

Ross River Virus Transmission, Infection, and Disease: a Cross-Disciplinary Review

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INTRODUCTION

Ross River virus (RRV) is a mosquito-transmitted *Alphavirus* that is endemic and enzootic in Australia and Papua New Guinea (59, 85, 92, 167, 179). It caused a large epidemic in 1979 and 1980 involving Fiji, New Caledonia, Samoa, and the Cook Islands (4, 56, 95, 123, 130, 162, 197). RRV causes the most common arboviral disease in Australia, a characteristic syndrome of constitutional effects, rash, and rheumatic

manifestations (60, 66, 122). Joint pain and disability may persist for several months (78). The number of reported cases in Australia during 1991 to 2000 averaged 4,745 per year, peaking at 7,823 in 1996 (Communicable Diseases Network—Australia New Zealand, <http://www.health.gov.au/pubhlth/cdi/nndss/year002.htm>).

The syndrome caused by RRV was initially referred to as epidemic polyarthritis (53, 95). Subsequently, the term Ross River fever has appeared and is commonly used by the public (130). More recently, the term RRV disease has come into use (59). The latter term was proposed by Marshall and Miles (130), then supported by Fraser (60), and is justified because not all people with symptomatic infection present with fever or

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joint pain (59): 20 to 60% of patients have the former, while 83 to 98% have the latter (33, 60, 82, 144, 181, 194, 218). An additional problem is that epidemic polyarthritides includes disease caused by both RRV and Barmah Forest virus (BFV) (124). Here we use RRV disease to refer to symptomatic infections and RRV infection to include both symptomatic and asymptomatic infections.

This article discusses all aspects of the biology of RRV, with an emphasis on human infection and disease. First, the biology of arboviruses is reviewed. Historical aspects of RRV research, and the structure of the virus and its relation to other alphaviruses are then discussed. Then immune response, pathogenesis, and laboratory diagnosis are discussed, followed by a section on RRV disease. Knowledge of vertebrate reservoir hosts and mosquito vectors is then considered, followed by sections dealing with the epidemiology and public health significance of RRV disease. The final section synthesizes the information to draw conclusions regarding the significance of RRV disease and directions for future research.

ARBOVIRUSES—IMPORTANT CONCEPTS

Definition

Arboviruses are “maintained in nature principally, or to an important extent, through biological transmission between susceptible vertebrate hosts by haematophagous arthropods; they multiply and produce viraemia in the vertebrates, multiply in the tissues of the arthropods, and are passed on to new vertebrates by the bites of arthropods after a period of extrinsic incubation” (226).

Vertebrate Reservoir Hosts

Most medically important arboviruses are transmitted to humans from other vertebrate species. To be an important reservoir for human infection, an animal must fulfill the criteria set forth in reference 178: “The host is present in large numbers and is readily accessible to vectors in time and space. The host is attractive to arthropod vectors and allows vectors to feed upon it. The host is susceptible to virus infection, experiences low mortality from infection, and becomes viremic with a titer of sufficient magnitude and duration to infect susceptible blood-feeding vectors. The life history of the host proceeds in such a way that immunologically susceptible individuals enter the population at times of active transmission. Host herd immunity remains low.”

To establish that a vertebrate is a reservoir host, the best evidence is frequent virus isolation from wild vertebrates. This is seldom possible because viremia is usually short-lived. Consequently, antibody prevalence is frequently used as an indicator of the prevalence of past infection. However, laboratory studies of infection are needed to confirm the role of a vertebrate as a reservoir host, because this is the only means of establishing the level and duration of viremia (178).

Vector Competence

Four criteria establish the vector competence of a mosquito species (193): (i) isolation of the disease-producing agent from wild-caught specimens, (ii) demonstration of its ability to become infected by feeding upon a viremic host, (iii) demonstra-

tion of its ability to transmit by bite, and (iv) field evidence confirming association of the infected arthropod with the vertebrate population in which the infection is occurring.

The overall risk for arbovirus disease in human populations is determined first by the presence of virus and vectors and second by the vector competence of the virus-vector system. Therefore, vector competence is central to the epidemiology of human arbovirus diseases.

EARLY OUTBREAKS AND DISCOVERY OF RRV

In 1928 there were two reports of an “unusual epidemic” in Narrandera and Hay in New South Wales (54, 148) (Fig. 1). These are now thought to have been due to RRV. In Hay, Nimmo (148) described a syndrome of “pain, skin eruption and general manifestations” in which “painful swelling of the joints” predominated. Fifteen years later, during the Second World War, several outbreaks of arthralgia and arthritis were described in the Northern Territory, Queensland, and the Schouten Islands off the northern coast of Papua New Guinea (53, 72, 76, 80, 186, 214) (Fig. 1). In 1946, Dowling (53) applied the name epidemic polyarthritides to the syndrome.

An epidemic occurred in the Murray Valley (Fig. 1) in 1956, with a rash, usually maculopapular, being common (36 of 36 [10], 15 of 16 [70], and 45 of 45 [222]) and joint pains less so (23 of 35 had joint or muscle pains [10], 12 of 16 had joint and limb pains [70], and slightly more than one-third of 45 complained of joint pains [222]). Shope and Anderson (184) noted the similarity of the outbreak to an outbreak of “acute virus polyarthritides” caused by Chikungunya virus in Tanganyika in 1952 (159, 163). Differences were also noted, particularly the sudden onset of severe arthritis and fever followed by rash in the African outbreak, contrasted with the gradual onset of mild symptoms in the Australian outbreak, with fever rarely noted and mild (<38.3°C) when present (184). Because Chikungunya was known to be a group A arbovirus (now the genus *Alphavirus*), Shope and Anderson (184) sought serologic evidence that a group A arbovirus might be the cause of epidemic polyarthritides in Australia. Sixteen pairs of acute and convalescent sera were tested, and six showed antibody to one or more of 10 group A arboviruses. On hemagglutination inhibition (HI) testing, two patient’s sera showed diagnostic rises in titer to Semliki Forest virus (SF), one to AMM2354 (now Bebaru) virus, and one to both viruses. Neutralization (N) testing for AMM2354 and AMM2021 (now Getah) viruses gave negative results for all acute-phase sera, but four convalescent-phase sera showed definite evidence of antibodies against one or both viruses. Complement fixation (CF) testing of two pairs of sera was negative for all viruses except Getah virus, against which convalescent sera fixed complement. On the basis of this evidence, Shope and Anderson (184) suggested that the cause of epidemic polyarthritides was an unknown group A arbovirus.

Next, Doherty et al. (51) isolated a group A arbovirus from *Aedes vigilax* mosquitoes trapped beside Ross River in Townsville, North Queensland (Fig. 1), in 1959, using intracerebral (IC) inoculation of 1- to 2-day-old mice. They tested sera from 20 patients with epidemic polyarthritides for antibodies against the virus, designated T48 (T for Townsville and 48 for the mosquito pool number [R. L. Doherty, personal communication, 2000]) as well as three other group A arboviruses (Sind-

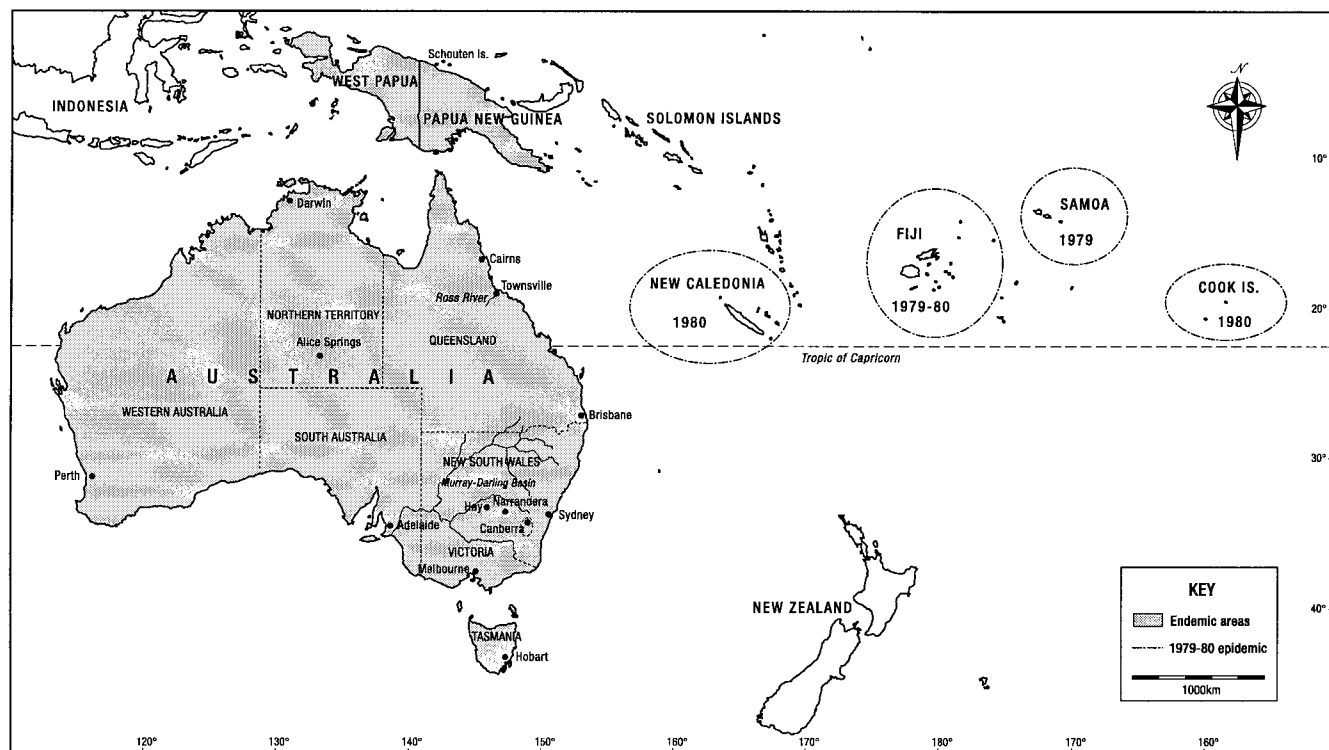


FIG. 1. Map showing cities, towns, and geographical features discussed in the text, areas where RRV is endemic, and the 1979 to 1980 South Pacific epidemic of RRV disease.

bis, Getah, and Bebaru). All 20 patients had significant changes in titer of HI and/or CF antibodies to T48. Antibody titer to T48 was at least equal to that against the other viruses, and in most cases was higher. Seven patients had no significant antibody response to Getah or Bebaru virus. It was hypothesized that the virus causing epidemic polyarthritis was related to, or was, T48 (51). The link between T48 and epidemic polyarthritis was further strengthened when Doherty et al. (47) demonstrated serologic evidence of T48 infection among epidemic polyarthritis patients in Queensland. Subsequently, the name Ross River virus was applied, and T48 became the type strain (42).

Finally in 1972, RRV was isolated using IC inoculation of human serum into 1- to 2-day-old mice and subsequent serial passage after IC inoculation of brain suspension from any mice found sick or dead (45). The serum used was from a 7-year-old aboriginal boy from Edward River, North Queensland, who had a headache and fever but no arthralgia or arthritis. The child's nonspecific illness meant that the association between infection with RRV and the clinical entity known as epidemic polyarthritis remained conjectural (45).

In 1979 RRV spread to the South Pacific, causing large epidemics in Fiji, the Cook Islands, and Samoa (4, 56, 95, 123, 130, 162, 197) (Fig. 1). Aaskov et al. (4), Fauran et al. (57), Rosen et al. (162), and Tesh et al. (197) isolated RRV from 1, 42, 51, and 1 patient, respectively, all with arthralgia. The Pacific epidemic was the first occasion on which virus had been isolated from patients with symptoms of epidemic polyarthritis. Then, in 1985, Aaskov et al. (7) isolated RRV from an Australian patient with symptoms of epidemic polyarthritis. This

was the first isolation of RRV from a patient with typical symptoms of polyarthritis in Australia.

STRUCTURE AND PHYLOGENETIC RELATIONSHIPS

Structure of the Virus

The genus *Alphavirus* contains small, enveloped viruses. The genome is single-stranded positive-sense RNA (90). The genome of the T48 strain of RRV is 11,853 nucleotides in length and codes for four nonstructural proteins (nsP1 to nsP4), a capsid protein, and three envelope glycoproteins (E1 to E3) (55). The E3 glycoprotein is not incorporated in the virion (26). The capsid protein and the genome form the nucleocapsid, which is about 400 Å in diameter, contains 240 copies of the capsid protein, and has $T = 4$ icosahedral symmetry (26, 191). T is the triangulation number, giving a multiplier of 60 that gives the number of subunits in the virus structure (81). The E1 and E2 viral glycoproteins are embedded in the lipid bilayer to form the envelope (191). Single E1 and E2 molecules associate to form heterodimers. The E1-E2 heterodimers form one-to-one contacts between E2 and nucleocapsid monomers (26).

