ARBOVIRUS RESEARCH IN THE BRAZILIAN AMAZON

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Studies on arboviruses in the Brazilian Amazon were initiated in 1954 as a joint project with the Servico Especial de Saude Publica, at the Instituto Evandro Chagas, Belem, Para, and the Rockefeller Foundation (Causey *et al* 1961). In the first 9 years this work was carried out under the direction of Dr Ottis Causey, with the assistance of his wife, Dr Calixta Causey. Later, other Brazilian and foreign organisations have contributed in terms of personnel and/or funds, towards this continuing success of the program (Woodall 1967; Pinheiro *et al* 1986).

STUDY AREAS AND GENERAL RESULTS

For 21 years (1954-1975), the Embrapa and Utinga forests, situated close to Belem, were studied, using multiple methods of detection for arboviruses. Oriboca forest, about 35 km to the east of Belem, was also explored in the early years of the study. This led to the isolation of over 50 arbovirus serotypes. In the early 1960's, the studies were extended to the Belem - Brasilia highway and 15 new virus types were discovered. In 1964 investigations were initiated in Serra do Navio, in the Central part of Amapa state where the first Brazilian arenavirus - Amapari - was isolated (Pinheiro *et al* 1966), along with other new agents, Serra do Navio and Araguari.

Since 1971, studies have been carried out in areas along the Transamazonica (Pinheiro *et al* 1974) and Santarem - Cuiabá highways. Pilot studies conducted in 12 of these areas revealed the existence of 41 new types of arboviruses. Five of these, derived from inoculation of materials collected from 1976 to 1978 at Sena Madureira, Acre state, were arboviruses new to the world. Virus isolation attempts in the area of Jari were made in 1980/81. Seven new arboviruses were isolated (Travassos da Rosa *et al* 1983, 1984).

In 1982 new areas were selected for study, which were associated with the construction of the hydroelectric dam in Tucurui, on the Tocantins river and the site of development of the important industrial complex in the large region which surrounds the areas of Serra Norte and Serra Sul and the domain along Carajas railway,in Maraba. Thirty-one arbovirus types from these new sites have already been identified. These are new to Brazil and at least 2 of them new to the world (Travassos da Rosa *et al* 1986).

In 1988, studies were conducted in Balbina, Amazonas state, and Samuel, Rondonia state, where new hydroelectric dams have been constructed. Two new viruses were isolated from material collected in Balbina.

ARBOVIRUSES TRANSMITTED IN THE BRAZILIAN AMAZON

Between November 1954 and December 1988, almost 9,000 isolations of at least 162 different virus types have been made (Fig 1). Human cases, wild and sentinel animals, and arthropods have been consistent sources of viruses. The use of sentinel mice was introduced in 1958, and continued until October 1975, proved to be the most efficient for surveillance of activity of these viral agents. Of these arboviruses, 137 were first isolated in Brazil, and 84 of them have been confirmed to be new to the world. The 53 remaining can be considered provisionally new, because their characterisation at the International Reference Center is still pending (Travassos da Rosa *et al* 1986 and unpublished data).

The arboviruses found in the Amazon are distributed in 20 serological groups containing 134 different serotypes. The remaining viruses are ungrouped. The Changuinola group, with 42 serotypes, is the largest in number, followed by the Phlebotomus fever group, with 18 types, of which 3 are pathogenic to man; and groups Bunyamwera and C, with 8 members each. The remaining groups, A, B, Guama, Capim, California, Anopheles A, Simbu, Turlock, Gamboa, VSV, Timbo, Mossuril, Kwatta, Hart Park, Tacaribe and Corriparta, contain 7 or fewer agents. Three groups - C, Guama and Capim - were initially formed of viruses exclusively from the Brazilian Amazon, but later, arboviruses from other regions of the Americas were discovered and incorporated into these groups.

