

Bluetongue surveillance methods in an endemic area: Australia

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Summary

Surveillance for bluetongue (BT) viruses (BTV) has been carried out in the Northern Territory, Australia since 1980. The number of sites, intensity of sampling and methods of testing have varied during this period. Monthly serology is conducted at a number of sentinel sites and intensive weekly sampling for virus isolation is conducted at the site of highest known arboviral activity. This has enabled the isolation of all eight BTV serotypes identified in Australia. Natural viraemias are between one and eight weeks. No additional serotypes have been isolated since 1986. However, genetic analysis of isolates has shown incursions of viruses of South-East Asian origin in 1992, 1994 and 1995. Trapping for *Culicoides* spp. has also been carried out at these sites on a regular basis. In recent years, an annual serological survey has supplemented the sentinel herds to more accurately define the BT zones described under OIE guidelines.

Keywords

Australia – Bluetongue virus – *Culicoides* – Epidemiology – Genetic analysis – Surveillance – Virus isolation.

Introduction

The Northern Territory of Australia lies in the semi-arid tropics with the more northerly areas experiencing hot, wet summers and warm, dry winters. Cattle raising is conducted on large properties ranging in size from 10 000 to one million hectares. There are no commercial sheep operations. Large areas in the north-east and south-west of the Northern Territory are unsuitable for raising cattle. The bluetongue (BT) endemic area is located between approximately 11°S and 17°S. Southerly extension to around 20°S occurs in some years, depending on seasonally variable risk factors.

Surveillance for BT virus (BTV) has been conducted at between one and fourteen sites since the first isolation of a BTV in 1975. This isolation of BTV-20 was made from a mixed pool of *Culicoides* species collected approximately 50 km south-east of Darwin at Beatrice Hill (14). The number of sites and intensity of monitoring has varied during this period. Since the commencement of the National Arbovirus Monitoring Program (NAMP) in 1993 (9), between six and fourteen sentinel herds have been monitored each year. The most intensive studies have been conducted at Beatrice Hill. This site has previously been identified as having intense and varied arbovirus activity (15). Light traps for insect

collections are also operated at most sites. In 2002 and 2003 sentinel herd monitoring was supplemented with an annual BTV serological survey at selected locations. This is designed to enable the BTV-free and surveillance zones to be more accurately defined under OIE guidelines.

Materials and methods

Sentinel herds

Each sentinel herd consists of 10 to 25 young cattle initially seronegative for BTV antibodies. Cattle are replaced annually or earlier if seroconversion occurs. Serum samples are collected at regular intervals, usually monthly or quarterly. At Beatrice Hill, cattle are bled weekly throughout the year. Monitoring sites are confined to the areas of commercial cattle operations.

Serological surveys

Serological surveys are conducted on selected properties along six north/south transects. Up to three properties are chosen along each transect, concentrating on the margins of the endemic area. Between 80 to 100 cattle are bled at each site. Selected cattle must be between 18 and 24 months old, have been bred on the property and have never

left the property. Between 10 and 15 locations are sampled each year.

Serology

Serum samples from sentinel animals and survey animals are tested for BTV antibodies by competitive enzyme-linked immunosorbent assay (c-ELISA) (10) and, if positive, by virus neutralisation (VN) tests (5).

Virus isolation

Lithium heparin blood samples collected weekly at Beatrice Hill are processed for virus isolation. Clots from serum samples collected at other sites are held for retrospective isolation following identification of seroconversion in individual animals. The isolation system uses embryonated chicken eggs (ECE) as described by Gard *et al.* (4), with the final cell passages through microtitre plates rather than cell culture tubes. The ECE homogenates are passaged onto mosquito cell cultures (*Aedes albopictus* C6/36). The second passage uses C6/36, BSR (13) and porcine stable equine kidney (PSEK) mammalian cell cultures. A third passage uses BSR and PSEK cell cultures to detect any cytopathology due to virus replication. Viruses are identified by a combination of BTV antigen capture ELISA (8) and VN (5).

Molecular studies

Each year, since 1992, a selection of BTV isolates have been subjected to genetic analysis (6, 7, 12). This has allowed identification of regional groupings of viruses and tracking of their movement (12).

Entomology

Light traps for insect collections are operated for three nights each month at most sentinel herd sites and other specific locations. Sampling is conducted throughout the year. Insects are collected into alcohol and the *Culicoides* species sorted and identified. More intensive monitoring by aspirating midges from cattle is also performed at Beatrice Hill.

Data management

Serological and entomological data are entered onto a web-based national database. This enables national zones to be developed for trade purposes (3).

Results

Virus isolation

Since 1981, BTVs have been isolated from sentinel cattle at Beatrice Hill every year except 1990 (11). The level of activity and number of serotypes has shown marked annual variation. All eight BTV serotypes identified in Australia have been isolated at

this site. BTV-1 is the most frequently observed. BTV-20, the original serotype isolated in 1975, was not seen again until 1992. Further isolates of BTV-20 were made in 1995, 1996 and 1997. BTV-21, isolated each year from 1981 to 1984, was not isolated again until 1994. Further isolates were made in 1995. BTV-16, first isolated in 1986, has reappeared at regular intervals (1988, 1992 and 2001). Table I demonstrates the marked annual variation in infection rates and activity.

