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Ross River Virus (RRV) Infection in Horses and Humans: A Review

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Abstract: A fascinating and important arbovirus is Ross River Virus (RRV) which is endemic and epizootic in nature in certain parts of the world. RRV is a member of the genus *Alphavirus* within the Semliki Forest complex of the family Togaviridae, which also includes the Getah virus. The virus is responsible for causing disease both in humans as well as horses. Mosquito species (*Aedes camptorhynchus* and *Aedes vigilax*; *Culex annulirostris*) are the most important vector for this virus. In places of low temperature as well as low rainfall or where there is lack of habitat of mosquito there is also limitation in the transmission of the virus. Such probability is higher especially in temperate regions bordering endemic regions having sub-tropical climate. There is involvement of articular as well as non-articular cells in the replication of RRV. Levels of pro-inflammatory factors viz., tumor necrosis factor-alpha (TNF-α); interferon-gamma (IFN-γ); and macrophage chemo-attractant protein-1 (MAC-1) during disease pathogenesis have been found to be reduced. Reverse transcription-polymerase chain reaction (RT-PCR) is the most advanced molecular diagnostic tool along with epitope-blocking enzyme-linked immunosorbent assay (ELISA) for detecting RRV infection. Treatment for RRV infection is only supportive. Vaccination is not a fruitful approach. Precise data collection will help the researchers to understand the RRV disease dynamics and thereby designing effective prevention and control strategy. Advances in diagnosis, vaccine development and emerging/novel therapeutic regimens need to be explored to their full potential to tackle RRV infection and the disease it causes.

Key words: Ross river virus, arbovirus, viremia, arthritis, mosquito, pyrexia, epidemiology, vector, diagnosis, control

INTRODUCTION

A self-limiting human rheumatic disease characterized by acute and chronic symmetrical peripheral polyarthralgia-polyarthritis, debilitating arthropathy, fever, myalgia and/or rash, transmitted by mosquitoes can be caused by a variety of alphaviruses such as chikungunya virus, Ross River virus, Barmah Forest virus, Sindbis virus, O'nyong-nyong virus and Mayaro virus (Suhrbier *et al*., 2012). A fascinating as well as important arbovirus is Ross River Virus (RRV) which is endemic as well as epizootic in nature in certain parts of the world like Australia as well as Papua New Guinea. The virus has been found to be epidemic in the South Pacific during the 1980s. In humans, infection with RRV may cause disease that typically presents polyarthralgia (or arthritis) along with fever as well as rashes. More outbreaks due to disease caused by this virus were recorded during the World War II thereby giving rise to the name epidemic polyarthritis. There is considerable burden of morbidity

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due to this virus and there can be spread of the virus to other countries as well. For justification as well as designing of prevention programs accurate data are required on costs of economy and to understand in a better way the transmission as well as behavioral and environmental risks (Anstey *et al*., 1991; Amin *et al*., 1998; Aaskov *et al*., 1997; Harley *et al*., 2001). The Ross River virus (RRV) is a member of the genus *Alphavirus* within the Semliki Forest complex of the family Togaviridae, which also includes the Getah virus. It is an arthropod-borne virus (arbovirus) that is transmitted by several mosquito species and is endemic and enzootic in Australia (Azuolas, 1998; Russell, 1994, 2002). RRV has similar cladistic classification, ecology and clinical signs in horses as that reported for Getah virus (Hinchcliff, 2007). Substantial sequence homology exists between Getah and RRV genomes (Strauss and Strauss, 1994). Getah virus causes disease in horses and pigs, whereas RRV causes disease in horses and humans (El-Hage *et al*., 2008). The horse is assumed to be an amplifying host of the virus since experimentally infected horses can infect mosquitoes (Kay *et al*., 1987). RRV infection has been suspected of causing musculoskeletal disease in performance horses in many riverland and northern regions of Australia for more than 25 years although documented cases are scarce. RRV has been isolated from horses exhibiting clinical signs such as reluctance to move, joint swellings and ataxia, in conjunction with positive RRV IgM serology (Pascoe *et al*., 1978; Azuolas *et al*., 2003; El-Hage *et al*., 2008). In humans, RRV is the most common Australian arbovirus disease and is often associated with a characteristic syndrome of debilitating polyarthritis (Fraser, 1986; Russell, 1994, 2002; Jeandel *et al*., 2004). In many cases, joint pain and disability may persist for several months or longer (Boughton, 1996; Suhrbier and La Linn, 2004; Colin de Verdiere and Molina, 2007).