Based on their physico-chemical properties, the arboviruses of the Brazilian Amazon are distributed into 5 families. Bunyaviridae, Reoviridae, Rhabdoviridae, Togaviridae, and Flaviviridae. Agents are also recognised in the families Arenaviridae, Poxviridae, Herpesviridae and Paramyxoviridae. 63 serotypes belong to the Bunyaviridae, 47 to the Reoviridae, 15 to the Rhabdoviridae, 8 to the Togaviridae

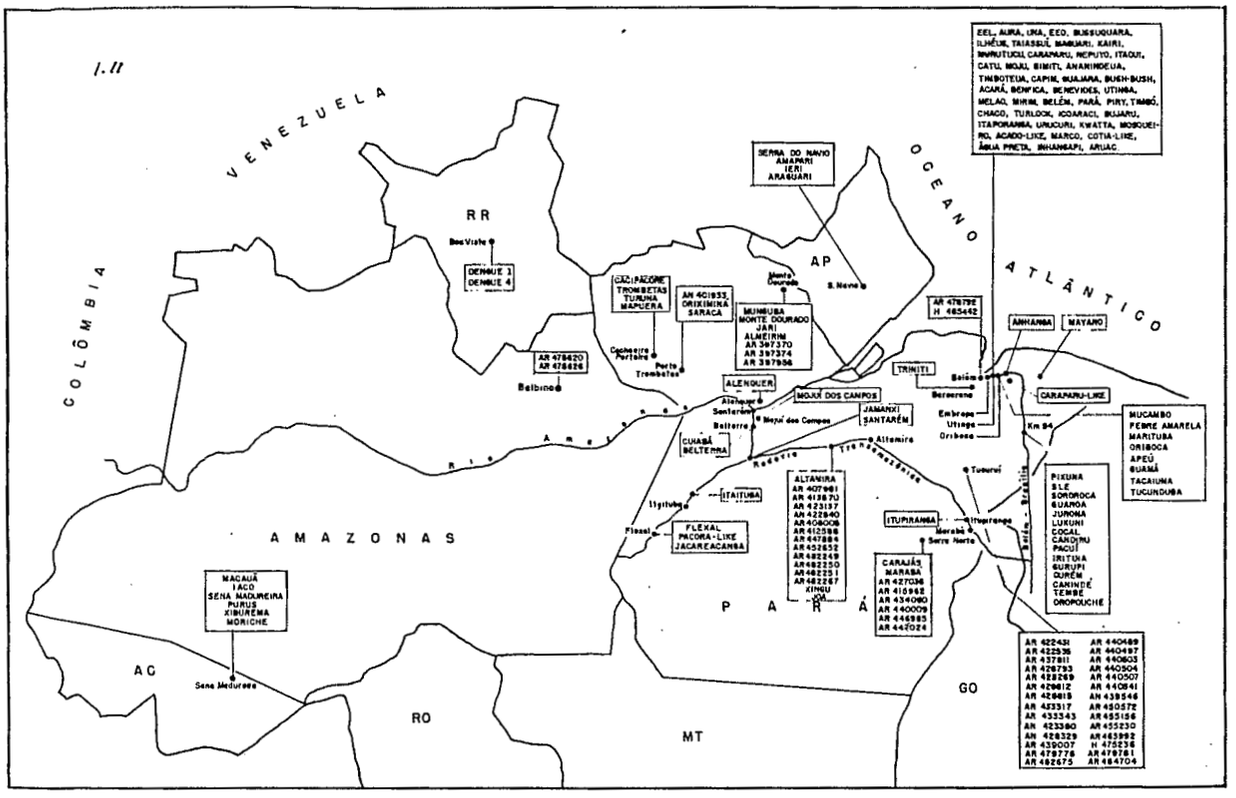

Figure 1. Map of Brazilian Amazonia showing the places of initial isolation of the 162 types of arboviruses and some other viruses of vertebrates.

and 7 to the Flaviviridae. There are also 2 members belonging to Arenaviridae and 1 each to Poxviridae, Herpesviridae and Paramyxoviridae. The remaining 17 have not been assigned to any taxonomic group.

The general pattern of annual rates of these isolations is shown in Figure 2. Although a slow decrease is obvious during the period of study, some periods were more productive than others, for example, 1967-70, 1975, 1978-80, 1984-85 and 1988. The greater number of strains isolated during the first half of the period (1954-75) was probably due to the use of sentinel mice. Conversely, the isolations of new viral types did not follow the same pattern, most strains being isolated only during the first 4 and last 13 years. This was probably due to the very low proportion of new viral types isolated from sentinel mice (5/137, or 3.6%).

