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Douglas and Tinaroo Viruses: Two Simbu Group Arboviruses Infecting *Culicoides brevitarsis* and Livestock in Australia

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Abstract

Two Australian members of the Simbu group, Douglas and Tinaroo viruses, were found to be distinct, by virus-neutralization tests, from three previously known Simbu group viruses isolated in Australia, namely Akabane, Aino and Peaton viruses. A low-titre, two-way, cross-reaction was noted between Akabane and Tinaroo viruses. Antibody to Tinaroo and Douglas viruses was detected in serum from cattle, buffalo, sheep, goats and deer but not in humans, pigs, kangaroos and wallabies. The results for horses were inconclusive. The distribution of antibodies to each virus falls mainly within the geographical distribution of the biting midge *Culicoides brevitarsis*, an insect from which each virus has been isolated.

Introduction

The family Bunyaviridae, to which the Simbu group belongs, contains more than 160 members and is therefore the largest known taxonomic subset of arboviruses (Bishop and Shope 1979). The Simbu group contains at least 20 viruses from Africa, Asia, Australia, South America and North America, two of which have been associated with naturally acquired or laboratory-acquired human infections (Oropouche and Shuni viruses; Bishop and Shope 1979). Another two members (Akabane and Aino viruses) have been associated with a disease of new-born calves in Japan and Australia, characterized by arthrogryposis/hydranencephaly (Kurogi *et al.* 1975; Coverdale *et al.* 1978, 1979).

The isolation of three additional Simbu group viruses in Australia—Peaton, Douglas and Tinaroo viruses—has been reported by St George *et al.* (1979) and Peaton virus has been discussed in detail by St George *et al.* (1980). Douglas and Tinaroo viruses were shown to be new to Australia (St George *et al.* 1979), and were then sent to the Yale Arbovirus Reference Centre for comparison with other arboviruses of the world. Both viruses were shown to be related to the Simbu group by complement fixation, but were shown by neutralization tests to be new to science (Kinney *et al.* 1981). This paper defines the distribution and host range of Douglas and Tinaroo viruses and shows serological relationships between five Australian members of the Simbu group of viruses.

Materials and Methods

Viruses

The type strain of Douglas virus (CSIRO 150) was isolated from bovine blood in baby hamster kidney (BHK21) cells (St George *et al.* 1979). The type strain of Tinaroo virus (CSIRO 153) was isolated from a pool of *C. brevitarsis* inoculated into BHK21 cells. Both viruses were passaged three times in BHK21 cells for use in neutralization tests. The other viruses used in the cross-neutralization tests were the B8935 strain of Akabane virus, the B7974 strain of Aino virus (Doherty *et al.* 1972) and the CSIRO 110 strain of Peaton virus (St George *et al.* 1980). The viruses were all grown in BHK21 cells.

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Antibody Surveys

Cattle sera used for antibody surveys were obtained from various parts of Australia between 1975 and 1983, and were in part from a sentinel herd scheme established and described in detail by St George (1980). Some cattle were bled only once, while others were bled weekly, monthly or quarterly. The locations of the herds are shown in Fig. 1. Eighty of these sera, comprising two blood samples from each of 40 New South Wales cattle, taken in November 1982 and June 1983, were tested for Akabane virus to investigate cross-reactivity between Akabane and Tinaroo viruses. Sera from other species were also collected when convenient, and only those sera were tested which were collected in areas where antibodies to Douglas and Tinaroo viruses were detected in cattle sera. The ages of the cattle were generally less than 1 year on the first bleeding, while the ages of the other species varied considerably.

Neutralization Tests

Antisera against Akabane, Aino, Peaton, Douglas and Tinaroo viruses were prepared in rabbits by the method described by Cybinski and St George (1982). The method used for neutralization testing was essentially the same as that described by St George *et al.* (1980) with the substitution of 100 50% tissue culture infective doses ($TCID_{50}$) of Douglas, Tinaroo, Akabane, Aino or Peaton virus. The virus–serum mixtures were incubated for 1 h at room temperature and Vero cells were used at a concentration of 3×10^5 cells/ml. Survey sera were diluted 1 in 4 for testing in duplicate while titrations were carried out using four wells per dilution. A positive serum was one which completely prevented the formation of cytopathic effect (CPE) in both the test wells at a 1 in 4 dilution although when only one well showed CPE, this was recorded as a trace. Titres were calculated by the method of Reed and Muench (1938).

