Excretion of bluetongue virus in cattle semen: a feature of laboratory-adapted virus

P.D. Kirkland[(1)], L.F. Melville[(2)], N.T. Hunt[(2)], C.F. Williams[(1)] & R.J. Davis[(1)]

(1) Elizabeth Macarthur Agricultural Institute, NSW Agriculture, Camden, NSW 2570, Australia
(2) Department of Business, Industry and Resource Development, Darwin, NT 0800, Australia

Summary

A series of experiments was conducted over a period of four years and involved both young (2-4 years) and old bulls (5-15 years) that were both naturally and experimentally infected with bluetongue virus (BTV). Several different virus serotypes were studied. In the Northern Territory, young bulls were exposed to natural infection with BTV over three wet seasons. During this time, bulls were infected with BTV-1, BTV-3, BTV-16 and BTV-20. In New South Wales, semen samples were examined from a large group of bulls of mixed ages that were naturally infected with BTV-1. Experimental infections in both young and old bulls (5-8 animals per group) employed both 'wild-type' and laboratory-adapted viruses from serotypes 1 and 23. A total of 41 bulls were included in the studies of natural BTV infection and 52 bulls in experimental infections.

There was no evidence of BTV in any of the semen samples collected from naturally infected bulls or experimentally infected young bulls. BTV was detected intermittently in semen from a number of old bulls infected with both laboratory-adapted BTV-1 and BTV-23. These detections occurred during or immediately after the period of detectable viraemia. Virus was also detected in a few semen samples from very old bulls infected with 'wild-type' BTV-23. These samples were collected during the period of viraemia and there was usually evidence of blood in the semen. Viraemia varied in duration between 17 and 38 days. Following immunosuppression, there was no evidence of resurgence of viraemia, or excretion of virus in semen, even in animals in which virus had been previously detected in semen. When the bulls were slaughtered, virus was not detected in any tissues.

Keywords

Australia – Bluetongue virus – Bull – Cattle – Laboratory-adapted virus – Semen – Viraemia – Virus excretion – Wild virus.

Introduction

Reports of the excretion of bluetongue virus (BTV) in the semen of bulls have influenced international policy for the movement of both live animals and semen between countries, and sometimes, within countries. In the United States of America (USA), Bowen *et al.* (1) were able to isolate BTV from the semen of bulls experimentally infected with US serotypes and suggested that this phenomenon may be related to the age of the bull. Trials in Australia (Darwin) with naturally infected bulls failed to detect any evidence of BTV in semen, even while bulls were viraemic (2). This project was conducted to investigate the effect of laboratory passage of virus, age of bulls and BTV serotype on the excretion of BTV virus in bovine semen. The study also investigated the possibility of persistence of virus after natural and experimental infection.

Methods

Experiments were conducted to monitor the duration of viraemia and excretion of virus in semen in both naturally and experimentally infected bulls. For both blood and semen, a large volume of samples was examined to maximise virus detection.

Natural infection

Each year in January, a group of 10 seronegative young bulls was introduced to Beatrice Hill, approximately 50 km south-east of Darwin in the Northern Territory of Australia (Fig. 1). Bulls were bled and ejaculated twice weekly from January to June. Whole blood and semen samples were collected for virus isolation and sheep inoculation. Sera from these bulls were checked monthly for seroconversion to BTV.

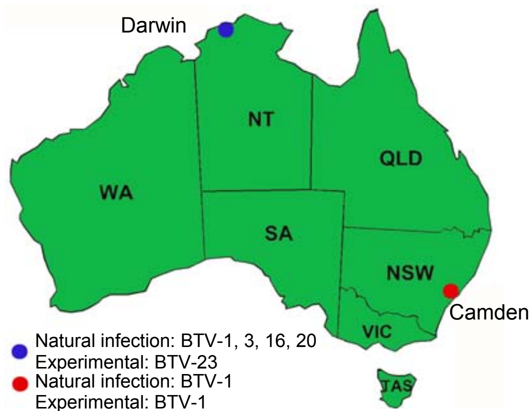

Figure 1
Location of bluetongue virus sample populations in Australia

In New South Wales, frozen semen samples were examined from a group of bulls of mixed ages which were naturally infected with BTV-1. A total of 130 batches of semen were collected from 19 bulls during the period of viraemia and up to three months later. All semen samples were examined by sheep inoculation.