ARBOVIRUSES PATHOGENIC TO MAN

Thirty of the 162 agents are known to be pathogenic to man, causing fever, fever with rash, haemorrhagic disease and, possibly, encephalitis (Table 1). Twenty-five of them have been

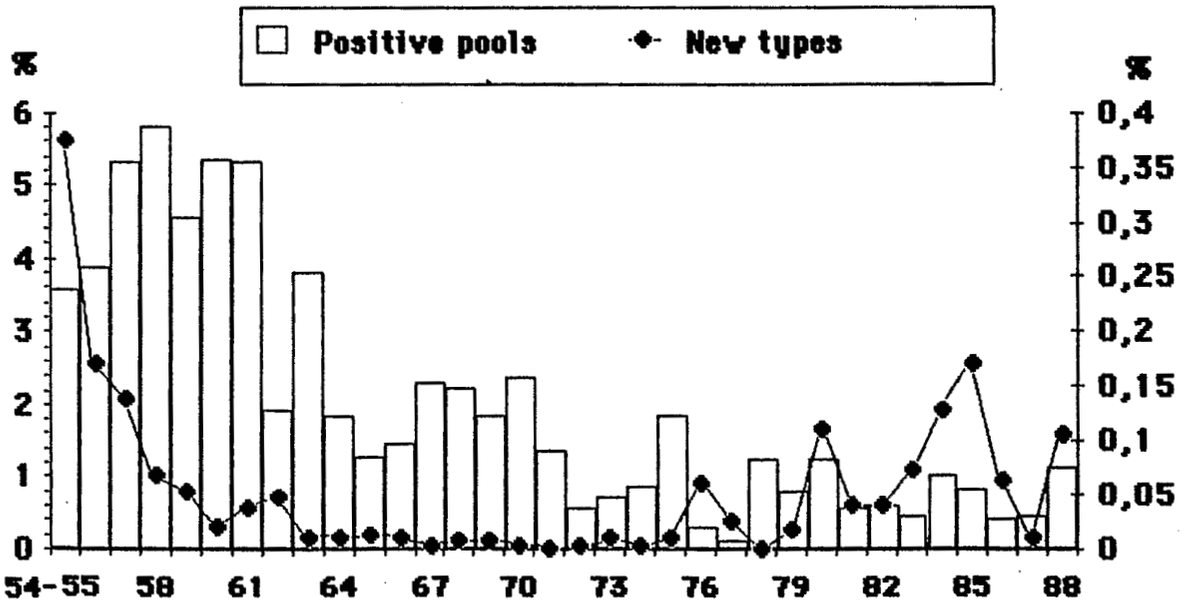

Figure 2. Annual rates (No. positive pools/total inoculated x 100) of isolations of arbovirus strains (left scale) and new serotypes (right scale) in Brazilian Amazonia, 1954-88.

TABLE 1

Pathogenic arboviruses for man in the Amazonian region, 1954–1988

Type of disease	*Number of viruses*	*Antigenic group*	*Virus isolation**	*Serology*
Febrile disease	23	A	Mucambo, Pixuna†	
		B	Ilheus, Bussuquara	
		Bunyamwera	Tucunduba, Xingu	
		C	Apeu, Caraparu, Caraparu-like, Marituba, Murutucu, Oriboca and Itaqui	
		Guama	Catu, Guama	
		California	Guaroa	
		Anoph. A	Tacaiuma	
		Simbu	Oropouche	
		Phlebotomus	Alenquer, Candiru, Morumbi	
		VSV	Piry †	
		Tacaribe	Flexal †, ‡	
Fever with rash	3	A	Mayaro	
		B	Dengue 1, 4	
Hemorrhagic fever	1	B	Yellow fever	
Encephalitis	3	A		EEE, WEE
		B	SLE	
TOTAL	30		28	2

* Antibodies for the majority of viruses have been found in human residents of Brazilian Amazonia.
† Laboratorial infections
‡ Arenavirus, not an arbovirus.

isolated from natural infections and 3 from laboratory infections. Of the 3 arboviruses responsible for encephalitis (EEE, WEE & SLE), only SLE has been isolated twice from persons in the region with no signs of encephalitis. Antibodies to these agents have been found at low rates in the majority of the towns in Para state (Pinheiro 1982). Of these 30 arboviruses hazardous to man, we give emphasis only to 4 of them - YF, Mayaro, Oropouche and DEN - because of their public health importance.