Table I
Characteristics of natural BTV infections, 1992-2003

Year	Bluetongue serotype	Sentinels infected	Detectable period of viraemia (weeks)	
			Min.	Max.
1992	16	54/75	1	6
	20	2/75	1	1
1993	1	33/74	1	3
1994	1	45/70	1	6
	21	3/70	2	3
1995	21	59/68	1	6
	20	45/68	1	4
1996	20	19/32	1	5
1997	1	28/47	1	5
	20	23/47	1	4
1998	1	19/24	1	5
1999	1	29/43	1	4
	20	18/43	1	5
2000	1	22/25	1	4
2001	1	6/24	1	4
	9	6/24	1	4
	16	23/24	2	8
2002	1	23/24	1	6
2003	1	36/48	1	5

Differences in the biological behaviour of these viruses are also demonstrated in Table I. In 1992, BTV-20 was isolated once from each of two animals of 75 monitored. Serology confirmed these were the only two of 75 infected. In 1995, BTV-20 was isolated from 45 of 68 animals monitored, with periods of viraemia up to four weeks, and in 1996, from 19 of 32 animals monitored with periods of viraemia lasting up to six weeks. Similar differences have been observed with BTV-21. During the period 1981 to 1984, isolations of BTV-21 were made from a small proportion of animals sampled and the periods of viraemia were less than two weeks. In 1995, BTV-21 was isolated from 59 of 68 animals monitored with periods of viraemia up to four weeks.

No additional serotypes have been isolated since 1986. However, genetic analysis of isolates obtained since 1991 has shown incursions of BTVs of South-East Asian origin in 1992, 1994 and 1995. Virus isolation and genetic analysis has also been performed on selected samples from sentinel sites other than Beatrice Hill. BTV-1 and BTV-16 have been isolated from sites within 300 km from Darwin. BTV-1 isolates from these sites were also shown to be the same South-East Asian genotypes as the viruses isolated at Beatrice Hill. BTV-1 isolated from a site 600 km from Darwin was an Australian genotype.

Serology

Monitoring of sentinel sites within 300 km of Darwin shows BTV activity in most years. At inland sites and in lower rainfall areas, activity is observed infrequently. In these areas, serological surveys have been utilised to detect low levels of infection. Combined data from sentinel herds and serological surveys have shown that the boundaries of the endemic area can move considerable distances between seasons. Current sentinel and survey sites are shown in Figure 1. These sites are confined to the areas of commercial cattle enterprises.

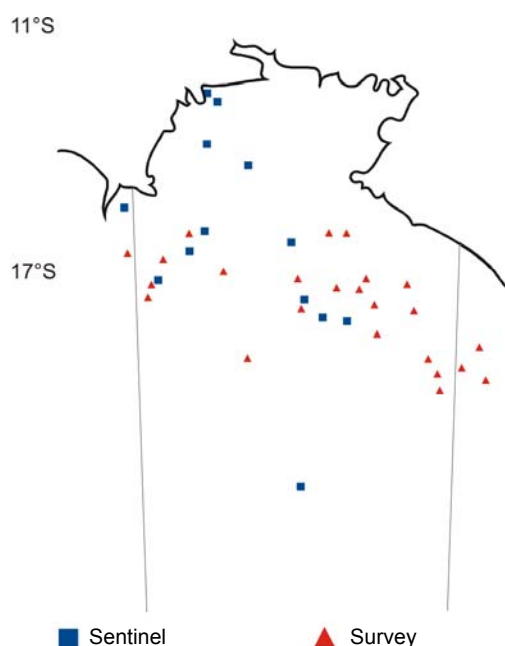


Figure 1
Bluetongue virus isolation and serology monitoring sites in the Northern Territory, Australia

Entomology

Five species of *Culicoides* are currently recognised as proven vector species in Australia (16). These species are *C. brevitarsis*, *C. actoni*, *C. fulvus*, *C. wadai* and *C. dumdumi*. All species except *C. dumdumi* have been

identified at the sites within 300 km of Darwin, although *C. wadai* is present only in very low numbers and in occasional years at sites other than Beatrice Hill. *C. dumdumi* has only been identified at Beatrice Hill on limited occasions. Only *C. brevitarsis* has been identified at sites further inland. *C. actoni*, *C. brevitarsis* and *C. fulvus* are present at Beatrice Hill most months. *C. fulvus* numbers are higher during the wet summer months but *C. actoni* and *C. brevitarsis* numbers do not show any seasonal dependence. Ongoing studies aspirating midges from cattle have shown that peak activity of *C. actoni* occurs before sunset (1). This species is clearly under-represented in light traps.

Discussion

Over the past 25 years, monitoring and surveillance programmes in Australia have identified the BTV serotypes temporarily or permanently present and their distribution. While sentinel herds have been the basis of this monitoring, additional information, such as vector distribution, genetic analysis and results from serological surveys, has been incorporated to give a more detailed understanding of the viruses and their distribution. Data from an intensively monitored site at Beatrice Hill has demonstrated marked annual variation in the activity and infection rates of BTV. Despite the intensity of monitoring, the ecology and epidemiology of BTV is still poorly understood. Changes in the biological behaviour of the viruses have also been observed. These changes coincided with the detection of incursions of BTV viruses for which there is evidence in South-East Asia. Continued genetic analysis of a selection of BTV isolates obtained each year has shown at least one of these genotypes has become established in the Northern Territory. Analysis of isolates from elsewhere in Australia has shown these genotypes are currently confined to the area within 300 km of Darwin.

In localised areas of Australia, where *C. brevitarsis* is the only vector species, attempts to model vector activity have been largely successful (2). In northern Australia where multiple vector species are present and incursions of viruses from South-East Asia are occurring, prediction of virus activity is very difficult. Similar problems are encountered when attempting to model BTV activity on a large scale where complex climatic and geographical interactions influence vector behaviour. Flexible surveillance methods are required to describe a dynamic BTV situation and to meet changing requirements for international trade.

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