Results

Cross-neutralization Tests

Dilutions of antisera against Akabane, Aino, Peaton, Douglas and Tinaroo viruses, were each tested against 100 $TCID_{50}$ of each virus. The results in Table 1 show that the viruses were distinct except for a low two-way cross-reaction between Tinaroo and Akabane viruses.

Table 1. Cross-neutralization titres for five Australian Simbu group viruses
Values given are the reciprocals of antiserum dilution that neutralized the virus in 50% of the wells. —, no reaction at 1 in 2, the minimum serum dilution tested

Virus	Rabbit antiserum				
	Akabane	Aino	Peaton	Douglas	Tinaroo
Akabane	**4096**	—	—	—	**16**
Aino	—	**2560**	—	—	—
Peaton	—	—	**32 768**	—	—
Douglas	—	—	—	**2455**	—
Tinaroo	**16**	—	—	—	**8192**

Serological Surveys

The distribution of cattle with antibody to Douglas virus and to Tinaroo virus is shown in Fig. 1. Both distributions lie approximately within the known range of *C. brevitarsis*, the only insect from which the viruses have so far been isolated. One animal in one South Australian herd had a serum in which antibody to Douglas virus was detected. There was a 100% conversion rate to Douglas virus in four herds, while 62% was the highest conversion rate noted for Tinaroo virus. In herds which were monitored regularly, neither virus infected cattle every year and no disease could be associated with either virus. Antibody conversions to both viruses occurred mainly in the summer months, although in at least one herd, Swans Lagoon, located in a dry part of the north Queensland coast,

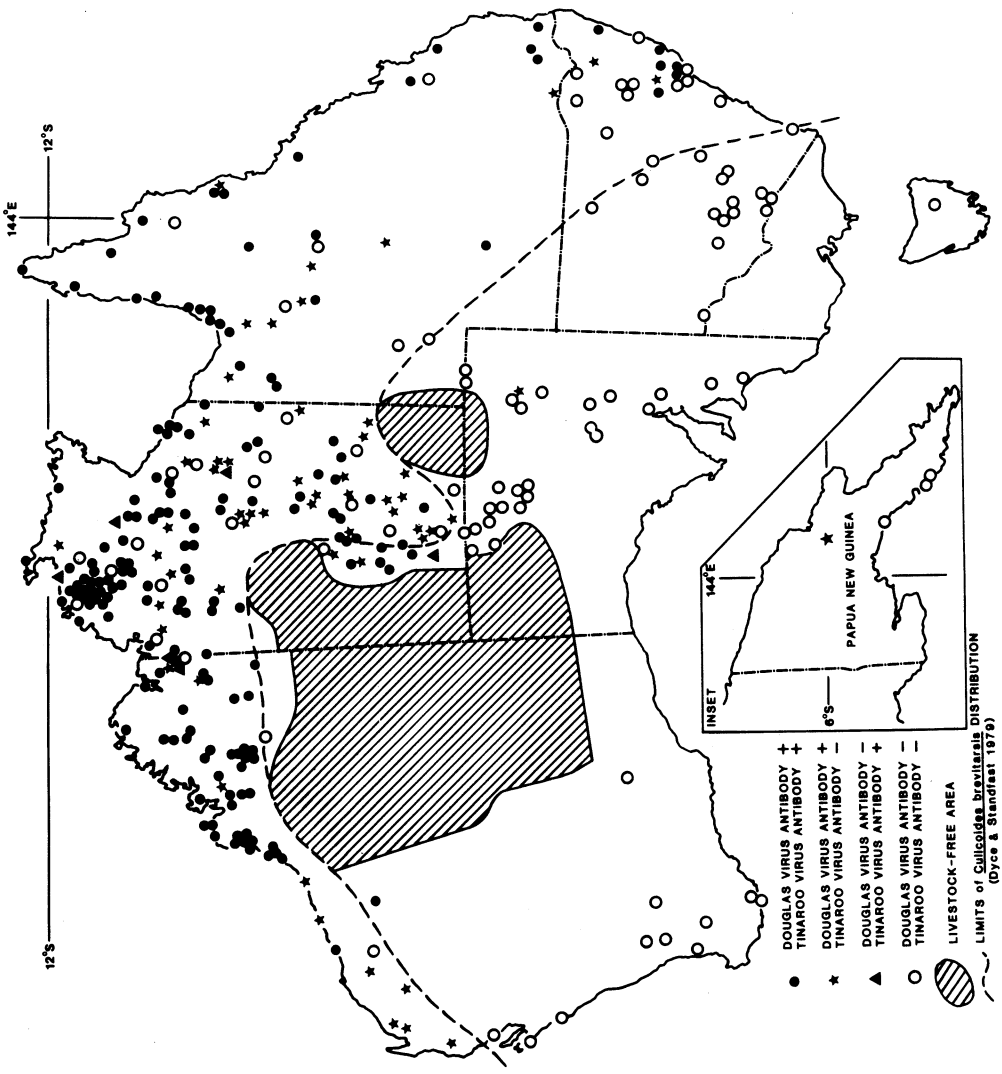

Fig. 1. Distribution of antibodies to Douglas and Tinaroo viruses in herds of Australian cattle shown in relation to the approximate southern limit of distribution of the biting midge *Culicoides brevitarsis*.

