

## **Peaton Virus: a New Simbu Group Arbovirus Isolated from Cattle and *Culicoides brevitarsis* in Australia**

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### *Abstract*

A new member of the Simbu group of arboviruses, for which the name Peaton virus is proposed, has been isolated from midges and cattle in Australia. Nine isolates were obtained from 101 pools of the biting midge *Culicoides brevitarsis* collected at Peachester, Qld, (26·51°S., 152·53°E.) between 30 November and 8 December 1976. Three isolations of the same virus were made from the blood of sentinel cattle collected at Grafton and Tamworth, N.S.W., on 20 January and 13 April 1977, respectively. Peaton virus was shown to be a member of the Simbu group of arboviruses by complement-fixation tests using antisera prepared against Australian strains of Akabane and Aino viruses. It was readily distinguishable from these viruses in cross-neutralization tests in tissue cultures and mice. A serological survey of sentinel cattle showed that neutralizing antibody was detectable only in cattle within the recorded limits of the suspected vector *C. brevitarsis*. Neutralizing antibody in blood serum was detected in 22 of 157 sheep, 21 of 137 horses, 7 of 18 buffaloes, 7 of 20 goats and 3 of 62 pigs, but not in 22 camels, 34 dogs, 3 cats, 76 human beings, 240 marsupials, 19 reptiles or 31 wild birds. The pathogenicity of Peaton virus has yet to be determined. The Yale Arbovirus Research Unit and the Center for Disease Control, Fort Collins, U.S.A., found that Peaton virus was distinguishable from all other Simbu group viruses and thus is a new virus.

### **Introduction**

In recent years arboviruses of the Simbu group (Bunyaviridae) have been recognized as pathogens of livestock. Of this group, two viruses, Akabane and Aino, were isolated in Australia in 1968 (Doherty *et al.* 1972). However, little attention was paid in Australia to Akabane virus until Hartley *et al.* (1975), following the lead of Kurogi *et al.* (1975) in Japan, associated it with congenital arthrogryphosis-hydranencephaly in calves. Since then, Coverdale *et al.* (1968) have provided serological evidence that Aino virus was associated with a similar syndrome in the New England area of New South Wales in 1976.

A number of viruses, other than Akabane, was isolated from insects in late 1978 in the course of a project designed to gain more field evidence on the relationship of cattle and insects with Akabane virus (St George *et al.* 1978). Further investigation of these viruses allow us to report the isolation in Australia of a third virus of the Simbu group. Some facets of the epidemiology of this virus have been explored, but no attempt has yet been made to determine its pathogenicity. The detailed study of the virus itself will be the subject of a separate communication.

## Materials and Methods

### *Insect Collections at Peachester*

Insects were collected by light traps, animal bait collections and truck traps on a farm and surrounding roads at Peachester, Qld, 100 km north of Brisbane, on 12 nights between 24 November and 8 December 1976. Poor weather prevented collections on the remaining nights. The insects were identified and sorted into species pools the morning after collection. They were then prepared for suckling mouse or tissue-culture inoculation by methods described by St George *et al.* (1978).

### *Virus Isolations from Sentinel Cattle*

For some years, blood samples have been obtained from cattle in various sentinel herds scattered throughout Australia in a pattern described by St George *et al.* (1977). The blood samples were collected from the same identified cattle a number of times during a year. The interval between samples varied considerably. Many herds were bled monthly, some quarterly, and others less often because of local circumstances. The scheme provided for a new group of animals to be bled each year so that susceptible animals would become available to replace those which may have been infected by arboviruses. The group size in a particular herd was 10–20. One of these sentinel herds was located at Grafton, N.S.W., and another at Tamworth, N.S.W. The samples arrived in some instances as separated serum, but in most cases with the clot and serum in the one bottle. Since August 1976, about 0.25 ml of serum and cells from the base of the clot have been cultured in baby hamster kidney tissue cultures (BHK21, Macpherson and Stoker 1962) in roller tubes. The technique of cell preparation was essentially that of French and St George (1965). The medium was changed to Eagle's Basal Medium (C.S.L., Melbourne) plus 3% (v/v) foetal calf serum a day later.

All tissue cultures were scraped down, pooled, and passaged 7–10 days after inoculation if no specific changes were noted up to the time they degenerated with age. This procedure was repeated at least once more. Where cytopathic effect (CPE) in a cell sheet was noticed earlier, the particular cell sheet was passaged separately.