Phylogenetic Relationships and Evolution

RRV belongs to the family *Togaviridae*, which contains the genera *Alphavirus* and *Rubivirus*. *Alphavirus* contains 24 registered members, all of which are arboviruses (211), and a 25th member, Trocara virus, has recently been added (199). The genus *Rubivirus* contains only rubella virus (177).

On the basis of the nucleotide sequence of the nonstructural proteins, the genus *Alphavirus* segregates into New World (American) and Old World (Eurasian-African-Australasian) viruses (212). An estimate of the time of divergence of the Old and New World alphaviruses was made on the basis of studies of the Venezuelan equine encephalitis virus (VEE) complex. Limited portions of the nsP4, E1, and 3' untranslated regions of the genome were sequenced and phylogenetic trees were constructed (210). Assuming an evolutionary rate of 5×10^{-4} substitutions per nucleotide per year, the New World eastern equine encephalitis virus (EEE) and VEE complexes diverged only about 1,400 years ago (213). In a later study, phylogenetic analysis of the nsP1, nsP2, and nsP4 proteins was carried out. On the basis of the phylogeny and the estimate for the time of divergence of the New World EEE and VEE complexes, the Old and New World alphaviruses diverged as recently as 2,000 to 3,000 years ago (212).

On serologic criteria, RRV is classified in the SF antigenic complex of the genus *Alphavirus*, one of seven serologic complexes in the genus (22). The other complexes are EEE, Middleberg, Ndumu, VEE, western equine encephalitis virus (WEE), and BFV (22). The alphaviruses can be grouped into three larger groups on the basis of genome sequence, the VEE-EEE group, the SF virus (SFV) group, and the Sindbis virus (SIN) group (191). The SF group contains RRV, Sagiya virus (SAGV) (211), SFV, Middleburg, Chikungunya, BFV, Getah, Bebaru, Mayaro, Una, and O'nyong-nyong (ONNV) viruses (191). Of these, complete nucleotide sequences have been published for RRV, SFV, ONNV, and SAGV, with RRV and SFV more closely related to each other than to ONNV (112, 183, 191). Analysis of the nsP4 and E2 genomic RNAs suggest that SAGV is most closely related to RRV (183), and it is now classified as a subtype of RRV (211). Given that RRV and SFV are thought to have diverged between 1,000 and 2,000 years ago (192), RRV and SAGV presumably diverged more recently. The relatively recent divergence of these three Old World alphaviruses from an ancestral virus in Australia, Africa, or Japan makes dispersal by birds seem likely.

IMMUNE RESPONSE, PATHOGENESIS, AND LABORATORY DIAGNOSIS

Immune Response to Infection and Pathogenesis of Arthritis

Following the bite of an infected mosquito, RRV particles are inoculated and attach to a cell surface receptor, possibly an integrin (105). The virus then penetrates and is uncoated within the cell (161). Primary replication occurs in skeletal muscle (73, 89, 146, 147). RRV then enters the blood, where clearance is mediated principally by neutralizing antibodies and type 1 interferon (86, 106, 229). At the time that symptoms commence, virus usually cannot be cultured from peripheral blood (124), so presumably it has been cleared by the mechanisms discussed above but has infected other tissues. The most prominent and disabling symptom of RRV disease is arthralgia. Therefore, the following discussion is focused on joint infection and the pathogenesis of arthralgia and arthritis.

Immune complexes are involved in the pathogenesis of some viral arthritides (65, 225), but Fraser et al. (65) could not find

immune complexes in the serum or synovial fluid of RRV disease patients. Immune complexes deplete complement and attract neutrophils, but Fraser et al. (65) and Clarris et al. (28) found normal C3 and C4 in serum of RRV disease arthritis patients. Synovial effusions in acute RRV disease consist mainly of monocytes and activated macrophages (28, 64, 84), which are attracted and activated by monocyte chemoattractant protein 1 (MCP-1), which is produced by RRV-infected synovial fibroblasts in vitro (134). Thus, direct and indirect evidence argues against immune complexes as the cause of RRV arthralgia and arthritis.

Viable RRV has not been isolated from the joints of patients with chronic joint symptoms following RRV infection (28, 60). Viral antigen had been demonstrated in leukocytes from joint effusions, and viral RNA has been demonstrated by PCR testing of synovial biopsy specimens (64, 188). Human synovial cells can be productively infected with RRV in vitro (34, 91). La Linn and coworkers (105, 107) showed that macrophages could be persistently and productively infected with RRV and suggested this might play a role in persistence through the phagocytosis of dying cells by other macrophages. Neutralizing antibody cannot clear RRV from infected macrophages (106, 107). Patients with RRV disease have a marked virus-specific T-lymphocyte proliferative response (3), and cytotoxic T lymphocytes can clear RRV-infected macrophages (106, 107). La Linn et al. (106) suggested that the development of chronic arthralgia and/or arthritis could be explained through the inability of some individuals to generate RRV-specific cytotoxic T lymphocytes and hence clear infection. This hypothesis fits well with earlier observations of Fraser and his group (62, 67). They found that CD8⁺ lymphocytes (usually cytotoxic T lymphocytes [160]) predominate in the skin rashes of patients who have made rapid recoveries from RRV disease (67). CD4⁺ lymphocytes (usually helper T lymphocytes [160]) predominate in the mononuclear synovial effusions of patients with chronic RRV disease symptoms (62). Also, human leukocyte antigen DR7 is more common among RRV disease patients than among controls (68). Human leukocyte antigen restriction of the ability to generate specific cytotoxic T lymphocytes and consequent persistent infection of joint macrophages or synovium may offer a mechanism for the persistence of symptoms (105, 188). La Linn et al. (107) demonstrated that RRV-infected macrophages secrete nitric oxide, an inflammation mediator. Interferon gamma, a cytokine produced by T lymphocytes, is elevated in synovial effusions of patients with RRV disease (105). One or both of these substances may mediate inflammation in RRV-induced synovitis. Synovial fibroblasts infected with RRV in vitro produce MCP-1, which has been implicated in the pathogenesis of some other viral arthritides and rheumatoid arthritis (134). RRV arthritis is probably an infectious arthritis, possible inflammatory mediators have been identified, and an immunological mechanism for chronic symptoms occurring in some people has been suggested.

Laboratory Diagnosis

Virus isolation from humans is rarely achieved, probably because RRV does not persist beyond the early stages of disease. Diagnosis is usually made on serologic grounds (124).

Because of its production early in the course of *Alphavirus*

infection and the fact that it usually does not persist at high titer (23, 25) the detection of immunoglobulin M (IgM) in an acute-phase specimen provides a presumptive diagnosis of recent infection (121). Sucrose gradient ultracentrifugation is required to separate IgM for HI testing, and only reference laboratories use the technique (D. Phillips, WHO Collaborating Centre for Arbovirus Reference and Research, Brisbane, Queensland, Australia, personal communication, 1999). In most laboratories HI cannot distinguish IgM and IgG. Enzyme-linked immunosorbent assay (ELISA) allows measurement of IgG and IgM separately, enabling presumptive diagnosis by a single IgM-positive specimen (121, 136). False-positive ELISA results may be caused by BFV, rubella (157), Q fever (150), and noninfectious causes such as rheumatoid factor, although the last is normally controlled for in the testing procedure.

A confirmed diagnosis cannot be made with a single serum specimen. Two sera obtained 10 to 14 days apart should be collected and tested in parallel by the same laboratory. The acute-phase serum should be collected within 7 days, and the convalescent-phase serum within 8 to 28 days of onset of illness (121). The standard for confirmed diagnosis of *Alphavirus* infections is a fourfold or greater increase or decrease in antibody titer determined by HI, CF, or N testing (23, 121).

Until 1999, most testing for RRV infection in Australia was done using indirect ELISA with a kit manufactured by PanBio; a second kit manufactured by Biocene is also now available (J. Kapeleris, PanBio, personal communication, 2001). Flexman et al. (59) suggest that IgG seroconversion, that is, an IgG-negative acute and IgG-positive convalescent specimen on ELISA testing, confirms the diagnosis. Based on ELISA testing of 124 HI-positive patients infected in South Australia from 1996 to 1997, the sensitivity of the PanBio ELISA kit is 98.5 and 84.6% and the specificity is 96.5 and 97.6% for IgM and IgG, respectively (120; M. Qiao, personal communication, 2001).

RRV DISEASE

Incubation Period

The incubation period for most arboviruses in 5 to 15 days (185). RRV usually incubates for 7 to 9 days, based on the experience of 20 patients who lived in areas of no risk, visited transmission zones, and developed symptoms with diagnosis confirmed by HI testing (63). Incubation may be as long as 21 days or as short as 3 days. The first estimate is based on a questionnaire-survey of notified cases (143) and the latter on a single case report (162).

Clinical Manifestations

RRV disease causes a characteristic syndrome, including constitutional effects, rash, and rheumatic manifestations (60). Several less common presentations have also been described.

Fraser (60) described the clinical features of a series of 43 patients. At first he actively sought cases receiving medical or surgical care in the Murray Valley town of Echuca, Victoria, for any symptom that could be attributed to RRV or other viral infections. Later, cases were referred from the casualty department of the Royal Melbourne Hospital, rheumatologists, fam-

TABLE 1. Joints affected by RRV disease

Joint	Frequency of joint involvement (% of cases)				
	Seglenieks and Moore (180) (n = 115)	Aaskov et al. ^a (4) (n = 36)	Mudge and Aaskov (144) (n = 400)	Condon and Rouse (33) (n = 189)	Westley-wise et al. (218) (n = 80)
Fingers		50 ^c	80	81	63
Hand	45				
Thumb			53	58	
Wrist	36	70	80	100	61
Elbow	17	40	44	71	
Shoulder		38	47	62	
Hip	4	10	27		
Knee	39	80	80	100	64
Ankle	50	78	88	97	64
Feet	49	36			
Toes			47		
Back	14 ^b	36	37	56	
Neck		36	12	70	
Jaw			10	15	

^a Percentages taken from a bar graph.

^b Includes back or neck.

^c Includes fingers and hand.

ily practitioners, and infectious disease and other consultant physicians (J. R. E. Fraser, personal communication, 1997). Patients referred from rheumatologists and other consultant physicians might have been unusual. However, this description is still the best account of RRV disease and is especially useful because it was done by a single clinician with appropriate skills. Unless otherwise specified, the description that follows is based on Fraser's observations.

Joint manifestations. Joint symptoms and signs are usually symmetrical and acute in onset, ranging from tenderness with minor restriction of movement to extreme redness and swelling. Effusions are common and peripheral joints are predominantly involved. Fraser (60) does not report the frequency of involvement of specific joints, but this information is noted in five other less direct studies of RRV disease (Table 1). These studies were carried out between 1971 and 1992 in Western Australia (33), New South Wales (218), South Australia (180), the whole of Australia (144), and Fiji (4). The methods for case ascertainment differ, but the results clearly show that ankles, fingers, wrists, and knees are the joints most commonly affected. The metacarpophalangeal joints are also very frequently affected by pain (78).

Rash. Rash affects about 50% of people with RRV disease, usually lasts 5 to 10 days, and may be the sole manifestation of infection. The rash appears mainly on the limbs and trunk, but may also occur on the palms, soles, digits, face, and even the scalp. Most commonly the skin eruption is maculopapular, but it may be vesicular or purpuric (60, 66).

Constitutional effects. Fever affects from one third to one half of patients. Rash, fever, and arthralgia may occur in any sequence. Myalgia is common, reported by up to 58% of patients (144). Lymphadenopathy occurs quite often, sore throat and coryza less frequently, and diarrhea is rare (60, 66). In a small study, Bennett et al. (17) noted that fatigue and malaise rather than anxiety and depression accompanied RRV disease. Fatigue typically affects over 50% of patients.

Other presentations. Other manifestations reported include splenomegaly, hematuria, and glomerulonephritis (11, 39, 60,

65, 222). Paresthesia may occur due to entrapment neuropathy. Headache, neck stiffness, and photophobia may occur. So far there are only three case reports suggesting meningitis or encephalitis caused by RRV infection, indicating that these must be rare. None of the three cases were proven to be caused by RRV, and more effort should be made to isolate RRV from cerebrospinal fluid if future cases occur. Because of the serious nature of such manifestations, these cases are reviewed in detail, although the evidence that RRV infection caused these cases is not strong.

The first report was of a 27-year-old man who died of encephalitis in Papua New Guinea during 1980 or 1981 (the date of onset is not stated in the paper) and had RRV antibody titers of 1:160 1 week after hospital admission. No other cause of encephalitis could be found, but he did not have arthralgia or arthritis, RRV was not isolated, and autopsy could not be performed, so the evidence that RRV caused his death is weak (179).

Lucas and Qiao (120) reported a 33-year-old male who was admitted to the intensive care unit of the Alice Springs (Fig. 1) hospital with encephalitis. There was no rash or evidence of arthritis. Cerebrospinal fluid Gram stain, latex agglutination for bacterial capsular antigens, cryptococcal antigen, and India ink stain tests were negative. There was no serologic evidence for other causes of acute and postinfectious meningoencephalitis. On admission, HI and ELISA testing for IgG and IgM to RRV were negative. IgM on ELISA was positive after 8 days and remained so for 34 days postadmission. IgG was negative on both of these occasions, but became positive 90 days postadmission. A diagnostic (≥ 4 -fold) rise in total antibody titer was not observed using HI testing. The IgG seroconversion after 90 days was later than would be expected, even allowing for the slow seroconversion noted by Qiao (120; M. Qiao, personal communication, 1999).