conversions to Douglas virus occurred during the winter months while conversions to Tinaroo occurred in the spring. Antibody titres ranged from 4 to 512 for both viruses.

The number of sera from cattle and other species which were positive for antibody to Douglas and Tinaroo viruses, together with the total number of sera tested, are listed in Table 2. One horse serum had an antibody titre of 8 to Douglas virus, while two horse sera had antibody titres of 16 and 6 respectively to Tinaroo virus.

Table 2. Antibodies against Douglas and Tinaroo viruses in serum from cattle and other species

Species	Douglas virus		Tinaroo virus	
	Total[A]	Percentage positive	Total[A]	Percentage positive
Cattle	1350/4755	28	705/5150	14
Buffalo	40/81	49	15/81	19
Sheep	7/116	6	11/119	9
Goat	3/54	6	2/30	7
Deer	140/396	35	195/432	45
Horse	1/125	1	2/115	2
Human	0/20	0	0/20	0
Pig	0/29	0	0/25	0
Kangaroo	0/11	0	0/36	0
Wallaby	0/54	0	0/44	0

[A] Numerator = number of animals with antibody to the virus; denominator = number of animals tested.

Forty cattle whose sera were negative for antibody to both Akabane and Tinaroo viruses in November 1982, developed antibody titres greater than 32 to Akabane virus during the following seven months. Five of the cattle also sero-converted to Tinaroo virus in the same time interval when tested at a 1 in 4 dilution, while another seven developed only traces of antibody to Tinaroo virus in their sera. On titration of the five positive sera, two had titres greater than 32 while the remaining three had titres less than 6.

Discussion

The surveys showed that antibodies to Douglas and Tinaroo viruses were found mainly in sera from the northern half of Australia (Fig. 1). In north-eastern South Australia, one animal in one herd was positive for Douglas virus, while no antibody to Tinaroo virus was detected in any sera from that State. In this remote area with extensive husbandry conditions there is no possibility of checking whether this animal was actually born in the herd where it was bled. In Queensland, the herds positive for antibodies were concentrated mainly in the north and east, although some central western herds were also positive. The only antibodies found in New South Wales cattle were in the north-eastern quarter of the State. In the Northern Territory, herds positive for Douglas and Tinaroo antibodies covered the whole of the State; however, the concentration was higher in the north. In Western Australia positive herds were again concentrated in the north, with some positive herds down the west coast. Antibodies to Douglas virus were detected in cattle sera from New Guinea. This is in contrast to all other Australian Simbu group viruses for which no antibodies have yet been detected in that country (Cybinski and St George 1978; Cybinski *et al.* 1978; St George *et al.* 1980).

Antibodies to Douglas and Tinaroo viruses were found in sera from cattle, buffalo, sheep, goats and deer, while sera from humans, pigs, kangaroos and wallabies were negative. Sera from species other than cattle were only tested for this study if they came from areas where antibodies to Douglas and Tinaroo viruses were present in cattle sera. Some difficulty

was experienced in finding sufficient numbers of these sera from some species and the testing of additional sera in the future, as they become available, would give more conclusive results for certain species which were antibody-negative. The prevalance of antibodies to both viruses in serum from horses was low (1 out of 125 for Douglas virus and 2 out of 115 for Tinaroo virus) and the antibody titres were also low (6–16). Therefore the possibility cannot be ruled out that these are cross-reactions with other unknown Simbu group viruses rather than homologous reactions. Akabane and Peaton viruses are known to infect horses (St George *et al.* 1979), so it is not unlikely that other Simbu group viruses may also do so.

There was no evidence in this study that Douglas or Tinaroo viruses cause disease in adult animals. However, antibody to Tinaroo virus has been found in a lamb with arthrogryposis, which did not have antibodies to the other Simbu group viruses, Akabane, Aino, Peaton and Douglas viruses (St George, personal communication). Both Douglas and Tinaroo viruses have been shown to cause death and deformities in experimentally inoculated chicken embryos (McPhee *et al.* 1982).