### *Sentinel Herd at Peachester*

Blood samples were obtained from 12 heifers aged 7–17 months in one sentinel herd at Peachester (26.51°S., 152.53°E.) about 100 km north of Brisbane. The heifers were bled at various intervals: weekly, from 22 August to 30 September 1976, twice weekly until 18 November, then daily for 15 days, then again weekly until 25 March 1977. The blood samples were cultured in BHK21 tissue cultures for virus and the separated sera assayed for specific antibodies as described below. Certain sera from an accumulated bank of samples collected from sentinel herds of cattle, plus serum samples from other species bled on a single occasion, were tested for antibody in a microneutralization test described below.

### *Antiserum Preparation*

A specific antiserum against one isolate, designated CSIRO 110, of the new virus was prepared in rabbits by giving a series of three intramuscular injections with a mixture of virus and Freund's complete adjuvant (C.S.L., Melbourne) at weekly intervals, and then one intravenous injection with virus alone 4–6 weeks later. The rabbits were bled after 7–10 days and the serum stored at –20°C. The antiserum against CSIRO 110 virus and others prepared similarly against Akabane and Aino viruses were used in cross-neutralization tests in tissue cultures and mice.

### *Serum Neutralization Tests*

A microtitre neutralization test was developed in sterile Cooke flat-bottomed microtitre plates (Cooke Engineering Co., U.S.A.) using Vero cells at a concentration of  $2 \times 10^5$  cells/ml. Medium 199 plus 10% (v/v) foetal calf serum was used as diluent throughout the test.

Survey sera were diluted 1:4 before testing, but the hyperimmune rabbit serum was used undiluted as well as being titrated in twofold steps. All sera were inactivated by heating at 56°C for 30 min. Similar tests on all sera were carried out with Akabane virus (strain R7949) and Aino virus (strain B7974), both of which were isolated by Doherty *et al.* (1972).

The tests were conducted in duplicate. Serum controls were included with each individual serum tested to check for cell toxicity. Known positive and negative sera were titrated as part of the controls

for each day's test. To each 0.025 ml of a 1:4 dilution of serum in a flat-bottomed well in a microtitre plate was added an equal volume of diluent which contained 300 50% tissue-culture-infective doses of the CSIRO 110 or CSIRO 133 isolates of Peaton virus. After incubation for 45 min at 37°C, 0.1 ml of diluent containing Vero cells was added to the mixture which was then covered with paraffin oil which had been sterilized by filtration. Titration of serum was carried out in microtitre plates using 0.025 microdiluters (Cooke Engineering Co., U.S.A.) and allowing four wells per dilution.

After incubation for 5 days at 37°C, the wells were examined for CPE. A serum was taken as positive for antibody if CPE was completely prevented, and negative for antibody if CPE was present. Antibody titres were calculated by the method of Reed and Meunch (1938), and are expressed as reciprocals of the dilution of the serum added to the well.

#### *Identification of the Virus*

One isolate, CSIRO 110, was adapted to suckling mice and, together with antisera prepared in rabbits and mice, was forwarded to the Queensland Institute of Medical Research, in its role as a regional reference laboratory of the World Health Organization, for comparison with other Australian arboviruses. A complement-fixing antigen was prepared there and tests carried out with it by standard techniques as described by Doherty *et al.* (1975). An attempt was made to produce a haemagglutinin by sucrose-acetone extraction of infected mouse brain as described by Doherty *et al.* (1975). The virus was later forwarded to the Yale Arbovirus Research Unit, and from there to the Center for Disease Control, Vector Borne Diseases Division, Fort Collins, Colorado, U.S.A., for comparison with the full range of Simbu group viruses.

## **Results**

### *Insect Collections at Peachester Farm*

*C. brevitarsis* was the dominant biting midge in the collections. From 11 truck trap collections, 3783 females and 6690 males were processed. Six animal bait collections yielded 870 *C. brevitarsis* while 693 were taken in 16 light trap collections. Other species of insects collected were *C. bundyensis*, *C. victoriae*, *C. antennalis*, *C. cuniculis* and *C. marksi*, all in very small numbers, plus a total of 21 mosquitoes of the species *Culex annulirostris*, *C. bitaeniorhynchus* and *Mansonia uniformis*.