Experimental infection

Groups of 5-8 mature bulls, aged 5-15 years, were selected from BTV-free areas and inoculated with either 'wild' virus or laboratory-adapted virus. Two serotypes were used: BTV-1 in New South Wales and BTV-23 in the Northern Territory. The laboratory-adapted strains of BTV-1 and BTV-23 had undergone 22 passages in BHK-21 cells. Bulls were inoculated subcutaneously with BTV-1 (6.57 $\log_{10}TCID_{50}$/bull) or BTV-23 (5.1 $\log_{10}TCID_{50}$/ bull). The inoculum for the 'wild' BTV infections was passaged so that a titre of virus similar to that used for the laboratory-adapted virus was obtained. The original 'wild' BTV consisted of unclotted blood from a sentinel calf. This virus stock was amplified by passaging 1-2 times in a calf and 1-2 times in a sheep. Each bull was inoculated subcutaneously with either 'wild' BTV-1, (6.29 $\log_{10}CEID_{50}$) or intravenously with 'wild' BTV-23 (5.4 $\log_{10}TCID_{50}$).

Following inoculation, whole blood and semen samples for virus isolation and serum for serology were collected twice weekly for 4 to 6 weeks then weekly for up to 8 weeks. Samples were held at 4°C, –80°C or in liquid nitrogen.

Persistent infection

To examine bulls for latent infection with BTV, nine seropositive bulls were immunosuppressed by corticosteroid administration. Blood and semen samples were collected for a further four weeks. Seropositive bulls were also slaughtered and a wide range of tissues was collected and checked for the presence of virus. Tissues included right and left testicles (upper and lower portion), head and tail of right and left epididymis, right and left seminal vesicle, right and left ampulla, prostate and spleen.

Virus isolation

Whole blood (2-5 ml) and undiluted semen samples (2-2.5 ml) were inoculated into separate sheep. The sheep were bled at the time of inoculation and four weeks later for serology. The presence of virus in a sample was indicated by seroconversion of the recipient sheep. Blood and semen samples were also inoculated intravenously into 11-day-old embryonated chicken eggs. The embryos were homogenised and the clarified supernatant inoculated onto *Aedes albopictus* cell cultures with up to three further passages on BHK-21 monolayers to detect virus (2).

Serology

Serum samples from all bulls (naturally and experimentally infected) and recipient sheep were tested for antibodies to BTV group antigens by agar gel immunodiffusion (AGID) and competitive enzyme-linked immunosorbent assay (c-ELISA) and for type-specific antibody by virus neutralisation (VN) tests. The AGID, c-ELISA and VN tests were performed using standard methods (3).

Results

Natural infection

At Beatrice Hill, 22 bulls were naturally infected during the study period as follows: 10 with BTV-1, 1 with BTV-3, 10 with BTV-16 and 1 with BTV-20. All sheep receiving blood from infected bulls seroconverted to the corresponding virus, but no sheep receiving semen seroconverted. In New South Wales, there was no evidence of BTV in any of the 130 batches of semen collected from 19 BTV-1 infected bulls around the period of viraemia.

Experimental infection: laboratory-adapted virus

BTV-1 in mature bulls (5-6 years)

All eight bulls became viraemic with the longest duration of viraemia being 27 days (Table I). BTV was identified in 22 semen samples from five bulls (1 to 6 ejaculates). In three (B, F and H) of these five bulls, virus was only detected in the semen during the period of viraemia (Fig. 2). Virus was detected in the semen of the other two bulls for up to three collections over a period of 10 days beyond the period of detectable viraemia. Although semen collections from the five bulls continued for a further 12 weeks, no further virus isolations were made. Titrations of the virus in semen and blood showed that the levels of virus were similar (Fig. 3).

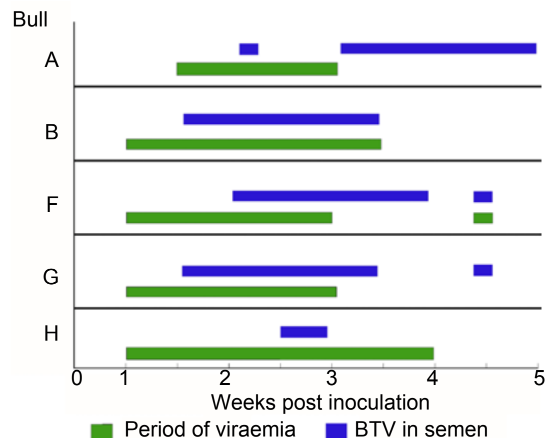

Figure 2
Duration of viraemia and virus detection in semen of mature bulls infected with laboratory-adapted bluetongue virus serotype 1

Table I
Detection of bluetongue virus serotype 1 in the semen of experimentally infected bulls