Penna and Irving (J. E. Penna and L. G. Irving, letter, *Med. J. Aust.* **159**:492–493, 1993) reported a possible case of meningitis caused by RRV. A 40-year-old male developed headache and neck stiffness 1 week after onset of fever and arthralgia. HI testing gave a result of 80 with RRV as the antigen and < 20 with Murray Valley encephalitis virus as the antigen. RRV IgM was detected in serum. Serologic tests for a number of other infectious causes of meningitis were negative.

Illness duration and prognosis. Recent estimates of the duration of symptoms with RRV disease have increased substantially compared with estimates made when the disease was first described. RRV disease has now become notorious in public discourse, with a reputation for inducing prolonged and disabling arthritis.

In the first described RRV outbreak, Nimmo (148) noted that “many individuals have been unable to carry on their employment for periods up to three weeks.” Subsequent reports focused on observations of soldiers in the second World War (53, 76, 186). Halliday and Horan (76) suggested that “joint enlargement subsides within a week, but painful limitation of joint movement persists for two or three weeks longer.” Dowling (53) found that joint swelling persisted for 4 to 7 days and pain usually disappeared a few days after swelling subsided, but 3 of 94 had joint pain persisting for 3 months after onset. Sibree (186) found that 17 of 28 soldiers were able to carry on normal army duties. Eleven others were admitted to

hospital, but eight of these resumed duties within 23 days. Three remained in hospital for 60 to 70 days due to persistently painful joints.

From the 1980s, reports on the clinical features began to suggest that some patients developed chronic disease lasting for months to years. Fraser (60) reported that 50% had recovered after 6 months, 75% in just over 12 months, and 5% had not recovered after 4 years. In a large New South Wales outbreak, Hawkes et al. (82) found that 11% were incapacitated for longer than 12 weeks. Three recent studies suggested that an even longer duration of symptoms was typical. Condon and Rouse (33) reported that 24 to 42 months after onset 57% of RRV disease patients had intermittent or continuous joint pain. Selden and Cameron (181) found that 30 months after diagnosis, 64% had joint pain. Westley-Wise et al. (218) found that 12 months after onset, 52.5% had joint pain. The validity of the last three studies is doubtful. They involved mailed questionnaires sent to notified cases months or years after disease onset, and the calculations are based on the fraction who responded. In the study of Selden and Cameron, only 179 of 814 responded at 30 months (181). This created a serious selection bias, as it is much more likely that those who were ill would respond, boosting estimated rates. Also, these studies used nonstandardized and nonvalidated questionnaires, lacked clinical review, did not exclude alternative diagnoses, and had no control groups.

A priori, we would not expect RRV disease morbidity to vary much. Evolution of the virus is slow (21), and it remains genetically homogeneous over large geographic areas (119, 176). This stability suggests that temporal or spatial changes in virulence are unlikely to occur. Methodological differences among the morbidity reports and substantial self-reporting bias in the three mailed questionnaire studies of the 1990s (33, 181, 218), together with cultural inflation of morbidity (88) over time, account for these differing estimates. The so-called cultural inflation of morbidity (88) results from increasing health expectations on the part of the public and cultural pressures on health professionals to treat an ever-growing set of nonfatal diseases, leading to a paradoxical increase in demand for health services as mortality declines.

The lack of clinical review and exclusion of alternative diagnoses may be a threat to validity. An analogous situation exists with chronic Lyme disease in the United States and Canada and suggests that caution should be exercised in ascribing chronic symptoms to an infectious disease of which there is a high level of public awareness and concern. Both infections have been implicated as causes of chronic rheumatic disease. In the case of Lyme disease, chronic illness directly attributable to *Borrelia burgdorferi* infection is considerably less common than previously thought. Burdge and O’Hanlon (20), working in Canada, assessed 65 patients sent to a multidisciplinary referral center for Lyme disease. By applying strict criteria for diagnosis and intensively investigating patients, major alternative diagnoses were made for 77%, probable diagnoses of chronic fatigue syndrome and fibromyalgia were made for 17%, and 6% remained undiagnosed. Only two patients were judged to have probable Lyme disease. An American study of Lyme disease produced similar results (190). Some intimation that the same phenomenon might be occurring with RRV disease is provided by Keary (P. J. Keary, Abstr. 40th

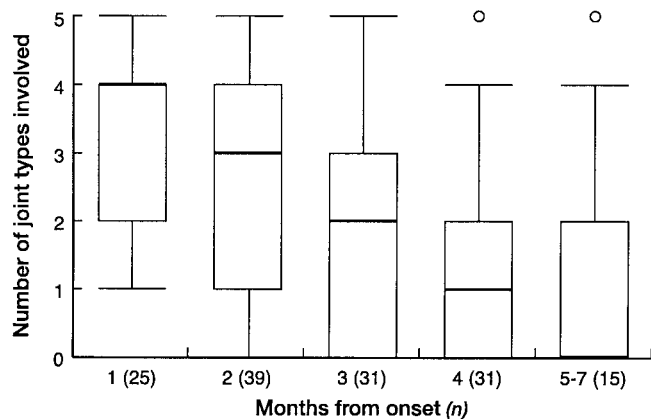


FIG. 2. Number of joint types that were painful over 7 months of postinfection follow-up for 47 cases of RRV disease, Cairns, 1998.

Annu. Sci. Conf. Aust. Rheumatol. Assoc., abstr. 16, 1997), who studied 103 patients with serologically confirmed RRV infection. Thirty either had preexisting or subsequently developed another rheumatic disease. The prevalence of rheumatic symptoms in the community is high (127, 206). It follows that prevalent symptoms not causally associated with RRV disease are likely to have been included in the results of the three 1990s RRV symptom studies (33, 181, 218), leading to an overestimate of symptom prevalence and duration. Experience with Lyme disease and the study by Keary both suggest that it is unwise to assume that the symptoms reported in these studies have been caused by RRV infection.

Until the natural history of RRV disease morbidity is better understood, the public health importance of this disease cannot be determined. This requires repeated clinical assessments of a community-based inception cohort of symptomatic persons with confirmed incident RRV disease, using standard measures of morbidity, and comparing results to expected levels of morbidity for people of that age and sex. One of us (D.H.) has completed such a study in North Queensland (78), and the information obtained will now be considered.

The inception cohort was a group of 47 family practice patients diagnosed with RRV disease in and around Cairns (Fig. 1) in 1998. One physician trained in rheumatological assessment (D. Harley) reviewed all patients three times in their homes from 7 to 210 days after diagnosis. The assessment included a clinical history, rheumatologic examination, and standard validated health questionnaires (Clinical Health Assessment Questionnaire [CLINHAQ] [69, 139, 223] and Short Form-36 [SF-36] [135]). The time course of symptoms and signs over approximately 7 months from onset is shown in the following table and graphs. There was a progressive diminution in symptom prevalence over time. The box plot (Fig. 2) quantifies the involvement, by pain within the week of review, of the five most commonly involved joint types (finger, metacarpophalangeal, wrist, knee, and ankle joints) in 141 questionnaire responses from 47 patients. The median number of painful joint groups decreased from four to one over the first 4 months and reached zero after 5 to 7 months. However, at least one joint remained abnormal on clinical examination for one quarter of the 15 examinations performed after 5 to 7 months

TABLE 2. Prevalence of joint abnormality over 7 months of follow-up, RRV disease, Cairns, 1998

Time postonset (mo)	No. of examinations ^a	Prevalence (%) of abnormal exams of one or more joints ^b
1	25	60
2	39	62
3	31	39
4	31	32
5-7	15	27

^a Forty-seven individuals were examined, each on three occasions, giving 141 examinations.

^b Objective signs on joint examination—tenderness, effusion, enthesopathy, swelling, heat, or other abnormal findings.

(Table 2). Of the four individuals who had abnormal findings in months 5 to 7, one was a 54-year-old woman with severe preexisting osteoarthritis who had swelling of the ankle joint and proximal interphalangeal joints of the fingers and grade I tenderness (195) of her knee joint. These findings reflected her underlying disease rather than having been caused by RRV disease and therefore inflate the prevalence of joint abnormalities at this review.

Figures 3 through 5 show the steady improvement in difficulties with activities of daily living (ADL), and physical, psychological, and social function over 6 months after infection. All analyses were linear regressions, with the study subject included as a random effect. Figure 3 shows the CLINHAQ Functional Disability Index (FDI) regressed against time since symptom onset. The FDI measures difficulties with ADL and can be interpreted as follows (187): 2.0 to 3.0, severely handicapped; 1.25 to 2.0, many major problems with ADL; 0.5 to 1.25, reasonably self-sufficient; and 0.0 to 0.5, self-sufficient. Figures 4 and 5 show physical and psychological dimensions of the SF-36 health status questionnaire, respectively. These are age- and sex-adjusted departures from the population standard for the Australian State of Queensland (207) in standard deviation units, regressed against time since symptom onset. It is clear that the group returns to normal physically over 4 to 6 months and psychologically over 2 to 5 months.

The results of this study indicate that RRV disease does cause pain and suffering for several months in a substantial

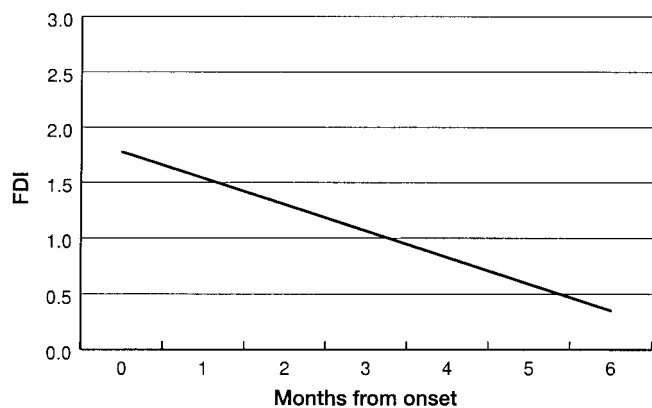


FIG. 3. Clinical Health Assessment Questionnaire Functional Disability Index (FDI) regressed against time since symptom onset for RRV disease cases, Cairns, 1998.

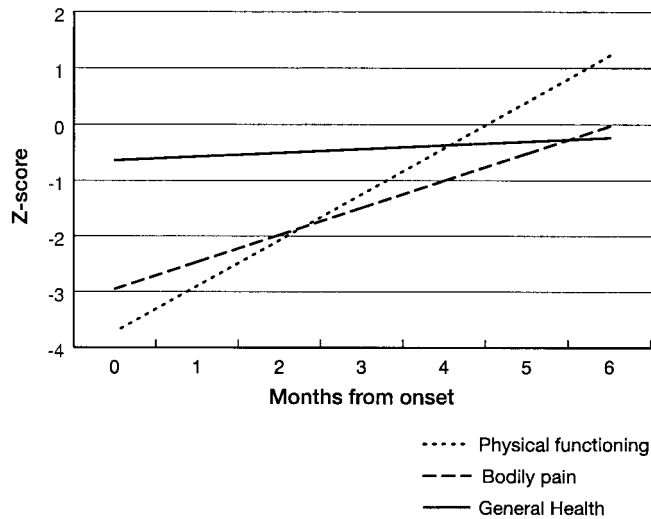


FIG. 4. Physical dimensions of the SF-36, age and sex standardized to the Queensland population and regressed against time since symptom onset for RRV disease cases, Cairns, 1998.

proportion of patients, but progressive resolution indicates that treating doctors are justified in taking an optimistic outlook regarding complete recovery over some months, whilst acknowledging the appreciable temporary disability that may result.

Treatment

Recommendations on treatment for RRV disease are not based on clinical trials. After extensive clinical experience with RRV disease, Fraser (66) reported that nonsteroidal anti-inflammatory drugs (NSAIDs) can give dramatic symptomatic relief. Condon and Rouse (33) found that of 255 patients, 36.4% felt that NSAIDs provided the “best and most effective relief,” and 16.4% felt that aspirin or paracetamol was the most

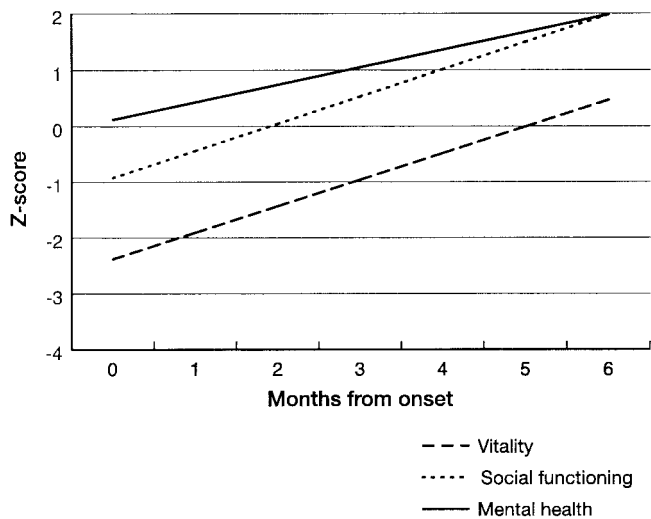


FIG. 5. Psychological dimensions of the SF-36, age and sex standardized to the Queensland population and regressed against time since symptom onset for RRV disease cases, Cairns, 1998.