YF is the only arboviral haemorrhagic disease found in the Amazon region, where only the sylvatic cycle is known to occur. From November 1954 to December 1988, 300 infections of YF have been diagnosed at IEC by isolation, histopathology and/or serology from humans, wild animals and arthropods. The maintenance of amarilic virus in the Brazilian Amazon forest is primarily by transmission among primate populations. This involves bites by infected sylvatic mosquitoes, especially those belonging to Haemagogus genus (Herve *et al* 1985). Thus, persons at risk are those who enter the forest to collect nuts, tap rubber trees or fell trees for timber. Human cases of YF have been recorded either sporadically or in outbreaks. In most of the cases, the infections are relatively benign or unapparent. The overall mortality is about 5 to 10%. However, among the more severe, hospitalised cases (classic clinical picture with haemorrhage, jaundice and albuminuria), mortality may reach 40 to 50%.

Mayaro Fever - Serological studies have shown that this virus is widespread in rural communities of the Brazilian Amazon. Two epidemics have been described among persons with close contact with the forest. The first one was in a small settlement of the Guama river in 1955, during which about 50 persons were infected. In the second, which occurred in Belterra near the confluence of both the Tapajos and Amazon rivers, some 800 persons, of a population of 4,000, were affected by this virus and exhibited intense arthralgia that affected predominantly the joints of the extremities (Pinheiro *et al* 1986). On the basis of available data, it could be postulated that the principal cycle of maintenance of Mayaro virus is similar to that of the jungle YF (Haemagogus-monkey). There is, however, the possibility of secondary cycles involving different hosts (Herve *et al* 1986).

Oropouche Fever (ORO) - caused by Oropouche virus and has been recognised as a major cause of human febrile illness in the Brazilian Amazon. Between 1961 and 1980 several outbreaks occurred in urban centres of Para state, in the eastern part of Amazon. It is estimated that at least 165,000 persons were infected. From May 1980 to February 1981, the first outbreaks of ORO were recorded in Amazonas state (Manaus and Barcelos). Nearly 97,000 out of the 650,000 inhabitants of Manaus were infected (Borborema *et al* 1982).

Based on serological studies, we were also able to detect, in 1980, an outbreak of ORO in Mazagao, a town in Amapá state. More recently, in 1988, 2 other outbreaks were studied in Maranhao state (Porto Franco) and Goiás state (Tocantinopolis) (Vasconcelos *et al* 1989).

Although some patients showed meningitis - like symptoms (Pinheiro *et al* 1981), neither death nor sequelae can be attributed to this viral infection in the Brazilian Amazon. The ORO virus occurs in 2 distinct cycles - urban and sylvatic. It is conceivable that the urban cycle involves a man-to-man transmission through *Culicoides paraensis*, while the sylvatic cycle may involve certain species of primates, sloths and wild birds which may act as vertebrate hosts for the virus (Pinheiro *et al* 1986). Little is known, however, on the forest vector(s) of ORO virus (Herve *et al* 1986).

DEN - The occurrence of an outbreak of DEN in Boa Vista, Roraima, the northern most part of Brazil, in late 1981 and early 1982 was the first recorded infection of DEN in Brazil. Serotypes 1 and 4 were found to be the causative agents of this outbreak. A total of 13 strains were isolated from patients and 3 others were recovered from *Ae aegypti* captured in the city (Travassos da Rosa *et al* 1982). The incidence was 22.6%, corresponding to 11,000 persons infected (Osanai *et al* 1983). Later, in 1986 and 1987, extensive outbreaks caused by DEN-1 occurred in Rio de Janeiro (Schatzmayr *et al* 1986), Ceara and Alagoas states (Houly *et al* 1988), with millions of infected persons. Although not included in the presently considered period of study, the recent isolation of DEN-2 virus from an imported case merits reporting here as an important factor in the possible appearance of DHF in Brazil and South America (Travassos da Rosa *et al* 1989).

ARBOVIRUSES OF UNKNOWN PATHOGENICITY TO MAN

The arboviruses of unknown pathogenicity to man constitute the majority of arboviruses in the Amazon region. It is difficult to appraise the importance of such agents as human pathogens. However, recent and progressive ecological changes due to large projects in Amazonia may lead man to a more intimate contact with the forest and, consequently, to a more frequent exposure to potential pathogenic arboviruses which circulate in the jungle.