### *Virus Isolations*

Thirteen isolations of viruses were made in suckling mice from 101 pools of 3783 female *Culicoides brevitarsis* collected at Peachester between 30 November and 8 December 1976. Of these 13 isolates, 3 were Akabane virus, 1 was a rhabdovirus as yet unidentified, and 9 were isolates of a single 'new' virus. One of these nine isolates was designated CSIRO 110. Six of the nine new viruses were also isolated in BHK21 tissue culture as well as in mice. Two of the six tissue-culture isolates did not produce CPE in the tissue-culture tubes originally inoculated but did so only on passage. The remaining four isolates produced CPE as small foci which developed on the fifth day post-inoculation. These foci did not extend on subsequent days, but, on the contrary, decreased in size so that in a further 3 days the cell sheet appeared normal when compared with control cell sheets of the same age. When the cell sheet was passaged after it became apparently normal, no CPE was detected in tissue cultures to which it was passaged up to three further times. Virus did transfer with supernatant fluid if it was removed at the time foci were present. Nine of the isolates of the new virus were obtained from parous female *C. brevitarsis* collected between 30 November and 8 December 1976 from a total of 5346 collected in the whole trapping period. Four of these isolations were made from 1498 female *C. brevitarsis*

collected in truck trap collections made on 7 and 8 December 1976. No isolations of virus were made from 6690 male *C. brevitarsis* from the same collections.

Three isolations of the same virus were made in BHK21 tissue cultures from the blood of three normal heifers in sentinel herds: one in a herd located at Grafton (isolate CSIRO 133) and two in the herd at Tamworth, N.S.W. CPE was noticed between the seventh and ninth days after inoculation of the BHK21 tissue cultures. The blood samples were collected on 20 January and 13 April 1977, respectively. Each of the donor animals had neutralizing antibody to CSIRO 133 virus in a blood sample collected 14–21 days after virus isolation but not in the serum samples collected at the time when virus was demonstrated to be present in the blood.

The common identity of the insect and cattle isolates was initially suggested by the use of paired serum samples from the animal from the Grafton sentinel herd from which CSIRO 133 virus was isolated. These results were later confirmed with specific rabbit antisera.

#### *Simbu Group Relationship*

The sucrose-acetone extract of mouse brain infected with the CSIRO 110 strain of virus did not agglutinate gander cells. It did fix complement in the presence of homologous antiserum, and also in the presence of antisera to two members of the Simbu group, Akabane and Aino viruses. This relationship with the Simbu group was confirmed by reciprocal complement-fixation tests. In contrast, no fixation of complement occurred when antisera against D'Aguilar, Warrego, Wallal and Mudjinberry viruses isolated from biting midges in Australia were tested.

**Table 1.** Tissue-culture cross-neutralization tests to Peaton, Akabane and Aino viruses  
Values are the reciprocal dilutions of serum which neutralized the dose of virus shown in parentheses

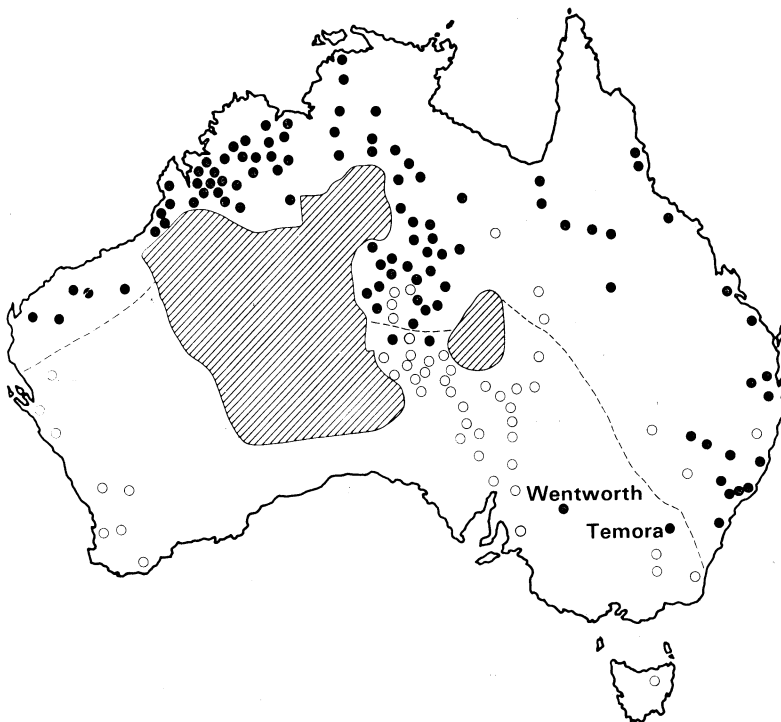
Virus	Rabbit antiserum prepared with		
	Peaton virus	Akabane virus	Aino virus
Peaton (300 TCID <sub>50</sub> )	> 256	< 1	< 1
Akabane (100 TCID <sub>50</sub> )	< 1	> 256	< 1
Aino (300 TCID <sub>50</sub> )	< 1	< 1	> 256

The comparison of the new virus with Akabane and Aino viruses in the tissue-culture microtitre neutralization tests is shown in Table 1. Similar results were obtained with neutralization tests in suckling mice in which the homologous antiserum had a neutralizing index of 3·0 or greater, but no cross-neutralization occurred. These results show that the virus is a member of the Simbu group of arboviruses, but is readily distinguishable from Akabane and Aino viruses by neutralization tests. The name Peaton virus is advanced as a provisional name for this virus and is used to facilitate further description and discussion.