TABLE 3. Isolates of RRV from nonhuman vertebrates

Species	No. of isolates	No. of animals tested	Reference
Placental mammals			
Horse (<i>Equus caballus</i>)	1	8	149
	1		24
Marsupials			
Agile wallaby (<i>Macropus agilis</i>)	2	17	49
Birds			
		775 (104 species)	221
Magpie lark (<i>Grallina cyanoleuca</i>)	1		
Flycatcher (<i>Microeca fascians</i>)	1		
Masked finch (<i>Poephila personata</i>)	1		

effective. Physical interventions (swimming, hydrotherapy, physiotherapy, or massage) were the most beneficial for 10.3% of patients, but for 24.1% rest was the only source of symptom relief. Some clinicians use oral corticosteroid therapy and have found that patients respond well (D. Harley, unpublished data), but it is doubtful whether steroids are needed, and the adverse effects should constrain their use. In theory, steroids could make RRV disease worse, but this has not been observed.

The available information is limited but suggests a stepwise approach would be best for treatment of RRV disease arthritis; first, a trial of rest and physical therapy, followed by simple analgesics, and then NSAIDs if necessary. Until more information is available, steroid treatment cannot be recommended.

ECOLOGY OF RRV

Vertebrate Reservoir Hosts

Theoretically, to establish the status of putative reservoir hosts, we require information on behavior, ecology, immunology, and reproductive cycles and the course of arbovirus infection. In practice, most knowledge of the likely vertebrate reservoir hosts for arboviruses has come from antibody prevalence surveys and experimental studies of infection. This is true for RRV.

Here we review knowledge of reservoir hosts for RRV, including the infrequent isolation of virus from vertebrates, extensive antibody prevalence surveys, the numerous experimental infection studies, and current thinking about reservoir hosts.

Seven RRV isolates have been reported from nonhuman vertebrates, remarkably few for such an important zoonotic infection (Table 3). The information base for antibody prevalence in vertebrates is much larger than for virus isolates (Table 4).

The results of experimental infection studies conducted on over 20 species are shown in Table 5. Largely on the basis of the data in Table 5, Kay and Aaskov (95) concluded that “marsupials are more competent hosts than placental mammals which, in turn, are more effective amplifiers than birds.” However, this is only true if level and duration of experimental viremia are equated with reservoir competence. Even then, with only a small number of experimental infection studies performed on a small number of animals, it seems premature

TABLE 4. RRV antibody prevalence surveys of nonhuman vertebrates^a

Species	Method and results				Reference	Species	Method and results				Reference		
	HI		N				HI		N				
	% Positive	n	% Positive	n			% Positive	n	% Positive	n			
Placental mammals						Swamp wallaby (<i>Wallabia bicolor</i>)			83	6	203		
Flying fox (<i>Pteropus</i> sp.)	0	7	0	8	48	Gray kangaroo (<i>Macropus giganteus</i>)	58	36	50	2	204		
Blackish flying fox (<i>Pteropus gouldii</i>)	50	6	100	3	49				100	13	48		
Little red flying fox (<i>Pteropus scapulatus</i>)	5	21			71				50	2	204		
	0	21	0	1	49				50	30	203		
Gray-headed flying fox (<i>Pteropus poliocephalus</i>)	25	261			71		40	10			137		
Pseudo-rat (<i>Pseudomys fumeus</i>)	0	1			132		89	28	100	5	49		
									74	39	168		
House mouse (<i>Mus musculus</i>)	0	4			71	Red kangaroo (<i>Macropus rufus</i>)	52	25	100	10	48		
			0	7	203	Northern nail-tail wallaby (<i>Onychogalea unguifera</i>)	100	1		0	2	204	
New Holland mouse (<i>Gyomys novaehollandiae</i>)	25	72			71						49		
Rats (unidentified)			3	33	197	Barred bandicoot (<i>Perameles gunnii</i>)	100	2				137	
	2	150	0	71	48	Brindled bandicoot (<i>Isodon macrourus</i>)	1	64	50	2	48		
Black rat (<i>Rattus rattus</i>)	0	18			71	Brown bandicoot (<i>Isodon obesulus</i>)					25	4	203
	0	3			132								
Brown rat (<i>Rattus norvegicus</i>)	0	1			71	Long-nosed bandicoot (<i>Perameles nasuta</i>)							
							100	7					
Eastern water rat (<i>Hydromys chrysogaster</i>)	12	26	100	1	49	Bilby (<i>Macrotis lagotis</i>)	0	40					48
Sombre Downs rat (<i>Rattus sordidus</i>)	7	14			49		0	1					48
Swamp rat (<i>Rattus lutreolus</i>)	9	11			71	Ringtail possum (<i>Pseudocheirus peregrinus</i>)	0	1					137
			0	72	203	Common brush tail possum (<i>Trichosurus vulpecula</i>)	66	3					71
Bush rat (<i>Rattus fuscipes</i>)			0	124	203		100	1					71
							50	2					49
Rabbit (<i>Oryctolagus cuniculus</i>)	0	7			132		77	13		4	243	14	137
	0	1			132	Common wombat (<i>Vombatus ursinus</i>)	43	7					137
			20	45	197	Potoroo (<i>Potorous apicalis</i>)	100	2					137
Dog (<i>Canis familiaris</i>)	8	24	11	36	48	Bettong (<i>Bettongia gaimardi</i>)	100	1					137
	53	30	40	5	49	Tasmanian devil (<i>Sarcophilus harrisii</i>)	100	4					137
	33	33	50	34	162	Sugar glider (<i>Petaurus breviceps</i>)	50	2					71
Domestic cat (<i>Felis catus</i>)			0	16	197	Koala (<i>Phascolarctos cinereus</i>)					16	93	168
Feral pig (<i>Sus scrofa</i>)	50	4	100	2	49	White-footed dunnart (<i>Sminthopsis leucopus</i>)					0	3	203
Domestic pig (<i>Sus scrofa</i>)			15	27	197								
	43	70	77	47	162	Monotremes							
Goat (<i>Capra hircus</i>)			0	2	204	Short-beaked echidna (<i>Tachyglossus aculeatus</i>)					0	6	203
	0	8	38	8	48								
			17	6	203	Birds							
						Birds (mixed species)	1	145	6	16	48		
Sheep (<i>Ovis aries</i>)	11	36	24	17	48		50	48	63	8	49		
			0	4	203	Frogmouth owl (<i>Podargus strigoides</i>)	17	6					71
Cow (<i>Bos taurus</i>)			20	40	31	Azure kingfisher (<i>Alcyone azurea</i>)	0	1					71
			19	187	203	Raven (<i>Corvus coronoides</i>)	0	1					71
	54	41	62	21	48	Kookaburra (<i>Dacelo gigas</i>)	0	1					71
	33	12	100	11	162	Yellow-faced honeyeater (<i>Melithaga chrysops</i>)	0	4					71
	6	102			137	Fowl					5	20	197
	67	66	100	21	49		9	147					52
Horse (<i>Equus caballus</i>)			65	23	31	Reptiles							
			62	120	203	Unidentified	0	15					49
	86	36	89	37	48								
	~60	1,300 ^b			14								
Marsupials													
Agile wallaby (<i>Macropus agilis</i>)	85	169	19	12	49								
Brush wallaby (<i>Macropus rufogriseus</i>)	67	21	100	5	48								
			86	7	203								
			0	2	204								
	67	33			137								
Black-striped wallaby (<i>Macropus dorsalis</i>)	27	11	100	1	48								
Southern whip-tail wallaby (<i>Macropus parryi</i>)	66	3	100	1	48								
Red-necked pademelon (<i>Thylogale thetis</i>)	0	3			48								
Tasmanian pademelon (<i>Thylogale billardieri</i>)	56	50			137								

^a Adapted from reference 208.

^b IgG ELISA.

TABLE 5. Results of experimental infection of nonhuman vertebrates with RRV^a

Species	Maximum titer of viremia (log ₁₀ /LD ₅₀ ^b or PFU/ml)	Duration of viremia (days)	No. tested	Reference
Placental mammals				
Adult laboratory mouse (<i>Mus musculus</i>)	5.4 ^b	1–3		130
Adult wild mouse (<i>Mus musculus</i>)		0		130
New Holland mouse (<i>Pseudomys novaehollandiae</i>)	7.6	1–5		130
Black rat (<i>Rattus rattus</i>)	4.6	3–6		130
Brown rat (<i>Rattus norvegicus</i>)	7.4	1–3	4	220
Swamp rat (<i>Rattus lutreolus</i>)		0		130
Gray-headed flying fox (<i>Pteropus poliocephalus</i>)		0–1		130
Red flying fox (<i>Pteropus scapulatus</i>)		0		130
Rabbit (<i>Oryctolagus cuniculus</i>)	4.7 ^b	0–3	4	220
Horse (<i>Equus caballus</i>)	6.3	0–5	11	95
Cow (<i>Bos taurus</i>)		0		95
Sheep (<i>Ovis aries</i>)	5.0 ^b	2–5		95
Juvenile domestic pig (<i>Sus scrofa</i>)	4.2	1–3		130
Juvenile feral pig (<i>Sus scrofa</i>)	4.8 ^b	0–5		95
Marsupials				
Bandicoot (<i>Isodon macrourus</i>)	7.2 ^b	1–6	4	220
Marsupial mouse (<i>Antechinus stuartii</i>)	4.4	1–4		130
Marsupial mouse (<i>Antechinus sp.</i>)	8.0 ^b	1–6	6	220
Tammar wallaby (<i>Macropus eugenii</i>)	5.3	2–3		130
Agile wallaby (<i>Macropus agilis</i>)	7.4 ^b	0–5		95
Gray kangaroo (<i>Macropus giganteus</i>)	6.1 ^b	6		95
Birds				
Adult domestic fowl		0	4	220
Day-old domestic fowl	5.0 ^b	1–5	12	220
Rufous night heron (<i>Nycticorax caledonicus</i>)		0		130
Australian magpie lark (<i>Grallina cyanoleuca</i>)		0		130
Pigeon (<i>Columba livia</i>)		0	5	220
Black duck (<i>Anas superciliosa</i>)	2.3 ^b	0–5		95
Little corella (<i>Cacatua sanguinea</i>)	<2.3 ^b	0–1		95

^a From references 95, 130, and 220. Only Whitehead (220) gives detailed methods. The T48 strain of RRV was used as a 20% suspension of suckling mouse brain in rabbit serum-saline. Experimental hosts were inoculated subcutaneously with virus diluted 10^{-4.5}. To determine viremia, blood from experimental hosts was diluted 1:10. Blood was titrated by intraperitoneal inoculation of suckling mice. Virus titers were expressed as the minimum dose sufficient to kill 50% of inoculated mice (LD₅₀) per gram of undiluted blood. Marshall and Miles (130) and Kay and Aaskov (95) reproduce results from Whitehead (220) as well as their own unpublished data, but do not provide detailed methods.

^b Assayed in suckling mice. LD₅₀, lethal dose 50% endpoint.

to rank vertebrate hosts for their importance as reservoirs. As outlined in the introduction to this section, many factors other than level and duration of viremia must be taken into account to determine reservoir status.

The antibody prevalence and laboratory infection and transmission data suggest significant roles for macropods as reservoir hosts. *Macropus agilis*, the agile wallaby, is one of the few vertebrates from which RRV has been isolated (Table 3), and antibody prevalence surveys also suggest high rates of infection (Table 4). In 1971, in a paper on arbovirus infections of mosquitoes and mammals at Mitchell River mission (now Kowanyama) on Cape York Peninsula in remote North Queensland, Doherty et al. (49) concluded that “accumulating evidence suggests that the association of large marsupials, especially wallabies, with Ross River and Kokobera viruses is ecologically significant, and that the basic epidemiology of the human disease epidemic polyarthritis may therefore involve cycles of transmission between these large macropods and mosquitoes.”

Later, Doherty (43) qualified this opinion for urban areas, saying “it is difficult to see what mammal hosts could maintain epidemics in, for example, suburbs of Brisbane where wallabies, kangaroos and rodents are not in high population.” Russell (167, 168) considers that the natural vertebrate hosts of RRV are native mammals such as kangaroos. Mackenzie et al. (122) and Mackenzie and Smith (124) consider that macropods are the major vertebrate hosts. Before the white invasion of Australia, macropods may have been the reservoir for natural enzootic RRV transmission cycles, but it does not follow that they are the major reservoir for the large numbers of human infections that occur in Australia. Macropods are rare in urban Australia, and they cannot be the usual source of metropolitan human infections, the main component of reported RRV disease. Other animals must be involved in urban areas.