Bull	Age (years)	Duration of viraemia (days)	Virus	Ejaculates with BTV-1/ ejaculates tested
A	6	13	Lab* BTV-1	5/20
B	5	20	Lab* BTV-1	5/20
C	6	23	Lab* BTV-1	0/12
D	6	13	Lab* BTV-1	0/12
E	5	16	Lab* BTV-1	0/12
F	5	27	Lab* BTV-1	5/12
G	5	16	Lab* BTV-1	6/20
H	6	27	Lab* BTV-1	1/12
I	6	17	Wild BTV-1	0/12
J	6	13	Wild BTV-1	0/12
K	6	13	Wild BTV-1	0/12
L	6	10	Wild BTV-1	0/12
M	5	7	Wild BTV-1	0/12
N	6	17	Wild BTV-1	0/12
O	6	13	Wild BTV-1	0/12
P	5	23	Wild BTV-1	0/12

* Laboratory-adapted virus

BTV-23 in mature bulls (14-15 years)

All five bulls became viraemic with the maximum period of viraemia being 27 days (Table II). Virus was detected in six semen samples from three bulls during the period of viraemia (1 to 3 ejaculates) but never after the cessation of viraemia (Fig. 4). Three bulls died during the observation period from unrelated causes.

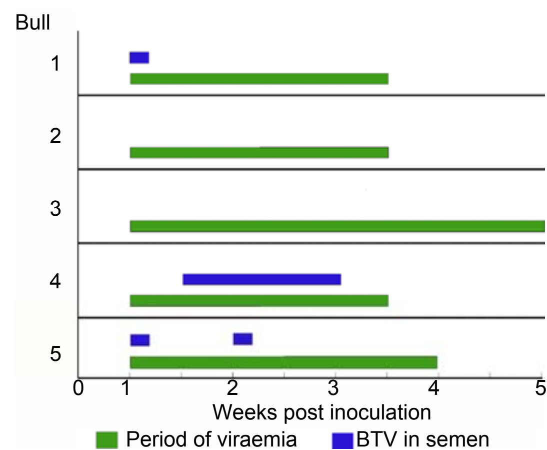

Figure 3
Duration of viraemia and virus detection in semen of mature bulls infected with laboratory-adapted bluetongue virus serotype 23

Table II
Detection of bluetongue virus serotype 23 in the semen of experimentally infected bulls

Bull	Age (years)	Duration of viraemia (days)	Virus	Ejaculates with BTV-23/ ejaculates tested
1	14	16	Lab(a) BTV-23	1/10
2	14	16(b)	Lab(a) BTV-23	0/5
3	15	27	Lab(a) BTV-23	0/10
4	14	16(b)	Lab(a) BTV-23	3/4
5	14	20(b)	Lab(a) BTV-23	2/6
6	12	27	Wild BTV-23	1/10
7	6	21	Wild BTV-23	0/10
8	12	27	Wild BTV-23	0/10
9	11	21	Wild BTV-23	2/10
10	10	24	Wild BTV-23	2/10

a) Laboratory-adapted virus
b) Died from unrelated causes

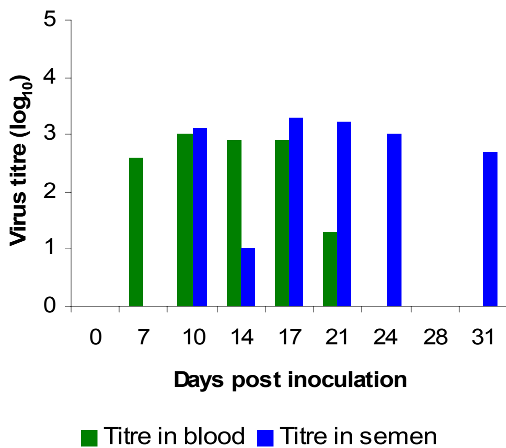

Figure 4
Comparison of titres of bluetongue virus in the blood and semen of Bull G (Table I), a mature bull infected with laboratory-adapted BTV-1

BTV-1 and BTV-23 in young bulls (2-3 years)

Each of the 13 young bulls inoculated with laboratory-adapted virus became infected, with patterns of viraemia similar to the old bulls (up to 27 days for BTV-1 and 38 days for BTV-23). Virus was not identified in the semen of any of these bulls.

Experimental infection: 'wild' virus

BTV-1 in mature bulls (5-6 years)

All eight bulls became viraemic with the longest duration of viraemia being 28 days. No virus was detected in any of the semen samples (Table I).

