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## Ross River Virus: An Arthritogenic Alphavirus of Significant Importance in the Asia Pacific

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### Abstract

Ross River virus is a mosquito transmitted disease endemic in tropical Australia, Papua New Guinea, East Timor, adjacent islands of Indonesia and the Solomon islands. It occurs epidemically in temperate Australia and sporadically in Pacific Islands, such as Fiji, Tonga, Samoa and the Cook Islands. It is the most common arbovirus disease in Australia. The disease occurs mainly in adults, with clinical symptoms rare before puberty. The symptoms are rash, joint pain and general effects such as fatigue, fever and muscle pain, which appear from 3 to 21 days post-infection and can persist for 3–6 months. In Australia, Ross River virus is responsible for an average of 8,000 cases annually. The long-term effects of Ross River virus disease are thought to be due to the virus's ability to evade the patient's immune system. Antibodies produced against the virus may be insufficient to neutralise it and may even improve the ability of the virus to infect host cells (antibody-dependent enhancement). The virus is also capable of persisting for long periods in macrophages and may be reactivated during times of stress. Various human host proteins may also increase Ross River virus infection rates and contribute to disease symptoms.

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Ross River virus (RRV) belongs to the *Togaviridae*, a family that consists of RNA viruses, and are primarily arboviruses (arthropod-borne viruses) [1]. The family comprises of two genera, *Rubivirus*, a genus containing only one recognised

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**Table 1.** Alphaviruses and other recognized RNA viruses

Virus name	Family/genus	Distribution/characteristics
Semliki Forest	<i>Togaviridae/Alphavirus</i>	Africa, Eurasia; encephalitic
O'nyong-nyong	<i>Togaviridae/Alphavirus</i>	Africa; arthralgia, rashes
Ross River	<i>Togaviridae/Alphavirus</i>	Australia, Oceania; arthritis, rashes
Barmah Forest	<i>Togaviridae/Alphavirus</i>	Australia, Oceania; arthritis, rashes
Chikungunya	<i>Togaviridae/Alphavirus</i>	Africa/Asia; arthralgia, rashes
Sindbis	<i>Togaviridae/Alphavirus</i>	Australia, Oceania; arthritis, rashes
WEE	<i>Togaviridae/Alphavirus</i>	North/South America; encephalitis
EEE	<i>Togaviridae/Alphavirus</i>	North/South America; encephalitis
<b>Southern Elephant Seal</b>	<b><i>Togaviridae/Alphavirus</i></b>	<b>Antarctic territories, Macquarie Is.</b>

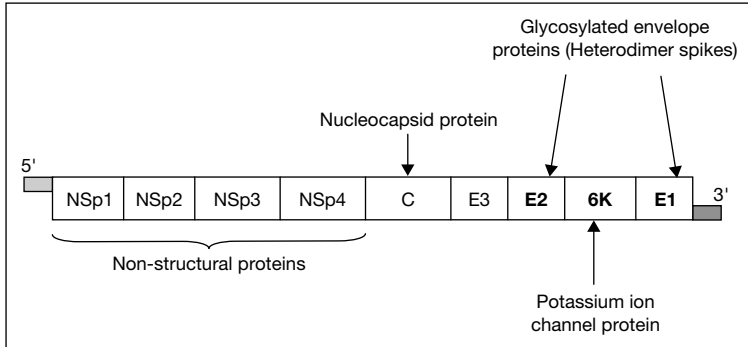
There are currently 26 known alphaviruses which are distributed on all the inhabited continents. RRV is limited to Australia and the neighbouring Pacific Islands. Some alphaviruses arose following a genomic recombination event, while others known as Old World viruses share ancient origins. Most alphaviruses cause diseases in humans, but animals such as horses, marsupials, birds and fish can also be carriers. The most recently identified Alphavirus, Southern Elephant Seal Virus SESV (in **bold**), was found in tick louse (*Lepidophthirus macrorhini*) carried by Southern Elephant seals, marine mammals indigenous to marine Antarctic territories.

species, rubella virus, and *Alphavirus*, the genus which includes 26 recognised members classified antigenically into seven complexes [2, 3] (table 1). These complexes are segregated into New World (American) and Old World (Eurasian-African-Australasian) viruses depending on the nucleotide sequence of their non-structural proteins [4]. An Old World virus, RRV belongs to the genus *Alphavirus* and is a subtype of Getah virus (GETV) in the Semliki Forest virus (SFV) serological complex. Many members of this genus are important causative agents of disease in humans and other animals [5] (table 1). Most alphaviral infections give rise to transient, debilitating diseases such as arthritis, whilst others may infect the central nervous system, potentially causing encephalitis [6].