Antibody to RRV has been detected in brush tail possums, a common urban marsupial, albeit in very few animals. Three studies using HI testing have tested between 1 and 13 animals and found antibody prevalences of between 50 and 100% (49, 71, 137). A larger study of 243 animals used N testing but detected antibody in only 4% (14) (Table 4). Given the large numbers of possums in many Australian cities, these animals warrant further investigation as potential reservoir hosts; indeed, recent experimental work at the Queensland Institute of Medical Research (QIMR) suggests they could be important reservoir hosts (A. Boyd, personal communication, 2000).

Outbreaks in major metropolitan centers (9, 113, 122, 152) have led Mackenzie et al. (122) to speculate that horses may act as amplifying hosts. RRV has been isolated from horses on two occasions (24, 149). Prevalence of IgG was 60% in a survey of 1,300 horses in Victoria during 1995 to 1996 (14). Horses can experimentally infect *Culex annulirostris* (a major vector of RRV) (103). They also develop relatively high-titered viremias (6.3 PFU/ml) which may last up to 5 days (Table 5). These data indicate that horses may play a role as amplifying hosts when present in appreciable numbers.

Given their close association with humans and their reported high RRV antibody prevalences in the case of dogs (Table 4), domestic dogs and cats should be studied further. Unpublished work at QIMR suggests that dogs and cats may

not be important reservoir hosts (A. Boyd, personal communication, 2000).

RRV is relatively homogeneous over large geographical areas, suggesting that humans or livestock transported by air or possibly flying foxes are able to transport the virus long distances (119, 123, 125, 176). If birds act as reservoir hosts, they could also account for these observations.

Serologic surveys and virus isolations from mosquitoes trapped near flying fox camps suggest that flying foxes might be reservoir hosts (49, 71, 79, 158). However, experimentally infected gray-headed (*Pteropus poliocephalus*) and red (*Pteropus scapulatus*) flying foxes failed to develop detectable viremias (130). Also, Ryan et al. (175) concluded that the gray-headed flying fox, *Pteropus poliocephalus*, was not an important reservoir host because only 10 of 510 (2%) *A. vigilax* mosquitoes fed on infected flying foxes were infected with RRV after an extrinsic incubation period, and because RRV could not be detected in any of 122 blood-fed *A. vigilax* immediately after feeding on infected *P. poliocephalus*. RRV has been isolated frequently from mosquitoes collected at flying fox camps in Brisbane and Cairns (79, 158). Harley et al. (79) obtained 26 (79%) of 33 isolates of RRV in Cairns during 1996 to 1998 from within 1 km of a spectacled flying fox (*Pteropus conspicillatus*) camp. The number of positive pools from within 1 km of the camp was significantly greater than for mosquitoes trapped elsewhere, for all species trapped, and for the important vector species *C. annulirostris*. The spectacled flying fox, *P. conspicillatus*, is limited in its geographical range, but occurs in large colonies close to human habitation in Cairns, a city with a high incidence of RRV disease (75, 152). No studies of antibody prevalence or experimental infection exist for this species. The role of flying foxes as RRV reservoir hosts remains unclear.

It has been suggested that birds do not play a role as reservoir hosts, largely based on experimental studies of infection (Table 5) (95). But birds are ubiquitous in Australia and relatively few species have been studied (95). RRV antibody prevalences in birds have generally been low (Table 4). One exception to this is from Doherty et al. (49), who found that 50% of 48 and 63% of 8 birds of mixed species collected at Mitchell River mission (now Kowanyama) on Cape York Peninsula from December 1967 to April 1969 had HI and N antibodies, respectively. There is serologic evidence for infection of various species of birds (48–50, 71, 197, 221). In 1972, Doherty (43) suggested that despite the variable and often low antibody prevalence found in birds, their role as reservoir hosts should receive critical attention. He noted that antibodies might be short-lived, not accurately reflecting past infection. There is some evidence for this. Kay et al. (103) found that 14% of *C. annulirostris* mosquitoes that fed on little corellas (a species of parrot) with viremias of maximum titer 2.3 suckling mouse infectious dose LD₅₀/ml subsequently became infected. However, the birds did not show serologic evidence of infection on HI testing. The first isolates of RRV from vertebrates were from the heart muscle of three birds (Table 3), but the only birds in which experimental viremia has been induced are day-old chicks (130). The genetic homogeneity of RRV isolates from geographically widespread areas (119, 176) suggests that the role of birds as reservoir hosts should be reevaluated. The close relationship between RRV in Australia, its subtype

SAGV in Japan, and SFV in Africa (112, 183) makes it likely that a recent ancestral virus used birds as a reservoir host.

Overall, the search for reservoir hosts of RRV has placed too much reliance on the results of experimental infection studies. Data do not exist linking infection in vertebrates with human disease. This information is technically and logistically difficult to obtain and needs to measure exposure to infected suburban horses, cattle, dogs, cats, possums, and birds. There are good grounds for reappraising the role of birds as RRV reservoir hosts. Also, there is evidence from epidemics in the South Pacific and Western Australia that humans can act as reservoir hosts for RRV. This is discussed more fully in Transmission Cycles.

Mosquito Vectors

This section examines information on mosquito vectors of RRV infection. We consider the prevailing opinion that the main vectors of human RRV infection in Australia are *A. vigilax*, *Aedes camptorhynchus*, and *C. annulirostris* (95, 123, 125, 167–169). This opinion arose because (i) there have been more RRV isolates from these species than from others; (ii) these species are infected with and transmit RRV in laboratory studies; and (iii) these species have been found to be abundant, often with RRV isolated from them, in temporal and spatial association with outbreaks of RRV disease.

This evidence is tabulated and discussed below. Other species that might be vectors are also considered. RRV has been isolated from over 30 mosquito species in Australia (27, 79, 114, 158, 167, 173). The vector status of most of these is unknown, because data on experimental infection and transmission and association with human infection, which are requisites to establish vector status, are lacking (123, 193).

Table 6 shows isolates of RRV from mosquitoes. Data for male and blood-fed female mosquitoes have been excluded from the table. Male mosquitoes do not blood feed (30) and therefore cannot transmit RRV to humans. Isolation of virus from males implies transovarial transmission (200). While this is likely to be important for maintenance of RRV in nature, it has no direct impact on transmission of RRV to humans, the primary focus of this review. Virus isolation from blood-fed females is not strong evidence for vector competence. It means only that they have taken a blood meal with virus in it; barriers to transmission, the mesenteron infection, dissemination, and salivary gland escape barriers may prevent such mosquitoes from acting as vectors (77).

Sudia et al. (193), in a paper on VEEV in Dade County, Florida, listed four criteria for establishing the vector status of a mosquito species (see earlier section on Vector Competence). These criteria will be used below to assess the evidence on mosquito vectors of RRV.

Research to isolate RRV from wild-caught mosquitoes, the first criterion to incriminate a vector, has been extensive (Table 6). Most of this work has been done in the last 10 years, reflecting the growing realization of the remarkable complexity of RRV ecology and epidemiology and growing public concern about RRV disease. Attempts to show that mosquito species can be infected by viremic hosts and transmit by biting others—the second and third criteria for incriminating vectors—have also required extensive work. The evidence spans 20 years

TABLE 6. RRV isolates from mosquitoes

Genus and species ^a	No. of mosquitoes examined	No. of RRV isolates	State, territory, or country ^b	Reference(s)	Genus and species ^a	No. of mosquitoes examined	No. of RRV isolates	State, territory, or country ^b	Reference(s)
<i>Aedes</i>					<i>E. N. Marks's species 85</i>	268	1	WA	118
<i>A. alboannulatus</i>		2	WA	114					
<i>A. alternans</i>	3,321	1	NSW	170					
	60	3	WA	118					
	1	1	QLD	158	<i>Anopheles</i>				
<i>A. bancroftianus</i>			VIC	129	<i>A. amictus s.l.</i>	1,205	1	QLD	46,93
<i>A. camptorhynchus</i>		4	VIC	Aldred et al., 1990 ^c				QLD & NSW	130
	1,848	1	TAS	138	<i>A. annulipes s.l.</i>			VIC	129
			NSW	168			4	NSW	27
		4	WA	117	<i>A. farauti s.l.</i>	30,551	1	WA	116
		79	WA	116				Western Province, PNG	87
		2	NSW	27					
		36	WA	114					
<i>A. carmentis</i>	6,146	14	QLD	79					
<i>A. clelandi</i>		1	WA	116	<i>Coquilletidia</i>				
<i>A. daliensis</i>	1,106	1	WA	118	<i>C. linealis</i>		1	NSW	32
<i>A. flavifrons</i>	248	2	TAS	137		202	1	NSW	71
<i>A. funereus</i>		2	NSW	27		17,119	1	NSW	170
	2,943	12	QLD	158			3	WA	116
		1	QLD	171			4	NSW	27
	11,094	1	QLD	173					
<i>A. imprimens</i>	99	1	QLD	79	<i>Culex</i>				
<i>A. kochi</i>	99	1	QLD	173	<i>C. annulirostris</i>		3	QLD	49
	11,405	2	QLD	79			8	NSW	224
<i>A. lineatus</i>	4,644	1	QLD	79		6,153	1	NSW	71
<i>A. multiplex</i>	2,531	1	QLD	173				VIC	131
<i>A. normanensis</i>	13,305	8	QLD	46,93		136,077	16	NSW & VIC	133
	≅40,000	15	NT	219		84,010	9	QLD	46,93
	28,909	6	WA	19		2,847	13	WA	228
<i>A. notoscriptus</i>		1	NT	219				VIC	128
		1	NSW	27		≅190,000	23	NT	219
	7,087	13	QLD	158			47	NSW	27
	637	1	QLD	79		9,475	23	QLD	158
<i>A. phaecasiatus</i>		1	NT	219			2	QLD	171
<i>A. polynesiensis</i>	267	6	Cook Islands	162		2,751	1	QLD	173
						30,541	9	QLD	79
<i>A. procax</i>		2	NSW	27	<i>C. australicus</i>			VIC	129
	4,266	4	QLD	158		759	2	WA	118
		1	QLD	171	<i>C. gelidus</i>	257	1	QLD	79
	1,478	1	QLD	173	<i>C. globocoxitus</i>		1	WA	114
<i>A. ratcliffi</i>		1	WA	116	<i>C. quinquefasciatus</i>	2,608	7	New Caledonia, Vanuatu, Wallis, and Horne Islands	58
<i>A. sagax</i>			VIC	129					
		2	WA	116					
<i>A. theobaldi</i>			VIC	129					
<i>A. tremulus</i>		1	WA	116					
<i>A. vigilax</i>	88	1	QLD	51					
	34,843	9	NSW	71		1,572	2	WA	118
		1	NSW	202	<i>C. sitiens</i>	1,207	1	WA	118
	≅40,000	2	NT	219		97	1	QLD	158
	119,817	77	NSW	170					
		30	WA	116	<i>Mansonia</i>				
	13,325	40	WA	118	<i>M. uniformis</i>		1	NSW	224
	5,517	9	QLD	158		898	1	QLD	173
		23	NSW	27	<i>M. septempunctata</i>	913	3	QLD	79
		5	WA	114	<i>Tripteroides</i> sp.		1	WA	116
		1	QLD	171					
	2,077	1	QLD	173					
	3,308	1	QLD	79					

^a The subgenera *Verrallina* and *Ochlerotatus* of the genus *Aedes* have recently been elevated to genus rank (155, 156). We have used the older nomenclature to avoid confusion with the existing literature.

^b Australian states and territories: NSW, New South Wales; NT, Northern Territory; QLD, Queensland; TAS, Tasmania; VIC, Victoria; WA, Western Australia. PNG, Papua New Guinea.

^c J. Aldred, J. Campbell, G. Davis, N. Lehmann, and J. Wolstenholme, letter, Med. J. Aust. 153:434, 1990.

and involves experiments with five genera and 20 species conducted in Australian and American laboratories (Table 7). To weigh the evidence on putative vector status, we use the first three criteria as well as the fourth criterion of field evidence linking infected mosquitoes with infected vertebrates, humans in the current context. We consider the major human vectors first, noting their distribution in Australia.

A. vigilax is absent from Tasmania and the Victorian coastline, but is otherwise continuously present along the Australian coastline and will disperse tens of kilometers from breeding sites, having been recorded as far as 320 km west from the Queensland coast (110). It appears intermittently in plague proportions in urban areas around much of the Australian coastline (110). Most commonly, this mosquito breeds in temporary bodies of saline to brackish water (110). It is mainly an evening biter and, in Australia, is exophilic, with indoor biting being exceptional (110). *A. vigilax* will feed on mammals and also on birds and reptiles when available (30, 110, 166). *A. vigilax* has yielded about 200 RRV isolates from a variety of areas (Table 6). RRV isolation rates from *A. vigilax* have been as high as 1 in 43 during epidemics in the Pilbara region in Western Australia; the timing of isolates is consistent with this species' being a vector for humans (118, 123). An outbreak of RRV and BFV infection occurred in Nhulunbuy, Northern Territory, in February 1992, and almost all the mosquitoes trapped were *A. vigilax* (140).