MATERIALS AND METHODS

Etiology: RRV is a small, enveloped virus with the genome being single-stranded positive-sense RNA (Johnston and Peters, 1996). The RRV T48 strain genome is as long as 11,853 nucleotides coding for nonstructural proteins (nsP1 to nsP4) (four in number), a capsid protein and envelope glycoproteins (E1 to E3) (Faragher *et al*., 1988). The E1 and E2 viral glycoproteins are embedded in the lipid bilayer to form the envelope (Strauss and Strauss, 1994). Single E1 and E2 molecules associate to form heterodimers. The E1-E2 heterodimers form contact side-by-side between E2 and nucleocapsid monomers. The E3 glycoprotein is not incorporated in the virion (Cheng *et al*., 1995). The capsid protein and the genome form the nucleocapsid of about 400 Å in diameter containing several copies of capsid protein (240 in number) having icosahedral symmetry (Strauss and Strauss, 1994; Cheng *et al*., 1995). T is the triangulation number that gives copies equivalent to a multiple of 60 giving the number of subunits in the structure of the virus. Geographic genetic variability has been reported among RRV isolates (Lindsay *et al*., 1993). Given the range of virulence within RRV genotypes (Fraser, 1986; Russell, 1994), it is likely that most strains produce only subclinical or no disease.

Epidemiology and disease transmission: The RRV is endemic and enzootic in Australia and Papua New Guinea (Russell, 1994; Hii *et al*., 1997; Frances *et al*., 2004). The virus is found in most areas of continental Australia, Tasmania, West Papua and Papua New Guinea, New Caledona, Fiji, Samoa and the Cook Islands (Russell, 2002; Frances *et al*., 2004; Klapsing *et al*., 2005; Ryan *et al*., 2006). The virus caused a large epidemic in 1979 and 1980 involving Fiji (Aaskov *et al*., 1981), New Caledonia, Samoa (Fauran *et al*., 1984) and the Cook Islands (Rosen *et al*., 1981). In Australian humans, RRV causes the most common arboviral disease that has characteristically having constitutional effects, rash and rheumatic manifestations (Fraser, 1986). Mild pyrexia and constitutional signs initially occur, with rash on the skin and oral lesions later. Arthritis or arthralgia affects primarily the wrists, knees, ankles and small joints of the extremities. The signs and symptoms like joint pain and disability may persist for months and the disease can relapse (Harley, 2000; Harley *et al*., 2001; Jeandel *et al*., 2004; Suhrbier and La Linn, 2004; Colin de Verdiere and Molina, 2007). The syndrome caused by RRV was initially referred to as epidemic polyarthritis (Dowling, 1946). In Australia, RRV associated disease is common in humans with nearly 5000 cases per year and much larger numbers during disease epidemics (Russell, 2002; Hinchcliff, 2007). The vertebrate hosts include a large number of eutherian, marsupial and monotreme mammals and birds (Russell, 2002). Kangaroos and wallabies (Macropod species) are thought to be the most imperative amplifying hosts, although this is debated (Old and Deane, 2005).

The RRV is an arthropod borne virus, maintained in the mosquito-vertebrate-mosquito-host and transmitted via the bite of an infected mosquito. The virus is annually active in most regions of Australia but exists as strains of varied virulence. Native macropods are thought to be the natural vertebrate hosts but there may be involvement of horses and humans during epidemic activity and mosquitoes are vertically infected. Different mosquito