SUMMARY AND CONCLUSIONS

Arbovirus studies conducted in many areas of the Brazilian Amazon region over the past 35 years, revealed the presence of a large number of agents. Thousands of virus strains were isolated from many sources, including man, comprising 162 distinct serological types. Thirty of these serotypes are known to cause human illness, of which YF, Mayaro, Oropouche and DEN viruses are those of greater importance in public health. These results have shown that the arboviruses of the Amazon region are focal, with transmission cycles intimately related to natural vectors and vertebrate hosts. Moreover, many areas of this vast region remain to be explored for the presence of arboviruses, particularly those pathogenic to man and domestic animals. The rapid colonisation of the Amazon region and the consequent increase of human populations, many of which are in constant contact with forest areas, together with environmental changes in the region, raises the concern that this may lead to an exacerbation of arboviruses hazardous to man. Thus, it is important to continue to expand the study of arboviruses in the Amazon region, especially to assess their impact on human health.

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REFERENCES

Borborema CAT, Pinheiro FP, Albuquerque BC, Travassos da Rosa APA, Travassos da Rosa JFS and Dourado HV (1982) Primeiro registro de epidemias causadas pelo virus Oropouche no Estado do Amazonas. Rev Inst Med Trop Sao Paulo 24: 132 - 139.

Causey OR, Causey CE, Maroja OM and Macedo DG (1961) The isolation of arthropod borne viruses, including members of 2 hitherto undescribed serological groups in the Amazon region of Brazil. Am J Trop Med Hyg 10: 227-249.

Herve JP, Degallier N, Travassos da Rosa APA and Sa Filho GC (1985) A febre amarela silvestre no Brasil e os riscos de propagacao urbana. Hileia Medica, Univ Fed Para 7: 31-40.

Herve JP, Degallier N, Travassos da Rosa APA, Pinheiro FP and Sa Filho GC (1986) Arboviruses - Aspectos Ecologicos. In: Instituto Evandro Chagas, 50 Anos de Contribuicao as Ciencias Biologicas e a Medicina Tropical 1: 409-437.

Houly CAP, Ramos-dos-Santos Eliziario L, Oliveira FM, deNeves-dos-Santos M, Simoes AT, Vergetti G, Santa Cruz LAM and Travassos da Rosa APA (1988) Seroepidemiological study of an epidemic of Dengue in Delmiro Gouveia, Alagoas, Brazil. In: 4o. Encontro Nacional de Virologia, 26 a 30 de outubro de 1988, Hotel Primus, Sao Lourenco, MG, Soc. Brasil. Virol., Resumos, p. 40.

Osanai CH, Travassos da Rosa APA, Tang AT, Amaral RS, Passos ADC and Tauil PI (1983) Surto de Dengue em Boa Vista, Roraima. Nota Previa Rev Inst Med Trop Sao Paulo 25: 53-54.

Pinheiro FP, Shope RE, Paes de Andrade AH, Bensabath G, Cacios CV and Casals J (1966) Amapari, a new virus of the Tacaribe group from rodents and mites of Amapa Territory, Brazil. Proc Soc Exp Biol Med 122: 531-535.

Pinheiro FP, Bensabath G, Paes de Andrade AH, Lins ZC, Fraiha H, Tang AT, Lainson R, Shaw JJ and Azevedo MC (1974) Infectious diseases along Brazil's Trans Amazon highway: surveillance and research. Bull Pan Am Health Organ 8 : 11-122.

Pinheiro FP, Rocha AG, Freitas RB, Ohana BA, Travassos da Rosa APA, Rogerio JS and Linhares AC (1981) Meningite associada as infeccoes por virus Oropouche Rev Inst Med Trop Sao Paulo 24: 246 - 251.

Pinheiro FP (1982) Situacao das arboviroses na regiao amazonica. In: Simposio inernacional sobre arbovirus dos tropicos e febres hemorragicas, Belem, 1980. Rio de Janeiro, Academia Brasileira de Ciencias, p. 24-48.

Pinheiro FP, Travassos da Rosa APA, Freitas RB, Travassos da Rosa, JFS and Vasconcelos PFC (1986) Arboviroses - Aspectos clinico-epidemiologicos. In: Instituto Evandro Chagas, 50 anos de Contribuicao as Ciencias Biologicas e a Medicina Tropical 1: 375-408.