A. camptorhynchus occurs in New South Wales, Victoria, Tasmania, South Australia, and Western Australia and is a common salt marsh mosquito in coastal areas in the southern part of its range. Adults bite humans, domestic animals, and birds during the day and particularly after sunset (110). Over 100 isolates of RRV have been made from *A. camptorhynchus* in Western Australia, Victoria, New South Wales, and Tasmania (Table 6). Two studies have shown that *A. camptorhynchus* can be infected with RRV and that it can transmit the virus to suckling mice (Table 7). In 1991, an RRV isolate came from *A. camptorhynchus* trapped on the east coast of Tasmania, where at least six human RRV infections had occurred at around the same time, a relatively large number of cases in Tasmania (138). Campbell et al. (24) trapped mosquitoes in southeast Gippsland, Victoria, during a large outbreak. *A. camptorhynchus* was the most common species trapped, constituting 96% of the entire catch, and yielded four isolates of RRV. *A. camptorhynchus* infection rates as high as 1:30 have been recorded before and during outbreaks in coastal southwest Western Australia (123). During the course of an outbreak in Perth and the southwest of Western Australia in 1991 to 1992, Lindsay et al. (113) obtained 10 of 13 isolates from *A. camptorhynchus*. During an outbreak in southwest Western Australia in 1995 to 1996, 36 of 44 isolates came from *A. camptorhynchus* (114).

Culex annulirostris is widely distributed in all Australian states except Tasmania. This mosquito breeds in temporary or semipermanent ground waters and effluent run-off and is a crepuscular feeder both in- and outdoors (108). *C. annulirostris* feeds upon humans, other mammals including marsupials, and (rarely) on birds (97, 108, 145, 164, 165, 205). About 140 RRV isolates have come from *C. annulirostris* (Table 6). Four studies have shown that this species can be infected with RRV, while two demonstrated that it could transmit RRV to suckling mice (Table 7). Marshall and Miles (130) suggest that *C. annulirostris*

may be more important to maintain primary cycles than as an epidemic vector. Russell (167), however, notes that *C. annulirostris* was probably an epidemic vector in a 1983 to 1984 outbreak in New South Wales. During that outbreak, *C. annulirostris* numbers were well above average during the summer months (167), when most cases occurred (82). Marshall and Miles (130) and Russell (167) agree that *C. annulirostris* is predominantly an inland rural vector. However, RRV isolates have been obtained from *C. annulirostris* trapped in Queensland coastal areas, and it undoubtedly also plays a vector role in these areas (79, 158, 173).

Interest in *Aedes notoscriptus* as a possible vector of RRV was aroused when 13 isolates were recovered from mosquitoes trapped in Brisbane during an outbreak (158), although two isolates had been obtained from this species in the Northern Territory and New South Wales previously (Table 6). Ritchie et al. (158) also speculated that *A. notoscriptus* might play a role in RRV transmission to humans during winter, as they obtained an RRV isolate in June 1994. *A. notoscriptus* uses peridomestic larval habitats, readily bites humans, and has been observed feeding on poultry, marsupials, horses, and cattle (111). It feeds at all times during the day and night, but with peaks of activity close to sunset and sunrise (111). Four studies have shown that this mosquito can be infected with RRV and one that it can transmit the virus (Table 7).

Rosen et al. (162) obtained six isolates of RRV from *A. polynesiensis* collected on the island of Rarotonga, Cook Islands, in 1980 at the time of an outbreak. These are the only RRV isolates from this species. *A. polynesiensis* has been shown to transmit RRV in laboratory experiments (74, 141). It occurs on most islands in the eastern Pacific but not in Australia and breeds in natural and artificial containers (109, 162). *A. polynesiensis* commonly feeds on humans (109). While only six isolates have been obtained from *A. polynesiensis*, these were obtained at the time of an outbreak, and isolates were not obtained from *C. annulirostris*, a known vector. It is probable that *A. polynesiensis* was a vector in human-mosquito-human RRV transmission in the Cook Islands and probably other parts of the Pacific in 1979 and 1980.

RRV has never been isolated from wild-caught *A. aegypti* (Table 6), but four studies have demonstrated that this species can be infected with and transmit the virus (16, 74, 99, 141) (Table 7). Harley et al. (79) did not isolate RRV from 121 adult female *A. aegypti* collected in Cairns, Queensland, during 1997. *A. aegypti* is adapted to breeding around human habitation and will oviposit in artificial containers and cavities in trees (109). It feeds primarily on humans, is endophilic, and has peaks of biting activity in the early morning and late afternoon (182). The distribution of *A. aegypti* in Australia was reviewed in detail by Lee et al. (109). For Queensland from 1981 to 1983, Kay et al. (96) reported *A. aegypti* along the Pacific coast around Cairns and Townsville, along the northern coast at Normanton and Karumba on the Gulf of Carpentaria, and in the Torres Strait Islands, and inland in northwestern zones around Cloncurry, Winton, Dirranbandi, and Roma.

The foregoing outlines descriptive evidence linking mosquito species to human RRV disease. Three recent studies have moved beyond this by testing causal hypotheses regarding the location or abundance of mosquito breeding and the measured risk for human disease. Using a cross-sectional design in

TABLE 7. Experimental infection with and transmission of RRV by mosquitoes^a

Genus and species	Location	Infection or infectious dose ^b	Transmission	Reference
<i>Aedes</i>				
<i>A. aegypti</i>	Fiji	4.2 PFU	+	141
	Townsville, QLD	4.9 LD ₅₀	+	99
	Jakarta	+	+	74
<i>A. alboannulatus</i>	Townsville, QLD	3.8 PFU	+	16
	Southen NSW	+		16
<i>A. albopictus</i>	Houston	+	+	142
	Beijing	2.5 PFU	+	141
<i>A. albopictus</i>	Hawaii	2.6 PFU	+	141
	Shanghai	2.4 PFU	-	141
<i>A. albopictus</i>	Singapore	4.0 PFU	+	141
	Sri Lanka	4.1 PFU	+	141
<i>A. alternans</i>	Batemans Bay, NSW	+	-	217
<i>A. australis</i>	New Zealand	+	+	126
<i>A. camptorhynchus</i>	Batemans Bay, NSW	2.4 PFU	+	15
	Southern NSW	4.5 PFU		16
<i>A. funereus</i>	Brisbane, QLD	4.2 CCID ₅₀ V	+	175
	Redcliffe, QLD	+		100
	Maroochydore, QLD	4.5 CCID ₅₀ V	+	173
<i>A. multiplex</i>	Maroochydore, QLD	4.9 CCID ₅₀ V	-	173
<i>A. notoscriptus</i>	New Zealand	+	-	126
	Southern NSW	5.1 PFU		16
	Maroochydore, QLD	+	-	174
	Sydney, NSW	+	+	41
<i>A. notoscriptus</i>	Brisbane, QLD	3.2 CCID ₅₀ V	+	209
	Maroochydore, QLD	4.0 CCID ₅₀ V	-	173
	Tonga	+		104
<i>A. oceanicus</i>	Tonga	+		104
	<i>A. polynesiensis</i>	Raratonga	+	74
	American Samoa	+	+	74
	Fiji	<1.2 PFU	-	141
	Rarotonga	<1.2 PFU	+	141
	Samoa	<1.2 PFU	+	141
<i>A. procax</i>	Tahiti	<1.2 PFU	+	141
	Maroochydore, QLD	3.4 CCID ₅₀ V	+	173
<i>A. pseudoscutellaris</i>	Fiji	2.0 PFU	+	141
<i>A. rubrithorax</i>	Southern NSW	+		16
<i>A. tongae tabu</i>	Tonga	+	+	104
<i>A. vexans</i>	Tonga	+	+	104
	Batemans Bay, NSW	+		216
	Maroochydore, QLD	+	+	174
	Redcliffe, QLD	4.5 LD ₅₀	+	94
	Batemans Bay, NSW	3.4 CCID ₅₀ B		201
	Brisbane, QLD	+		98
	Maroochydore, QLD	3.8 CCID ₅₀ V	+	173
<i>Coquilletidia xanthogaster</i>	Brisbane, QLD	+		98
<i>Culex</i>				
<i>C. annulirostris</i>	Brisbane, QLD	+		98
	Brisbane, QLD	+	+	102
	Maroochydore, QLD	+	+	173
	Griffith, NSW	+		216
<i>C. australicus</i>	Maroochydore, QLD	4.5 CCID ₅₀ V	+	173
<i>C. quinquefasciatus</i>	Florida	-	-	74
	Brisbane, QLD	-		101
	Charleville, QLD	+		101
	Kowanyama, QLD	+		101
	Cairns, QLD	-		101
	Mildura, VIC	+		101
	Darwin, NT	+		101
	Maroochydore, QLD	+		173
	Tonga	+		104
	<i>Mansonia uniformis</i>	Brisbane, QLD	+	
Maroochydore, QLD		+	+	173

^a Adapted from reference 208. Also see Table 6, footnotes *a* and *b*.

^b Infection is indicated as + (positive virus infection or transmission to suckling mice) or - (negative virus infection or transmission to suckling mice). PFU, virus dose in log₁₀ Vero PFU per mosquito required to infect 50% (Vero cells are an African green monkey kidney fibroblast cell line). LD₅₀, virus dose in log₁₀ suckling mouse LD₅₀ per mosquito required to infect 50%. CCID₅₀, virus dose in log₁₀ CCID₅₀ per mosquito required to infect 50% in BHK (baby hamster kidney) cells, (B) or Vero cells (V).

the Peel region of Western Australia, Lindsay et al. (115) found that 99% of RRV cases occurred within 3 km of the breeding sites for *A. camptorhynchus* and *A. vigilax*. Dale and coworkers (37, 38; P. E. R. Dale, A. Muhar, L. Thalib, and E. Arito, Abstr. 3rd Nat. Conf. Mosq. Control Assoc. Aust., 1998) used remote sensing and Geographic Information Systems in the analysis of risk for RRV infection by mapping mosquito breeding sites and disease notifications. They showed that RRV notifications, age standardized by suburb, correlated with particular geographical features (wetlands, bushland, littoral vegetation, and riparian vegetation). Three of these vegetation types (wetlands, littoral, and riparian vegetation) are known to be suitable for mosquito breeding. Bushland would be expected to contain macropod reservoir hosts. While these studies cannot incriminate specific vector species, they do represent significant moves toward correlating human disease with environment. Ryan et al. (172) went further and related abundance of mosquito species trapped to standardized morbidity ratios for RRV notifications in one census district of Maroochy Shire in southeast Queensland from 1991 to 1996. They found a positive correlation between *C. annulirostris* and *Aedes funereus* numbers and square-root-transformed standardized morbidity ratios. This was not the case for *A. vigilax* or *Aedes procax*. Their study provides evidence linking the former two mosquito species to RRV human transmission in Maroochy Shire.

Taken together, the evidence regarding mosquito vectors for RRV points to the ecological heterogeneity of transmission settings and indicates that several vectors are involved in Australia. There is sufficient evidence to assume that *A. vigilax*, *A. camptorhynchus*, and *C. annulirostris* are vectors of human RRV infection. Other mosquito species, *A. notoscriptus* in particular, may be also be vectors in Australia. *A. polynesiensis* is a potential vector in the South Pacific.

EPIDEMIOLOGY OF INFECTION AND DISEASE

Antibody Prevalence Surveys

The 11 published antibody prevalence surveys include data from all Australian regions (except Tasmania and West Australia) and Southeast Asia and the Pacific Islands (Table 8); interpretation is complex because of different sampling methods and patchy geographical representation.

Only one survey used a formal population-based sampling scheme (61). The others used convenience samples, such as blood donors or patients with suspected virus or arbovirus infection (151, 215). Blood donor studies underrepresent children and may overestimate population antibody prevalence. Even in the one published study in which random, population-based sampling was used, children were underrepresented (61). Passive surveillance of submitted diagnostic sera, used in two of the surveys (44, 151), probably overestimated antibody prevalence, but this would depend on the symptomatology being investigated and the prevalence of other infections in the population sampled. These sources of bias must be considered when interpreting the published antibody studies.

Reported antibody prevalence differs among geographical areas. Doherty et al. (48) found an antibody prevalence of 19% in 78 sera from Brisbane, southeast Queensland, 64% for 258

sera from residents of Mitchell River Mission (now Kowanyama), Western Cape York Peninsula, Queensland, and 45% among 121 sera from aboriginal communities in northeast Queensland. Phillips et al. (151) found an antibody prevalence of 31.6% in 2,010 sera submitted for suspected viral or arboviral infections in Queensland. Boughton et al. (18) found an antibody prevalence of 12.8% in residents of New South Wales and Victoria. Weinstein et al. (215) found an antibody prevalence of 8.4% in South Australian blood donors. Among these studies there is a gradient of decreasing antibody prevalence from north to south. An exception was the study by Campbell et al. (24), showing relatively high antibody prevalence (25%) in residents of Gippsland, Victoria, for sera taken after an outbreak.