species are involved as vectors in various regions and in different seasonal and conditions of environment. The saltmarsh mosquitoes in coastal areas viz., *Aedes camptorhynchus* and *Aedes vigilax* are the most important vectors in southern and northern regions, respectively, whereas *Culex annulirostris* in inland areas is the most important vector (Russell, 1998, 2002). Recently, four other Australian mosquito species viz., *Verrallina carmenti*, *Verraullina lineata*, *Mansonia septempunctata* (Diptera: Culicidae) and *Verrallina funerea* (Diptera: Culicidae) have been shown to possess the potential to contribute to RRV transmission cycles (Jeffery *et al*., 2006, 2007; Webb *et al*., 2008). A study on mosquito feeding patterns and natural infection of vertebrates with RRV found that under natural conditions, mosquito feeding (including that of *Culex annulirostris*, *Aedes vigilax* and *Aedes notoscriptus*) was primarily on dogs (37.4%) but also on birds (18.4%), horses (16.8%), *brushtail possums* (13.3%), humans (11.6%) and cats, flying foxes and macropods, depending on site and that brushtail possums and horses played a role in the urban transmission of RRV (Kay *et al*., 2007). Rainfall, high tide and maximum temperature appears to play important roles in RRV transmission (Tong *et al*., 2004). The life cycle of mosquito as well as the host is dominated by the sets of climate. There is impact of temperature and rainfall; humidity as well as tides on replication of the virus substantially. They also affect the breeding as well as survival of mosquitoes and help in the RRV proliferation (Hu *et al*., 2004; Russell and Kay, 2004; Gatton *et al*., 2005). A systematic study on climatic, social and environmental factors and RRV disease revealed disease transmission cycles' sensitivity to climate and tidal variability, with rainfall, temperature and high tides being among the major determinants of the transmission of RRV disease at the macro level. The nature as well as magnitude of the interrelationship between variability of climate; density of mosquito and the RRV disease transmission varied with geographic area and socio-environmental condition. The analysis indicated the existence of a complex relationship between climate variability, social and environmental factors and RRV transmission, suggesting that different strategies may be needed for the control and prevention of RRV disease at different levels (Hu *et al*., 2007; Tong *et al*., 2008).

Climate and mosquito surveillance data have been used for epidemiological predictions for human RRV infections (Woodruff, 2003; Gatton *et al*., 2005; Williams *et al*., 2009; McIver *et al*., 2010), with climate data on their own being moderately sensitive (64%) for predicting epidemics during the early period of warning. Mosquito surveillance data when added increased the sensitivity of the early warning model to 90% and of later warning model 85% (Woodruff *et al*., 2006). Based on correlations revealing strong associations between monthly RRV infections in humans and climatic variables and also four implicated mosquito species populations. Jacups *et al*. (2008) have created three models to identify the best predictors of RRV infections for the Darwin area of Australia. The climate-only model which included total rainfall, minimum daily average temperature as well as maximum tide explained deviance of 44.3%. The vector-only variables, using average monthly trap numbers of *Culex annulirostris*; *Aedes phaecasiatus*; *Aedes notoscriptus* and *Aedes vigilax* explained 59.5% deviance. The third global model including rainfall, minimum temperature and three mosquito species was found to be best which explained 63.5% deviance and predicted RRV disease in humans accurately. A plausible association between mouse (*Mus musculus*) abundance and RRV incidence in northwest Victoria, Australia has also been suggested to be coinciding with the seasonal abundance and relative absence of the mosquito vector Culex annulirostris, suggesting that short-lived highly fecund amplification hosts may profoundly influence disease transmission.

In places of low temperature as well as low rainfall or where there is lack of habitat of mosquito there is also limitation in the transmission of the virus. In certain parts of the country where there is epidemic of the infection due to RRV because of annual pattern of the disease the chance of future epidemic may change. Such probability is higher especially in temperate regions bordering endemic regions having sub-tropical climate. During a year the temperate regions which are cooler in nature may experience RRV activity for a longer time of the year (McMichael *et al*., 2003; ABC Online, 2006; Carver *et al*., 2008; http://emedicine.medscape.com/article/233913). Molecular studies on the nsP3 and E2 genes of RRV indicated that intra-host multiple viral lineages were responsible for the long-term persistence of RRV at different geographical locations (Liu *et al*., 2011). The RRV shows a continuous low relative genetic diversity through time and the chances of rapid antigenic variation/ evolution in future as a result of vaccination is negligible (Jones *et al*., 2010; Aaskov *et al*., 2012).