At the Yale Arbovirus Research Unit, Peaton virus was found by cross-neutralization tests to be distinguishable from all other Simbu group viruses. The report from the Center for Disease Control, Colorado, stated that, from complement-fixation and neutralization tests, Peaton virus appears to be a new member of the Simbu group of viruses related to Sango virus (Calisher, personal communication). Sango virus is a Simbu virus isolated from cattle in Nigeria (Causey *et al.* 1972).

*Sentinel Herd at Peachester Farm*

No isolations of Peaton virus were made from blood samples from the Peachester herd. All of the sentinel group of 12 animals bled at Peachester developed antibody to Peaton virus between 1 December and 23 December 1976. There were two or three negative serum samples from each of these animals before the first date on which antibody to Peaton virus was detected. The time of antibody response in most individual animals could be estimated only approximately, as only a limited series of sera were available. The first animal developed antibody on 1–2 December and the last between 15–23 December 1976, that is, in a total time span of 2–3 weeks.



**Fig. 1.** Distribution of neutralizing antibodies to Peaton virus in sentinel cattle in relation to the southern limit (---) of *Culicoides brevitarsis*. Hatched areas represent desert which is free of domestic livestock. ● Antibody detected; ○ no antibody detected.

Fig. 1 shows the location of herds from which cattle serum was collected for testing for antibody to Peaton virus. The herds in which a change in antibody status from negative to positive for antibody to Peaton virus (seroconversion) occurred during the period of testing, and the time interval over which seroconversion took place, are shown in Table 2. In the sera which were titrated the titres ranged up to 64, with 90% reaching a titre greater than 16. No antibody was detected in 137 serum samples from three herds in Papua New Guinea. Some sera collected much earlier were tested to establish whether Peaton virus was new to Australia or not. Of 41 cattle sera collected in 1970, 18 contained antibodies to Peaton virus.

### *Serology of Other Species*

Neutralizing antibody was found in sera from 22 of 127 sheep, 21 of 137 horses, 7 of 18 buffaloes, 7 of 70 goats and 3 of 62 pigs, but not in 22 camels, 34 dogs, 3 cats, 76 human beings, 240 marsupials, 19 reptiles or 31 wild birds.

**Table 2. Occurrence of seroconversion of herds**

Location of sentinel cattle is shown, and the time interval during which seroconversion to Peaton virus occurred at these locations

Location	Date of seroconversion
Northern Territory	
Douglas Daly (13·51°S., 131·13°E.)	20.v.77–29.ix.77
Victoria River (16·24°S., 131·01°E.)	3.ix.77–7.x.77
Queensland	
Julia Creek (20·01°S., 141·56°E.)	28.vi.74–6.iii.75
Lowmead (24·32°S., 151·45°E.)	5.iv.77–21.vi.77
Peachester (26·51°S., 152·53°E.)	2.xii.76–13.i.77
New South Wales	
Scone (32·03°S., 150·52°E.)	21.ii.77–29.iii.77
Singleton (32·34°S., 151·10°E.)	22.ii.77–6.iv.77
Paterson (32·44°S., 151·34°E.)	23.ii.77–29.iii.77
Williamtown (32·49°S., 151·50°E.)	13.xii.76–31.iii.77
Camden (34·03°S., 150·42°E.)	21.ii.77–14.iii.77
Wentworth (34·10°S., 141·50°E.)	5.v.77–30.vii.77

### Discussion

Peaton virus appears to be a new member of the Simbu group and is of particular interest to Australia. The comparison with the other members of the Simbu group at the Center for Disease Control at Fort Collins has shown it to be distinguishable from all other members of the Simbu group (Calisher, unpublished data) though related to Sango virus. We have proposed the name of Peaton, which is an amalgam of the names of the localities from which the first cattle and insect isolates came. Though it is customary to name arboviruses according to the area from which they are first isolated, this practice can produce social or economic problems if the viruses are later found to be pathogenic.

The range of vertebrate species in which antibody to Peaton virus has been detected so far is narrower than that for Akabane virus, but the general pattern is similar in that domestic animals are infected rather than marsupials or human beings. Much more serology remains to be done to define the vertebrate host range.