An increase in antibody prevalence across the 1980s is apparent when comparing the studies of Boughton et al. (18) and Hawkes et al. (83). The population comparisons involved specific towns and areas using data from the first paper in the second. Overall, antibody prevalences rose from 13 to 39%, suggesting that infection may be becoming more common in parts of New South Wales.

Most of the studies demonstrated increasing antibody prevalence with increasing age. Exceptions are the reports by Weinstein et al. (215) and Tesh et al. (196). The prevalence of antibodies was consistently a little higher among males than females when this was reported (18, 44, 83, 151, 189, 215) (Table 9).

The studies above show that risk of infection differs geographically and the prevalence of past infection is generally higher in the north than the south of Australia. With two exceptions, the studies show that antibody prevalence increases with age. Unless this is an age cohort effect, the age pattern indicates a relatively constant age-specific risk for infection. This contrasts with risk for disease, which has generally been shown to be highest for young adults. Behavioral and environmental risks for disease would presumably be similar to those for infection, but host characters may modify the age-specific risk for disease relative to that for infection. If so, this would account for the different age distributions of infection and disease. Antibody prevalence surveys show that prevalence of past infection is slightly higher in males. Male and female risks may vary due to differing behavior.

Asymptomatic to Symptomatic Case Ratio

The first estimate of the asymptomatic to symptomatic case ratio, 50:1, comes from north and central Queensland in 1978 to 1979 (6). The second, 3.0:1, is from an epidemic in Fiji in 1979 (4). The third estimate, 0.3:1, is from a study by Hawkes et al. (82) of an RRV epidemic in New South Wales during 1983 to 1984. The fourth estimate, 1.2:1, comes from the experience of Marines in a joint U.S. and Australian military exercise at Shoalwater Bay, Queensland, during March 1997 (171). Only the second (3.0:1) and last (1.2:1) estimates are reliable.

The Queensland estimate by Aaskov et al. (6) was based on incidences of 0.37 and 15 to 35 per 1,000 per annum calculated for symptomatic and asymptomatic infection, respectively. The ratio was therefore between 41:1 (15/0.37) and 95:1 (35/0.37); it was originally given as 50:1 but more recently has been

TABLE 8. RRV antibody prevalence surveys in human populations

Study period	Population and sampling scheme ^a	Method ^b	No. tested	Antibody prevalence (%)	Reference
1957–1964	Aboriginal communities in north and other parts of QLD, including Brisbane and the Torres Strait, sampling scheme not specified	HI, N	636 ^c	50 ^d	48
1966–1971	Aboriginal communities in north QLD & NT, sampling scheme not specified; symptomatic patients from eastern QLD	HI	2,148	47 ^e	44
1958–1974	Surveys for melioidosis, arboviruses, parasites, and sera taken for other reasons in SE Asia and Pacific Islands	N	3,178	55 ^f 42 ^g 15 ^h	196
1974	Random sampling of households in Echuca, VIC	HI	739	15	61
1974–1975	Children under 11 years from north central and southeast QLD and Darwin, NT, sampling scheme not specified	HI	2,730	8	189
1978–1979	Residents of Yeppoon, Townsville, and Innisfail, QLD, sampling scheme not specified	HI	345	22 ^d	6
1981–1982	Sera from blood donors to NSW and VIC blood transfusion services, RFDS Broken Hill, hospital, commonwealth, and private pathology laboratories in NSW	HI	16,842	13	18
1988	Sera from residents of Raymond Island and Loch Sport, Gippsland, VIC, sampling scheme not specified	ELISA	523	25	24
1989	Sera referred to QLD Health Laboratory of Microbiology and Pathology from patients with suspected viral or arboviral infections	HI, ELISA	2,010	32	151
1991–1992	Sera from permanent residents of western NSW and northern VIC from selected districts and with equal representation of age groups 0–9, 10–19, 20–29, 30–39, 40–49, 50–59, and ≥60 yr	HI	2,635 ⁱ	39	83
1992	Donors to the SA Red Cross Blood Transfusion Service	ELISA	2,952	8	215

^a Abbreviations: QLD, Queensland; NT, Northern Territory; SE Asia, Southeast Asia; VIC, Victoria; RFDS, Royal Flying Doctor Service; NSW, New South Wales; SA, South Australia.

^b HI, hemagglutination inhibition; N, neutralization; ELISA, enzyme-linked immunosorbent assay.

^c Number tested for HI antibody; 308 of these were tested for N antibodies.

^d Prevalence on HI testing; 45% of 308 sera showed antibodies on N testing.

^e Result on HI testing of 507 sera from north Queensland aboriginal communities.

^f Result on HI testing of 178 sera from Northern Territory aboriginal communities.

^g Result of HI testing of 1,463 sera from eastern Queensland submitted from patients with “encephalitis, pyrexia, polyarthritis, hepatitis and occasionally other conditions” (44).

^h Antibody prevalence given for entire survey of sera from Taiwan, Malaysia, Phillipines, Indonesia, Papua New Guinea, Solomon Islands, New Hebrides, New Caledonia, Samoa, and Caroline Islands. Antibody prevalence as high as 64% in two Sepik villages in Papua New Guinea.

ⁱ 2,873 sera included in study, but results only given for 2,635 sera tested for RRV antibodies.

reported as 80:1 (95). Neither figure is justified. Incidence of symptomatic infection was calculated by dividing the number of cases recruited via family physicians by the estimated population of the study area. To determine the incidence of asymptomatic infection, 171 people were studied serologically

TABLE 9. Sex distribution of antibodies to RRV

% Positive (no. tested)		Male-female ratio ^a	Reference
Male	Female		
45 (727)	39 (736)	1.2	44
8 (1,532)	7 (1,198)	1.1	189
34 (1,046)	29 (964)	1.2	151
17 (—)	11 (—)	1.6	18
45 (871) ^b	36 (1,274) ^b	1.3	83
10 (1,447)	7 (1,505)	1.4	215

^a Overall mean male-female ratio, 1.3.

^b These do not add to the total 2,873 sera tested because no data on sex-specific antibody prevalences are given for two districts.

twice over 1 year to detect fourfold rises in RRV HI titer. Sixty-one percent of the asymptomatic subjects were soldiers from Townsville; they were not living within the area where cases were reported. Given their occupation, they were not likely to have resided in Townsville for a long period. So the populations from which symptomatic and asymptomatic infections originated were not comparable. Six of 171 (35 of 1,000) showed a fourfold rise in HI titer. With only six asymptomatic infections to estimate incidence, the confidence intervals would be wide. An incidence range of 15 to 35 asymptomatic infections per 1,000 per annum is reported, without stating how the lower estimate was obtained. The ratios estimated are likely to be too high because symptomatic cases were probably undercounted.

An RRV epidemic began in April 1979 on the Island of Viti Levu in Fiji, particularly around Nadi. In the nearby village of Nawaka, 97 of 416 people (23.3%) reported joint pain. Overall, 386 villagers had HI antibodies to RRV after the epidemic (4).

Assuming that antibodies reflect infections during the epidemic and that all symptomatic cases were counted, the asymptomatic-symptomatic ratio was 289 to 97 or 3.0:1. If the preepidemic prevalence of antibodies was 13%, as suggested by Aaskov et al. (4), the estimate becomes 2.5:1. However, other Fijian research suggests that preepidemic antibody prevalence would have been zero (12, 130), making 3.0:1 the correct estimate. This estimate is based on clinical and serologic assessment of a single population. The population is well defined, so this is likely to be an accurate estimate of the asymptomatic to symptomatic case ratio, at least for symptomatic disease with arthralgia in Fijians.

Hawkes et al. (82) estimate that 257 of an estimated 340 infections in Griffith Shire, New South Wales, during a 1983 to 1984 epidemic were symptomatic, an asymptomatic-symptomatic ratio of 0.3:1. The probable number of infections, 340, was extrapolated from the appearance of antibody in 2 of 94 blood donor sera after the epidemic. It was assumed that all cases of RRV disease were counted in Griffith Shire. No case definition for RRV disease was given. The small number of seroconversions in blood donors and other methodological problems invalidate this estimate.

In March 1997, 1,300 U.S. Marines were involved in Operation Tandem Thrust at Shoalwater Bay, Queensland. There were nine cases of RRV disease. On the basis of serologic study of 938 Marines, the infection rate was 1.5% (171). Therefore, there would have been 20 infections expected among the 1,300 Marines, making the asymptomatic-symptomatic ratio 11 to 9, or 1.2:1.

Superficially, there appears to be considerable variation in asymptomatic-symptomatic ratios. Indeed, Kay and Aaskov (95), noting the enormous difference between the estimates of Aaskov et al. (6) and Hawkes et al. (82), suggest that the asymptomatic-symptomatic ratio differs in endemic and epidemic transmission, but those two estimates were too unreliable to permit such conclusions. The only reliable estimates are those based on the Fijian data of Aaskov et al. (4), giving an asymptomatic-symptomatic ratio of 3.0:1 during epidemic transmission in Fiji in 1979, and the experience of visiting U.S. Marines in a high-risk area of Queensland in 1997, giving a ratio of 1.2:1 (171). The ratios in other epidemiological circumstances or among other human populations are unknown.

Notifications and Incidence

There have been no population-based studies of the incidence of infection or disease; notifications collected by state and federal health authorities in Australia are the only source of information. Notification to state health departments, the National Notifiable Diseases Surveillance System, and the Federal Department of Human Services and Health through the LabVISE system (a sentinel scheme collecting data on viruses and other infectious agents from virology and serology laboratories) requires at least a single positive test for IgM, a presumptive diagnosis (121). The majority of notifications in Australia are presumptive diagnoses (35, 36).

The average annual number of notified cases in Australia from 1991 to 2000 was 4,745, peaking at 7,823 in 1996 (Communicable Diseases Network—Australia New Zealand, <http://www.health.gov.au/pubhlth/cdi/nndss/year002.htm>). During

TABLE 10. Australian RRV notifications per 100,000 per annum^a by state or territory of notification, 1991 to 2000^a

Year	No. of notifications ^b							
	ACT	NSW	NT	QLD	SA	TAS	VIC	WA
1991	0	7	281	72	0	0	9	9
1992	1	5	134	137	5	0	3	44
1993	1	10	138	77	57	0	28	11
1994	0	5	171	99	2	0	1	6
1995	1	4	208	52	2	6	1	12
1996	0	17	69	149	2	16	3	86
1997	3	27	116	70	46	3	24	40
1998	2	8	62	56	4	2	3	19
1999	2	17	71	64	3	14	6	30
2000	5	12	76	41	26	2	7	67

^a Population denominators from Australian Bureau of Statistics, Census of Population and Housing, 1996, which includes 1991 and 1996 census data. Populations for intercensus and postcensus years calculated using simple linear interpolation. Numbers of notifications from Communicable Diseases Network, Australia New Zealand, (<http://www.health.gov.au/pubhlth/cdi/nndss/year002.htm>, updated 15/2/2001).

^b ACT, Australian Capital Territory; NSW, New South Wales; NT, Northern Territory; QLD, Queensland; SA, South Australia; TAS, Tasmania; VIC, Victoria; WA, Western Australia.

1992 there was a reported national incidence of 36.4 per 100,000 per annum (227). The reported incidence in Queensland was nearly four times higher, 139.6 per 100,000 per annum—the highest incidence for any Australian state (227). The zone of highest incidence within Queensland, 278.4 per 100,000 per annum, was in the Northern Statistical Division (local government areas of Townsville, Bowen, Burdekin, Charters Towers, and Dalrymple in North Queensland) (227). During 1992 the reported incidence in Cairns was 148.1, in Townsville was 321.3, and in Brisbane was 95.7 per 100,000 per annum (152). This figure was probably atypical for Brisbane—from 1989 to 1991, the highest incidence per 100,000 per annum was 28.7 (152). But for Cairns and Townsville, the incidence was always above 100, an order of magnitude greater than for Brisbane, reflecting the increased RRV risk for coastal areas in tropical compared with subtropical zones (152). In 1998 the national incidence was 16.5 per 100,000, and 63% of notifications came from the state of Queensland (198). As in 1992, the highest incidence occurred in the Northern Statistical Division, though the central Queensland Fitzroy Statistical Division had a similarly high incidence (over 150 per 100,000 per annum) (198).

Overall, over recent years the likelihood of a person's becoming infected has varied markedly in the temperate zone states and has been stable in the entirely tropical areas of Queensland and the Northern Territory (Table 10). High incidences of human infection must relate to high numbers of vector mosquitoes and immunologically susceptible human and other vertebrate hosts, with temperature, rainfall, and other environmental factors also playing a role. Season certainly is important, with far more cases occurring in summer and autumn (Table 11).