### Virion and Genomic Properties

Alphaviruses are small spherical, enveloped viruses with a single stranded, positive-sense RNA as the genome [5]. The virion of alphaviruses consists of four major components: the genomic RNA, the nucleocapsid core, the plasma membrane and the glycoprotein shell [7, 8].

The glycoprotein shell consists of 80 'spikes' with each spike comprising a trimer of heterodimers of two glycosylated envelope proteins: E1, the hemagglutinin (52 kDa) and E2, the neutralising antigen (49 kDa) [1, 9]. The E1 protein



**Fig. 1.** The genome of Ross River virus (prototype T48) is 11851 nucleotides long, and is flanked with a 5' cap and a 3'-poly-A tail. Structural proteins form the rest of the genome, encoding for viral envelope glycoproteins and nucleocapsid proteins. E1 (haemagglutinin) assembles with E2, the neutralising epitope, to form surface heterodimers across the lipid bilayer. E1 also forms heterotrimers, which assemble as hollow-base tripartite heads within the bilayer, and is believed to be involved in membrane fusion. The third glycoprotein, E3, is not involved in virion structure. The 6K protein has been associated with the formation of cation-selective ion channels in lipid bilayers. Non-structural proteins are encoded by genes nsP1, nsP2, nsP3 and nsP4, which later assemble as two polyproteins after post-translational cleavage.

forms the core of the trimeric spike, whilst the E2 protein is found primarily on the outer surface. The glycosylated spikes penetrate the host cell-derived plasma membrane and interact directly with the nucleocapsid core. The E1-E2 heterodimers, of which there are 240 in total, form 1:1 associations between E2 and nucleocapsid monomers across the lipid bilayer [1]. The nucleocapsid (40 nm in diameter) has an icosahedral symmetry and is surrounded by a 4.8-nm-thick lipid bilayer. Within the core structure are 240 copies of the single nucleocapsid protein, C (32 kDa), and a single copy of the genomic RNA [9].

The genome is approximately 11.7 kb in length, varying marginally between the different alphaviruses [10]. Encoded in the 5' two thirds (7.6 kb) of the genome are the non-structural proteins (nsP1-4) that are involved in genomic replication and mRNA synthesis, whilst the 3' third (4.1 kb) of the genome encodes the structural proteins of the virus (fig. 1). These consist of the capsid protein, three envelope glycoproteins and an additional 6K protein, which are all translated from the subgenomic mRNA as a single polyprotein in the order C-E3-E2-6K-E1 [1, 10]. In RRV-infected cells, the 6K proteins have been shown to form cation-selective ion channels in the lipid bilayer, thus increasing the permeability of the cells to monovalent cations and aiding the process of virion budding [10]. It should be noted that the E3 glycoprotein is not incorporated into the virion. The function of E3 and the reasons as to why it

is not incorporated are unknown [7]. The prototype RRV (T48) genome is an RNA molecule of 11.8 kb, excluding the poly (A) tail, and consists of three regions that are strongly conserved between the alphaviruses. These are believed to play roles in the regulation of viral replication and essentially are composed of a tract of 23 nucleotides situated next to the poly (A) tail at the 3' end, 21 nucleotides at the 3' terminus of the nsP4 gene and 50 nucleotides near the 5' end of the nsP1 gene [1].

## **Virus Transmission and Propagation**

Alphaviruses are largely mosquito-borne viruses, with mosquitoes the primary vectors for transmission and propagation of disease in nature. Propagation of these viruses occurs via a horizontal cycle involving mosquito vectors and vertebrate hosts, i.e. transmission from mosquito to vertebrate to mosquito. The main mosquito vectors are *Culex annulirostris* in inland areas and *Ochlerotatus vigilax*, *Verrallina funerea* and *Ochlerotatus camptorhynchus* in coastal regions [11].