There were more isolations of Peaton virus made from insects in suckling mice (9) than in BHK21 tissue cultures (6). However, the tissue-culture method in use may not have been optimal at that time as it was still being developed. Each of the three isolations from the blood of cattle was made in BHK21 tissue cultures but was not attempted in mice, so that no comparison can be made of the relative efficiency of the two methods for isolation of Peaton virus from blood.

*C. brevitarsis* occurs in Papua New Guinea (Murray 1975) but the cattle sera from there did not have Peaton antibody. This is a similar situation to that found with

other members of the Simbu group of viruses, namely Akabane virus (Cybinski *et al.* 1978) and Aino virus (Doherty *et al.* 1973; Cybinski and St George 1978). Within Australia, the distribution of Peaton antibody in cattle falls largely inside the known limits of the suspected vector *C. brevitarsis* (Fig. 1).

Two positive sites (Temora and Wentworth) lie outside the known limits of *C. brevitarsis*. Temora, it was suggested (Della-Porta *et al.* 1976), is within the range of *C. brevitarsis* in exceptional seasons. The one positive animal at Wentworth seroconverted during a period (May–July) when insect activity would be expected to be minimal (Dyce and Standfast, unpublished data), while the herd was 700 km from the nearest known centres of *C. brevitarsis* activity.

The results from the studies of insects collected at Peachester give a more direct association between the infection of *C. brevitarsis* and cattle with Peaton virus. The insects were all collected within a radius of 2 km of the cattle. Thus virus was detected in the midges between 30 November and 8 December and infection of cattle, as judged by serology, probably began some days prior to 30 November and continued for 2–7 weeks. No Peaton virus was isolated from the daily blood samples from the sentinel heifers cultured between 18 November and 2 December. Infection of both *C. brevitarsis* and the same group of sentinel cattle with Akabane virus was detected immediately prior to the period when Peaton virus was detected (St George *et al.* 1978). Though the studies were not definitive, it appears that Akabane virus infected the sentinel cattle in this herd over a period of approximately 1 month followed by Peaton virus as a separate wave over a period of 2–7 weeks. Infection of the sentinel cattle with Akabane virus and the development of antibody to this virus did not prevent infection of the same animals with Peaton virus and the subsequent development of high titres of antibody to Peaton virus.

Peaton virus does not appear to have a significant antigenic overlap with Akabane and Aino viruses as judged by cross-neutralization tests with rabbit antisera.

The timing of seroconversion of the herds listed in Table 2 is in accord with what is known of the seasonal variation in abundance of *C. brevitarsis* (Dyce and Standfast, unpublished data). Populations peak in the late summer in the southern coastal areas while peak densities are reached during winter in the north.

The virus infection (both Akabane and Peaton) in the cattle at Peachester farm followed an apparent marked increase in numbers of *C. brevitarsis* in the area. One possible explanation for the appearance of the virus could be transovarial transmission in the insect vector. If this virus followed the pattern of another Bunyavirus, La Crosse virus (Beaty and Thompson 1975), up to 1% of the insects might be expected to be positive for virus if transovarial transmission had taken place. To test the hypothesis, male *C. brevitarsis*, which do not take a blood meal and could only become infected transovarially, were processed for virus isolation and compared with females, which were judged to be parous (Dyce 1969) and therefore had taken a blood meal, from the same collection. No virus was isolated from the 6690 males processed while four isolations were made from the 1498 females. We cannot therefore advance any evidence to support the hypothesis of transovarial transmission in this instance.

At present, nine isolations of Peaton virus from *C. brevitarsis* have been made, a total which equals the combined totals of six isolations of Akabane virus (Doherty *et al.* 1972; Standfast, unpublished data) and three of Aino virus (Doherty *et al.* 1972) from that insect. Thus the evidence that *C. brevitarsis* is the important vector of Peaton virus is approximately the same as has been advanced in Australia for

the other Simbu group viruses, particularly for Akabane virus (Della-Porta *et al.* 1976; Cybinski *et al.* 1978a). To prove that *C. brevitarsis* is a vector of Peaton virus requires direct experimentation under laboratory conditions.

No evidence of the pathogenicity of Peaton virus is yet available from field or laboratory studies. Thus we have one more arbovirus 'in search of a disease'. Its status as a pathogen can be more readily assessed now a serological test is available. All precolostral samples collected from calves or lambs with congenital arthrogryphosis-hydranencephaly should be examined for antibody to all three of the Simbu group viruses now recognized in Australia to determine whether the new member is involved as a cause of this syndrome.

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