-	-	+	-	-	+
16	+	ND	+	+	ND	+	-	-	-	-	-	+	-	-	+	-	-	-

ND - Not Done due to insufficient sample

S - Suspect

768, 980 - 6 year old rams

1001, 1002, 1003, 1004 - 3 year old rams

(iv) Vector competence of *Culicoides victoriae*

A total of 509 *C. victoriae* from Loxton S.A. and 10491 from Bairnsdale, Vic. fed on viraemic sheep. Feeding rates were higher in insects from Bairnsdale. Post feeding survival rate (9%) was similar in insects from both locations. Only 45 *C. victoriae* from Loxton and 386 *C. victoriae* and 558 *C. multimaculatus* from Bairnsdale were processed for virus isolation.

The population of *C. victoriae* at Loxton was noticed to be atypical compared to populations in eastern Australia. Females did not develop any abdominal pigmentation during the gonadotrophic cycle of blood digestion and egg maturation whereas the *C. victoriae* from the Bairnsdale population did develop some pigmentation as expected with *C. victoriae*. This suggests variation within the species and illustrates the value of collecting *C. victoriae* from more than 1 site in order to produce a reliable assessment of its potential as a vector.

No virus was recovered from any of the insects.

By combining the Loxton and Bairnsdale insects these results estimate that for BLU 1, *C. victoriae* and *C. multimaculatus* have infection rates of less than 0.2%. For *C. brevitarsis*, infection rates as low as 0.44% have been recorded for other bluetongue isolates. Consequently these results cannot definitively preclude the possibility of a similarly very low infection rate occurring in either *C. victoriae* or *C. multimaculatus*. To do so would require processing of many more insects.

(v) Intellectual Property

No intellectual property resulted from or arose in the course of carrying out the project.

(vi) Recommendations for commercial exploitation of results

The results indicate that use of live attenuated bluetongue vaccines would pose considerable risks for the Australian livestock industries and therefore commercial production of such vaccines cannot be recommended.

(vii) Total funding and MRC contribution

Total corporation funds expended on the project were \$259,269.

(viii) Impact on Meat and Livestock Industry

The cessation of development of attenuated bluetongue vaccines means that the Australian livestock Industry has no vaccine available for use in the event of an outbreak of clinical bluetongue. There is no impact on the livestock industry if there is no outbreak of clinical disease. Alternative vaccine options could be progressed in the next 5 years.

The confirmation of *C. victoriae* and *C. multimaculatus* as non competent vectors of bluetongue viruses should enable Australia to more confidently negotiate bluetongue - free status for livestock producing areas beyond the range of *C. brevitarsis*. This should allow greater market access for live ruminants and ruminant genetic material from these areas.