In the cross-neutralization tests (Table 1) it was shown that although the five Australian Simbu Group viruses are distinct from one another, some antigenic overlap occurred between Tinaroo and Akabane viruses which showed a low two-way cross-reaction. This cross-reaction could cause problems when testing undiluted serum for antibodies to Tinaroo virus, as any antibody detected could be due to Akabane virus. Titrations performed for both cross-reacting viruses would eliminate one or the other as the cause. A study by McPhee and Della-Porta (1981) reported a much greater degree of cross-reactivity between seven Simbu group viruses tested by plaque inhibition. These results indicate that the microtitre virus-neutralization test is more specific than the plaque-inhibition, virus-neutralization test, for five Simbu group viruses at least.

Viruses from other parts of the world also show an antigenic overlap with Australian Simbu group viruses. Yaba 7, Shamonda and Sabo viruses cross-react with Akabane virus; Sabo and Sango viruses cross-react strongly with Peaton virus; Kaikalur and Shuni viruses cross-react strongly with Aino virus; Sabo virus cross-reacts with Tinaroo virus; and Douglas virus cross-reacts with Sathuperi virus (Kinney *et al.* 1981). These viruses, if they occur in Australia, would cause further problems in antibody testing and a search for other Simbu group viruses in Australia would therefore be advantageous.

Antibody titres in the field are rarely as high as those of the hyperimmune rabbit sera shown in Table 1 and the heterologous titres would therefore be expected to be less than the dilution at which the sera are tested, namely 1 in 4. Of a sample of 40 sera tested for antibodies to both Akabane and Tinaroo viruses, 100% seroconverted to Akabane virus. Only 5% definitely seroconverted to Tinaroo virus, while 25% of sera had either a trace (17·5%) or low levels (7·5%) of antibody to Tinaroo virus. The low titres and trace reactions in this instance probably represent cross-reactions with Akabane antibodies. This cross-reactivity is less of a problem when testing for antibody against Akabane virus, as the incidence of this virus is much higher than that of Tinaroo virus—50% (St George *et al.* 1979) as compared to 14% (Table 2). The problem is overcome in this laboratory by monitoring antibodies in cattle sera over a period of several months or more, and transient, low-level antibody results are considered to be due to cross-reactivity rather than specific infection. It is not known how accurately the cross-reactivity with rabbit antiserum reflects the cross-reactivity in naturally infected cattle. Nor is it known to what extent this cross-reactivity is influenced by previous infections with related viruses.

Thimiri and Facey's Paddock viruses, which are only distantly related to the other known Simbu group viruses of Australia by complement fixation (Kinney *et al.* 1981; McPhee and Della-Porta 1981), are also found in Australia (Bishop and Shope 1979; Standfast and Dyce 1982). However, antibodies to these viruses have not been found in domestic animals (St George *et al.* 1979; St George, personal communication) and they have therefore not been included in this study.

Five isolations of Douglas virus have been made in Queensland in addition to the type strain; two from *C. brevitarsis* collected at Peachester (152°53′E.,26°51′S.), two from *C. brevitarsis* collected at Kairi (145°33′E.,17°13′S.) and one from bovine blood also collected at Kairi (St George and Cybinski, unpublished data).

Six further isolations of Tinaroo virus have been made in addition to the type strain; three from *C. brevitarsis* collected at Kairi, one from *C. brevitarsis* collected at Peachester (St George and Cybinski, unpublished data), and two from bovine blood collected at Tortilla Flat in the Northern Territory (131°08′E.,13°13′S.) (Gard and Cybinski, unpublished data). Thus there is direct evidence that virus infection occurs on widely separated locations within the area delineated by the antibody survey.

The distribution of Douglas and Tinaroo viruses corresponds closely to that of *C. brevitarsis* (Fig. 1), the only insect species from which Tinaroo and Douglas viruses have been isolated, and according to the standards set up by the World Health Organization (Anon 1967), *C. brevitarsis* is considered to be a suspected vector of these viruses. However, taking into account (1) the multiple isolations from both *C. brevitarsis* and from cattle, (2) the fact that the viruses have not been isolated from any other insect, (3) the close association of the biology of *C. brevitarsis* with cattle (Muller *et al.* 1982), and (4) the close correlation between the distribution of Douglas and Tinaroo viruses and that of *C. brevitarsis*, there is sufficient evidence to consider *C. brevitarsis* as the major vector of these viruses in Australia, although not necessarily the only vector.

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