BTV-23 in mature bulls (6-12 years)

All five bulls became viraemic with the longest duration of viraemia being 27 days (Table II). Virus was detected in five semen samples from three bulls during the period of viraemia (1 to 2 ejaculates) (Fig. 5). Each of these infected semen samples was collected at the peak of viraemia and four showed visible contamination with blood.

BTV-1 and BTV-23 in young bulls (2-3 years)

Each of the 13 young bulls inoculated with 'wild' type virus became infected, with patterns of viraemia similar to the old bulls. Virus was not identified in the semen of any of these bulls.

Latent infection

BTV was not isolated from any tissues of the genital tract or spleen when five of the bulls inoculated with laboratory-adapted virus (not treated with corticosteroids) were slaughtered 56 days post inoculation (Bulls C, D, E, F and H) (Table II). Nine bulls were immunosuppressed with dexamethasone. Three of these bulls had previously been infected with laboratory-adapted BTV-1 in New South Wales (Bulls A, B and G). Four of the bulls were naturally infected with BTV-1 and two were experimentally infected with 'wild' BTV-23 in the Northern Territory. There was no evidence of a resurgence of viraemia and no evidence of BTV in the semen. BTV was not isolated from any tissues of the reproductive tract or spleen when these bulls were slaughtered.

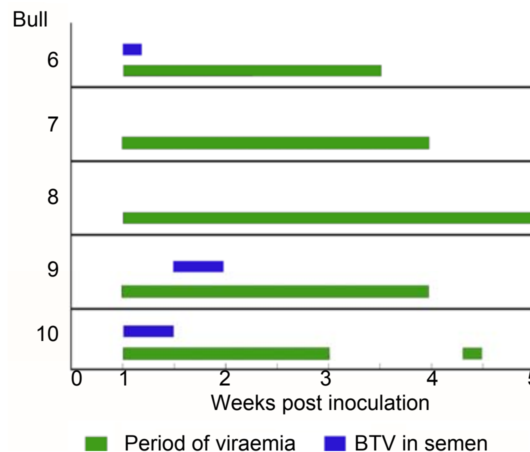

Figure 5
Duration of viraemia and virus detection in semen of mature bulls infected with wild-type bluetongue virus serotype 23

Discussion

These studies suggest that the use of laboratory-adapted BTV may be a major factor contributing to the contamination of bovine semen observed experimentally. More than half of the old bulls infected with laboratory-adapted BTV-1 and BTV-23, were found to excrete virus in their semen. These detections occurred during or immediately after the period of detectable viraemia. There was no evidence of virus in the semen or blood of these bulls during the subsequent 12 weeks of observation. The maximum duration of viraemia in any animal in these studies was 38 days.

When bulls were experimentally infected with 'wild' BTV, the results were more variable. Virus was detected in a few semen samples from very old bulls infected with 'wild' BTV-23. Each of these semen collections was made during the period of viraemia, in the first week, and probably near the peak of viraemia. There was usually evidence of blood in the semen. Virus was not found in the semen of any of the bulls infected with 'wild' BTV-1. The extreme age of the bulls infected with 'wild' BTV-23 was likely to have been a contributing factor to the detection of virus in their semen. Bulls aged between 10 and 12 years would not usually be used as donors for commercial collections of semen. It is believed that virus may be present in the semen of old bulls as

a result of inflammatory changes that occur in older animals (1).

There was no evidence of BTV in the semen of any of the 41 naturally infected bulls or 26 experimentally infected young bulls. The younger age of these bulls may have been a factor contributing to the lack of contamination of semen with virus. These findings are consistent with other data for the testing of semen for evidence of BTV contamination (4, 5). To date, there has been no published report of the isolation of BTV from the semen of a naturally infected bull.

Immunosuppression did not result in a resurgence of viraemia or excretion of BTV in semen, even in animals in which virus had previously been detected in semen. Virus was not detected in any tissue when bulls were slaughtered.

These studies indicated that unless semen is contaminated with blood, the excretion of BTV is confined to old bulls infected with laboratory-adapted virus. This may have implications for countries where live BTV vaccines are used because it is possible that vaccine virus may be spread in the field. There was no evidence of long-term persistence of the virus, even with laboratory-adapted strains.

Acknowledgements

Funding for this work was provided by Meat and Livestock Australia. Technical assistance in Darwin was provided by Shane Cross at Beatrice Hill and Richard Weir, Shelley Walton and Margaret Harmsen in the Virology Laboratory. The technical assistance of Alison Jugow, Yogini Lele, Christine Hornitzky and Ted Batty at the Elizabeth Macarthur Agricultural Institute in Camden, is appreciated.

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