RRV in particular is only transmitted in placental and marsupial mammals and the virus is maintained in the environment by the marsupials [1, 12]. Serological studies and laboratory investigations have indicated that native marsupials, specifically kangaroos and wallabies, are the primary natural reservoirs or hosts of RRV, however many others have also been considered [6]. In addition, RRV transmission from human to mosquito to human, without the involvement of a marsupial, has also been proposed. There is presently little doubt that such a cycle involving only humans and mosquitoes occurs frequently during periods of intense virus activity, e.g. during the summer and autumn months when mosquito vectors are most abundant [1, 13].

## **Ross River Virus Disease**

### *Discovery of RRV and Viral Activity*

Disease, thought to be caused by RRV, was first reported in 1928 when an unusual epidemic, resulting in temporary arthritis and rash, occurred in Narrandera and Hay in New South Wales [14, 15]. During the Second World War, epidemics of similar symptoms were described in the tropical regions of Australia and on the islands to the immediate north [13]. It was as a result of these reports, that in 1936 the name epidemic polyarthritis (EPA) was used to describe the disease caused by RRV [1]. In 1956, in the Murray Valley, the first large epidemic was recorded. However, the causative agent was not isolated until 1963 by Doherty and colleagues, from a pool of *Aedes vigilax* (now called

*Ochlerotatus vigilax*) mosquitoes collected near the Ross River in Townsville, North Queensland [1, 16]. Introduction of the virus to several islands of the South Pacific (Fiji, Cook Islands and Samoa) in 1979 resulted in an explosive epidemic, the largest recorded, with more than 50,000 cases of disease reported [17]. In Fiji the polyarthritis outbreak was dramatic and, following the epidemic, up to 90% of residents in some areas were serologically positive for RRV [18]. In American Samoa it was estimated, on the basis of serological tests, that at least 13,500 people were infected. Sera from 393 humans on Tutuila Island showed evidence of infection in 43.8% of the people sampled. In this same island, sera from 100 adults collected before the epidemic had no RRV antibodies, suggesting a recent introduction of the virus [19]. The Cook Islands were the easternmost reach of the epidemic, which affected the majority of the inhabitants of Rarotonga, the most populated island in the group. The virus was isolated from half of the serologically proven infections [20]. Although since 1981 no RRV outbreaks have been reported in the Pacific islands, these regions are not safe from new epidemics. At the beginning of 2004 two Canadian tourists contracted the disease while visiting Fiji. These 2 patients were the first cases notified since 1979–1981. Both were found serologically positive for RRV and displayed characteristic symptoms such as fatigue, arthralgia and general body pain [21].

Cases of RRV occur throughout the year in some areas, such as northeastern tropical Australia and Papua New Guinea. By contrast, in other areas, mainly Australian coastal regions, disease activity tends to occur in seasonal epidemic outbreaks [22]. Originally endemic in most rural areas, increased virus activity has resulted in an increase in the geographic spread. This has raised considerable concern, as emergence of the virus into major Australian population centres could induce a higher incidence of disease amongst the community [22]. In Australia, on average, RRV is responsible for approximately 8,000 cases of disease annually, directly costing the community an estimated AUD 1,018 per patient [23, 24].

### *Clinical Manifestations*

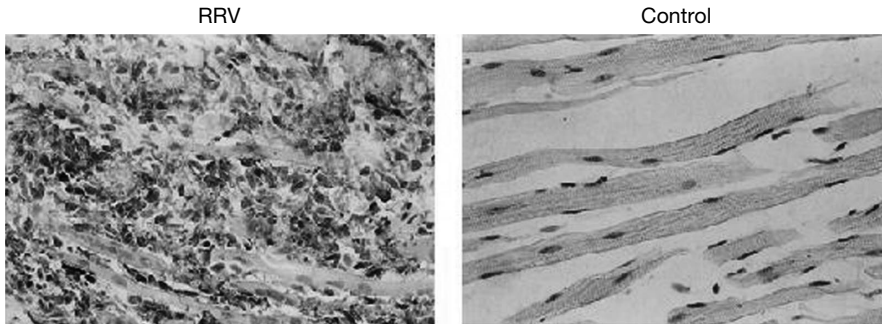
RRV disease, the most common arboviral disease in Australia, is primarily characterized by any or all of three major manifestations; these being rash, rheumatic pain (e.g. arthritis) and constitutional effects such as fatigue, fever and myalgia (muscle pain) [1]. Symptoms become evident from 3 to 21 days (average 9 days) post-infection with arthralgia (joint pain) usually constituting the initial, most prominent and incapacitating symptom of all, commonly affecting the joints of the extremities [13]. Approximately two-thirds of patients are affected by rash, usually lasting 5–10 days and occurring mainly on the limbs and torso [1, 13]. In addition, one third to one half of patients may experience fever, whilst myalgia and fatigue are reported to occur in over half of