Age and Sex of Cases

RRV disease occurs most commonly among adults aged between 25 and 39, with the age groups most commonly affected being 25 to 39 (144), 30 to 39 (33), 30 to 44 (181), 30 to

TABLE 11. Australian RRV notifications by month of onset, 1991 to 2000^a

Year	No. of notifications												Total
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
1991	128	605	800	885	501	201	119	74	44	64	46	65	3,532
1992	163	608	1,537	1,232	642	536	168	85	213	131	144	171	5,630
1993	254	820	1,809	827	539	230	299	123	124	118	140	145	5,428
1994	383	825	858	725	504	252	97	57	47	77	57	78	3,960
1995	182	226	261	439	631	433	99	59	63	60	96	53	2,602
1996	239	1,511	2,699	1,554	805	332	205	87	52	72	103	164	7,823
1997	498	1,116	1,263	1,625	1,107	463	195	107	59	63	74	116	6,686
1998	234	270	625	507	467	126	79	67	71	56	198	396	3,094
1999	390	650	871	935	617	277	100	110	72	94	112	179	4,407
2000	454	700	753	613	770	323	120	69	74	110	144	158	4,288

^a Source: Communicable Diseases Network, Australia New Zealand (<http://www.health.gov.au/pubhlth/cdi/nndss/year002.htm>, updated 15/2/2001).

39 (82), and 50 to 59 (218) years. There is no clear sex effect. The male-to-female ratio has been reported as 0.6:1 (144), 1.4:1 (33), 0.8:1 (82), 1.7:1 (218), and between 0.7:1 and 1.3:1 according to age (181). The sex ratios given above for notified cases and those reported for antibody prevalence surveys (Table 9; male-female ratio, 1.3:1) are very similar, suggesting that determinants of RRV infection and disease do not differ much by sex. As noted in the section on antibody prevalence surveys, RRV antibody prevalence usually increases with age, even though the preponderance of RRV disease cases occur in young adults. Age-specific risks for RRV infection and disease must differ, presumably because of age-related differences in the immune response to infection.

Transmission Cycles

Nonhuman vertebrates are usually involved in the maintenance of arboviruses (178). By analogy with the New World members of the genus *Alphavirus* (213), and on the basis of the evidence presented above, RRV is maintained as an enzootic infection and is transmitted to humans during epizootic and epidemic periods.

In certain areas, vertical transmission in mosquito vectors occurs. Field and laboratory data suggest that vertical transmission occurs in *A. vigilax* (94, 118), *Aedes tremulus* (118), and *A. camptorhynchus* (40). Dessication-resistant *Aedes* eggs can survive for several months away from water (29), so these could provide an important means of virus maintenance.

Rosen et al. (162) provided the first strong evidence for human-mosquito-human transmission by isolating RRV from 51 patients in the Cook Islands, demonstrating viremias high enough to contribute to the transmission cycle. Marshall and Miles (130) consider it a "virtual certainty" that human-mosquito-human transmission was maintained during the outbreak in the South Pacific in 1979 and 1980. They point to the low seroprevalence in nonhuman vertebrates, the rapid spread of the epidemic, and the demonstration of high-titered viremia in humans (162, 197). Tesh et al. (197) found a neutralizing antibody prevalence of 43.8% in 393 human sera collected early in the epidemic. Among animals tested for neutralizing antibodies, the highest prevalence was for dogs, with 20% of 45 being positive; pigs, chickens, and rats had lower prevalences, and no antibody was detected in banded rails (a flightless bird) or cats (197). It is implausible that such a high human seroprevalence, so soon after the onset of the epidemic, could have

been associated with zoonotic infection without higher seroprevalence among animals, unless the animal responsible for enzootic transmission was not studied.

Although there is some contention, RRV was probably not present in Fiji before the epidemic and appears not to have become established enzootically after the epidemic (4, 12, 130). This would fit most plausibly with the virus infecting humans largely or exclusively, and with the absence of suitable nonhuman vertebrate reservoirs. In the South Pacific, virus was isolated from humans with RRV disease symptoms by four groups, a feat not so easily achieved in Australia, suggesting higher viremias for the Pacific outbreaks (4, 57, 162, 197). Lindsay et al. (116) suggest that human-mosquito-human transmission was likely in an outbreak in suburban Perth from 1991 to 1992 because the first reported infection in several suburbs appeared to have been acquired outside Perth, but all subsequent infections were locally acquired. Overall, it seems that under appropriate circumstances RRV may be maintained for some time in a human-mosquito-human cycle, but that maintenance involves enzootic cycles and, in some environments, vertical transmission in mosquitoes.

Infection by nonmosquito transmission is probably unimportant. The evidence on human transplacental transmission is conflicting (5, 8). Transmission in blood transfusions has been suggested but has not been investigated (P. Weinstein, S. R. Weinstein, and R. J. Rowe, letter, *Med. J. Aust.* **163**:276, 1995).

PUBLIC HEALTH ASPECTS

Costs of RRV Disease

A conservative cost estimate for RRV disease in Australia can be made on the basis of the average number of notified cases from 1991 to 2000 (4,745 per year). Most notifications would have been made on the basis of a positive IgM test (35, 36), although generally IgG and IgM testing would be performed together. The cost of the testing is A\$23.80 (one Australian dollar is worth approximately US\$0.50) per test for IgG and IgM ELISA, plus a collection fee of A\$14.60, giving a total of A\$38.40. At times of low and high disease incidence, the proportion of tests that are positive varies between less than 5 and 25%, respectively, for a pathology laboratory doing a large amount of the testing in Queensland (I. Gardner, personal communication, 1999). Assuming that 5 to 25% of tests are

positive for the whole of Australia, the number of tests ordered to diagnose 4,745 cases will range from 18,980 to 94,900. The cost for testing will therefore be A\$728,832 to A\$3,644,160 in an average year, and this excludes testing performed to exclude other causes of arthralgia. Serologic testing for RRV clearly represents a considerable expenditure from the Australian health budget, and testing to exclude important differential diagnoses costs even more.

There are also costs for medical care. A consultation with a family physician costs A\$22.95. If each of 4,745 RRV disease cases attends a family physician twice, once to have the testing arranged and once for counseling and to arrange treatment, the cost will be A\$217,796 per annum, not including the cost of medication.

Westley-Wise et al. (218), Condon and Rouse (33), and Selden and Cameron (181) found that 62, 49, and 40%, respectively, of cases took at least 1 week off from work as a result of RRV disease. The average Australian weekly earning before tax for full-time work as of May 1999 was A\$749.40 (13). Therefore, lost earnings, if half of the cases of RRV disease in an average year take 1 week off work, will be A\$1,777,952.

The calculations above reveal that testing for RRV disease and treating it, and lost earnings as a result of it, are costing Australia between A\$2.7 and A\$5.6 million in an average year, without counting the substantial cost of other investigations, treatment, or decreased work output extending beyond 1 week. To calculate the full costs, one would also have to take into account the intangible costs of pain and suffering.

Potential for Epidemics and Dispersal

The factors determining human risk for RRV disease are complicated and poorly understood. Conditions necessary include adequate populations of reservoir hosts (potentially humans in some settings), vector mosquitoes, and appropriate climatic conditions for transmission. Within Australia, these conditions occur every year in tropical areas and less frequently in southern parts of the continent. To spread to other countries, these conditions must prevail and the virus must be introduced. This probably involved a viremic human in the South Pacific epidemic of 1979 and 1980. In late April 1979, eight cases of polyarthritides and rash, now known to have been RRV disease, were notified from a medical practice near Nadi International Airport in Fiji. For about 10 days reported cases were only from this area. Subsequently, the disease spread rapidly throughout the Fiji Islands, causing an estimated 50,000 clinical cases. In Fiji the epidemic occurred mainly from April to June 1979, with few cases being reported by September 1979, though laboratory-proven cases were reported until September 1980 (4, 130). In August 1979 the disease spread to American Samoa, where it was estimated 13,500 people were infected (130, 197). Then, between February and March and between January and June 1980, RRV disease cases occurred in the Cook Islands and New Caledonia, respectively (56, 130, 162). The number of cases in the Cook Islands is unknown; 32 cases were serologically confirmed in New Caledonia, but there were undoubtedly a larger number of infections (56, 130, 162). It is probable that a viremic traveler introduced RRV to Fiji, but introduction by a viremic animal is also theoretically pos-

sible, as may have occurred, for example, in the case of West Nile virus in New York (154).

The South Pacific epidemic suggests that RRV can be transported via viremic humans. However, the fact that RRV epidemics have not occurred elsewhere suggests either that suitable vectors and reservoir hosts are not present or that arrival of viremic humans from Australia and Papua New Guinea in receptive areas is an uncommon event. The history of the South Pacific epidemic suggests that even if an epidemic can be sustained for some time, endemicity will not be established unless reservoir hosts are present. While RRV disease cases present and are diagnosed in areas outside the endemic countries (153), the likelihood of arrival of a viremic person is small.

The arbovirus extrinsic incubation period is inversely related to temperature (200), so the risk for outbreaks is lower in colder countries. Medical practitioners in countries where RRV is not endemic should be aware of RRV, and if a case is diagnosed, this should be discussed with local public health authorities. Isolation of the patient from mosquitoes is appropriate. If enzootic cycles involving marsupials are necessary to maintain the virus, there is little likelihood of endemicity developing in countries without marsupials. However, the virus's ability to infect multiple mosquito and vertebrate species indicates its potential to become established in new tropical and subtropical areas of the world.

Population Health Interventions

Aaskov et al. (2) advocate blood donor surveillance for RRV, but there are no population health measures of proven effect to activate when increasing numbers of infections are detected. Mosquito control by spraying may decrease transmission and community concern. A candidate vaccine for RRV exists (1, 229), but the cost of vaccine trials is hard to justify for a disease that occurs only in Australia and Papua New Guinea and is never fatal.

A recent North Queensland case-control study suggested that individual efforts to decrease risk were effective, particularly the use of repellents, citronella candles, mosquito coils, and light-colored clothing (78). It also showed a significantly increased risk associated with camping in that setting, consistent with enzootic transmission involving macropods. There is evidence that *A. notoscriptus* is a vector of RRV (41, 209). This mosquito, as discussed in the section on mosquito vectors, breeds in containers around dwellings. In coastal parts of north Queensland, bromeliads (an ornamental plant that holds water in which mosquitoes breed) were found to increase risk for RRV disease (78). This observation is consistent with a vector role for *A. notoscriptus*. Therefore, reduction of mosquito breeding around dwellings should decrease risk. Health education regarding decreasing individual risk, particularly when in the bush in areas of high endemicity or during epidemics, should be given greater emphasis in campaigns to control RRV disease.

CONCLUSIONS

RRV causes at least 4,745 disease cases in Australia per annum, on average. Conservatively estimated, the yearly cost is \$2.8 to A\$5.7 million. The infection is also endemic in Papua New Guinea, but the number of cases there is not known and

other health problems are more important. RRV can spread to other countries; already it has caused large epidemics in several nonendemic South Pacific countries, but fortunately did not become established there permanently.

An enormous literature exists on the mosquito and vertebrate associations of RRV, but little is known about the human risks for infection and disease. Research should continue on the vectors and vertebrate hosts posing RRV risks for humans, especially in tropical Australia where the risk is high. We also need much more research on the environmental and behavioral determinants of infection. Such work could yield evidence for public health measures to reduce incidence by planning to avoid mosquito breeding or vertebrate hosts in suburban areas, or through behavioral measures to reduce the chances of being bitten by vector mosquitoes.

RRV disease causes considerable morbidity, and the limited data available on natural history suggest that many patients are unwell for weeks to months but then recover. Basic research on immunology and pathology of RRV disease may lead to better therapy in place of the current ad hoc approach to treatment. To enhance the prospects of generating funds for the research needed, more accurate information is needed on RRV morbidity along with better estimates of the overall economic burden.

ACKNOWLEDGMENTS

This review arose from a Ph.D. completed by David Harley at the Australian Centre for International and Tropical Health and Nutrition of the University of Queensland. An Australian National Health and Medical Research Council Dora Lush Biomedical Scholarship and the Royal Australian College of General Practitioners provided financial support.

David Bossingham trained D. Harley in rheumatologic examination. Di James is thanked for drawing the map (Fig. 1) and the graphs (Fig. 2 to 5). Comments from two anonymous reviewers were also greatly appreciated.

ADDENDUM IN PROOF

Tong et al. (S. Tong, P. Bi, J. Hayes, K. Donald, and J. MacKenzie, *Am. J. Trop. Med. Hyg.* **65**:171–176, 2001) have recently shown that the number of localities in Queensland giving notice of RRV cases increased significantly during the years 1985 to 1996. This fits with the pattern of increasing prevalence of past RRV infection noted in antibody prevalence surveys in New South Wales during the 1980s (C. R. Boughton, R. A. Hawkes, H. M. Naim, J. Wild, and B. Chapman, *Med. J. Aust.* **141**:700–704, 1984; R. A. Hawkes, J. Pamplin, C. R. Boughton, and H. M. Naim, *Med. J. Aust.* **159**:159–162, 1993) and suggests that both the incidence and the geographic distribution of RRV are increasing. Another significant discovery is that of a new species of *Alphavirus* in sub-Antarctic elephant seal populations, transmitted by seal lice (M. La Linn, M. J. Gardner, D. Warrilow, G. A. Darnell, C. R. McMahon, I. Field, A. D. Hyatt, R. W. Slade, and A. Suhrbier, *J. Virol.* **75**:4103–4109, 2001), further evidence of the extraordinary diversity and adaptability of this genus of arbovirus.

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