Schatzmayr HG, Nogueira RMR and Travassos da Rosa APA (1986) An outbreak of Dengue virus at Rio de Janeiro 1986 Mem Inst Oswaldo Cruz 81: 245-246.

Travassos da Rosa APA, Rocha JM, Silva OV and Lins ZC (1982) Surto de Dengue em Boa Vista, Territorio de Roraima Brasil. Boletim Epidemiologico da Fundacao SESP 14: 93-100.

Travassos da Rosa APA, Tesh RB, Pinheiro FP, Travassos da Rosa JFS and Peterson NE (1983) Characterization of eight new phlebotomus fever serogroup arboviruses (Bunyaviridae - Phlebovirus) from the Amazon region of Brazil. Am J Trop Med Hyg 32: 1164-1171.

Travassos da Rosa APA, Tesh RB, Pinheiro FP, Travassos da Rosa JFS, Peralta PH and Knudson DL (1984a) Characterization of the Changuinola serogroup viruses (Reoviridae: Orbivirus). Intervirology 21: 38-49.

Travassos da Rosa APA, Tesh RB, Travassos da Rosa JFS, Herve JP and Main AJ (1984b) Carajas and Maraba viruses, 2 new vesiculoviruses isolated from Phlebotominae sandflies in Brazil. Am J Trop Med Hyg 33: 999-1006.

Travassos da Rosa APA, Shope RE, Travassos da Rosa JFS and Vasconcelos PFC (1986) Arboviroses - aspectos virologicos. In Instituto Evandro Chagas, 50 anos de Contribuicao as Ciencias Biologicas e a Medicina Tropical 1: 365-373.

Travassos da Rosa APA, Vasconcelos PFC, Travassos da Rosa JFS and Guerreiro SC (1989) Primeiro isolamento do virus Dengue 2 no Brasil a partir de um caso oriundo de Luanda, Angola. In: I Encontro Regional Sul de Virologia - Virologica 89, Florianopolis, SC, 9 a 13 de outubro de 1989, Univ Fed Santa Catarina, in press.

Vasconcelos PFC, Travassos da Rosa JFS, Guerreiro SC, Degallier N and Travassos da Rosa APA (1989) Primeiro registro de epidemias causadas pelo virus Oropouche nos estados do Maranhao e Goias, Brasil. Rev Inst Med Trop Sao Paulo 31: 271-278.

Woodall JP (1967) Virus research in Amazonia. In: Lent, H ed Atas do Simposio sobre Biota Amazonica, Rio de Janeiro, Conselho Nacional de Pesquisa, p 51-63.

SEROPREVALENCE AND PATHOGENICITY OF CERTAIN AUSTRALIAN BUNYAVIRUSES

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ABSTRACT

A seroepidemiological study was carried out on 4176 human sera collected from all regions of NSW for the presence of antibodies to 9 Australian bunyaviruses viz AINO, AKA, BEL, GG, KOW, MAP, PEA, TIN, TRU and the orbivirus COR. Antibodies were found in titres up to 1280 to GG and to 640 to TRU viruses with prevalences of 4.7% and 2.1% respectively. Antibody prevalences to GG were highest in samples drawn from the north western plains zone; rates were 18.6% and 11.1% in males and females respectively. Antibody titres up to 40 were found to BEL, AINO, PEA and COR viruses; the significance of these low titres is uncertain. No antibodies were found to AKA, KOW, MAP or TIN, viruses in these NSW sera.

GG virus appeared to be pathogenic for man being associated with a self limiting acute epidemic polyarthritis like illness. TRU virus is suspected of being pathogenic but further observations are needed. This is the first report of the pathogenicity of these 2 Australian bunyaviruses.

CLINICAL AND SUB-CLINICAL BARMAH FOREST VIRUS INFECTION IN QUEENSLAND

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Many of the arboviruses found in Australia are not known to be associated with disease (Karabatsos 1985). In 1986, Vale *et al* (1986) provided the first serological evidence of human infection with an alphavirus known as BF virus. Hawkes *et al* (1987) subsequently detected antibodies to BF virus in blood donors from throughout NSW and this group also identified 3 patients with clinical BF virus infections (Boughton *et al* 1988).