infected individuals, with fatigue being the most consistent constitutional effect [1, 13]. While viral infection may potentially occur in any individual, clinical disease is very rarely observed in children. The reason for this is not clear but may be associated with differences in immune responses generated between adults and children. The disease has the most prominent incidence in adults of 20–50 years of age, particularly in those between the ages of 30 and 40 [25]. No clear gender effect has been established [13]. Although infection by the virus is not fatal, the resulting conditions can be debilitating and may persist in individuals for extended periods [25, 26]. Some controversy is present in the literature regarding the exact duration of disease, with reports of illness lasting from 3 months to several years [24, 27]. The variation is probably due to overestimates of symptom prevalence and duration, especially since established symptoms not solely associated with RRV disease may have been misdiagnosed and included in reported data [28–30]. Recently, a study performed by Mylonas et al. [24] demonstrated that although severe at onset, RRV disease usually resolved within 3–6 months. The researchers also showed that patients who suffered from disease beyond this time point were commonly afflicted with additional conditions. Furthermore, differences observed in the duration and severity of disease may be the result of diversity in the pathogenicity of RRV strains with which the individuals are infected [1].

Currently there is no cure for RRV and treatment is based on empirical anti-inflammatory regimes. As reported by Fraser et al. [31], symptomatic relief was achieved by the use of non-steroidal anti-inflammatory drugs. Other treatments, which have, at least to some extent, been found to provide relief, include physical interventions such as massage or physiotherapy, as well as rest [13]. A study by Yu and Aaskov [32] in 1994 reported the inactivation of RRV with binary ethylenimine (BEI) in mice and the ability to protect mice against later challenge with RRV. Similarly, an ethnobotanic study of Aboriginal medicinal plants by Semple et al. [33] showed that RRV infectivity was effectively reduced in vitro by 25% when treated with extracts of fruit, wood and leaf extracts of *Pittosporum phylliraeoides* var. *microcarpa* (Pittosporaceae). Developing superior treatment strategies will require better understanding of the immunology and pathology of RRV disease. A candidate RRV vaccine was developed by Aaskov and colleagues [32, 34] and was found to be effective in animal models, however no reports of human clinical trials of a RRV vaccine have been published.

### **Immunobiology of RRV Infection**

RRV has an extensive host range, including marsupials and humans, and has the potential to infect many different cell types within the host [17]. Early



**Fig. 2.** Differences in muscle tissue in uninfected and RRV-infected mice. Stained cells (monocytic cells) are present in high numbers in infected muscle. There is significant destruction of muscle fibres following RRV infection [38]  $\times 100$ .

studies on RRV demonstrated that certain cells and tissues of mice, such as muscle and brain, could readily be infected and facilitated the growth of the virus. However, recently, *in vitro* studies have identified the association of monocytes and macrophages with human RRV disease [35–37]. Both monocytes and macrophages have been identified as the predominant muscle-infiltrating cells at the height of clinical disease [38] (fig. 2). In addition, large mononuclear cell infiltrates, consisting mainly of monocytes and highly activated macrophages, have also been shown in biopsies obtained from rash lesions of infected patients as well as in rheumatic synovial effusions obtained from patients with EPA [39, 40]. Macrophages have been shown to be a primary cellular agent in the development, growth and persistence of RRV and have also been identified as playing a role in the pathology of RRV disease [38, 41].

In addition to the key monocyte/macrophage infiltrate, the primary cellular components identified in the synovial exudates are T lymphocytes, B lymphocytes and a small proportion of natural killer cells, while neutrophils are rarely present [37, 42, 43]. The lack of neutrophils in the synovial fluid of EPA patients distinguishes this condition from other arthritic conditions, such as rheumatoid arthritis. This deficiency is probably due to the absence of immune complexes in the serum of individuals suffering from RRV disease, as discovered by Fraser et al. [44]. Immune complexes attract neutrophils and are commonly involved in the pathogenesis of some viral arthritides [44]. Importantly, the *in vivo* T cell responses (both CD4+ and CD8+) in RRV disease are significant contributors in the resolution of the infection, where most patients experience a distinct virus-specific T cell proliferative response. In addition, infected