Pathogenesis: There is involvement of articular as well as non-articular cells in the replication of RRV as well as its dissemination. Experimental models to study the pathogenesis have shown that the disease occurs when cellular as well as tissue damage by replication of the virus as well as immune response indirectly activate in

target tissues in coordinated effort. There has been description of various types of cells as targets for replication of arthritogenic alphavirus that include cells from joints; bones and muscles; as well as cells of the immune system that gets infiltrated in the synovium as well as tissues that are infected. This highlights the association between the virus affected tissues where replication of the virus takes place and the local process of inflammation that contributes to the pathogenesis (Atkins *et al*., 1982; Lundstrom, 1999; Morrison *et al*., 2006; Al Kindi *et al*., 2012; Atkins, 2012). For investigation of the role of cellular immune response during RRV infection there has been development of several animal models. Inflammation of severe nature has been observed in bones as well as joints and muscle tissues in models of mice. The mice that is deficient in recombinase activating gene ($RAG^{-/-}$) are having the lacunae of functional T as well as B lymphocytes. Cluster of differentiation positive ($CD4^+$) T cells are involved in the swelling of joints. Such symptoms altogether suggest that immune response that is adaptive in nature has got a restricted role in the pathology of the disease caused by RRV. Interferon-gamma (IFN-γ) is expressed at a lower rate along with lower expression of tumor necrosis factor alpha (TNF-α); and interleukin-beta (IL-β) in tissues of muscles as well as joints. The clinical course of the disease in mice induced by RRV is reduced by IFN-γ and TNF-α neutralization. Reinforcement of such information has been done by observing that patients who use to develop chronic symptoms (like in case of *Chikungunya virus*) use to show activation of various cells of the immune system intensely in the phase of acute nature of the disease that include: dendritic cell (DC) as well as Natural killer (NK) cells; CD4+and $CD8^+$cells (Griffin *et al*., 1992; Linn *et al*., 1996; Mateo *et al*., 2000; Suhrbier and La Linn, 2004; Assuncao-Miranda *et al*., 2010, 2013). A small plaque variant Ross River virus (PERS) that grows persistently in macrophages produced comparatively marked increase in disease severity and mortality in mice (Lidbury *et al*., 2011). The seminal role of macrophage Migration Inhibitory Factor (MIF) and MIF receptor CD74 in determining the clinical severity of alphavirus-induced arthritis and myositis has been reported (Herrero *et al*., 2011a, 2013). Mannose binding lectin (MBL) may have a role in promoting RRV-induced musculoskeletal disease in both mice and humans and therefore, humans suffering from RRV-induced arthritis and myositis can be relieved by targeting the MBL pathway of complement activation (Gunn *et al*., 2012). The inflamed musculoskeletal tissues resulting from RRV activate a unique set of myeloid cells which prevent virus clearance and delay disease resolution in an arginase 1-dependent manner (Stoermer *et al*., 2012). The pathogenesis of RRV-induced disease and development of antiviral drugs against RRV has greatly been helped by the mouse model (Herrero *et al*., 2011b). Vital and distinct roles of determinants in both nsP1 and PE2 in the pathogenesis of RRV-induced musculoskeletal inflammatory disease in mice have been reported (Jupille *et al*., 2011).

It has recently been shown that RRV fitness in vertebrate and invertebrate cells is regulated by a tyrosine-to-histidine switch at E2 glycoprotein position 18 (E2 Y18H). This mutation led to the attenuation of wild type RRV and caused significantly less severe musculoskeletal disease in mice, with reduced viral loads in musculoskeletal tissues, less viremia and inefficient virus spread. Its replication in mammalian cells was also significantly reduced. However, the efficiency of replication of RRV E2 Y18H in C6/36 mosquito cells was better than the wild type RRV (Jupille *et al*., 2013).

Disease: In horses, several clinical features in RRV infection appears to be similar to Getah virus infection and characterized by pyrexia, lameness, stiffness, swollen joints, inappetence, reluctance to move and mild colic (Sentsui and Kono, 1980; Azuolas, 1998; Bennett *et al*., 1998; Brown and Timoney, 1998; Azuolas *et al*., 2003; Hinchcliff, 2007). El-Hage *et al*. (2008) observed clinical findings of submandibular lymphadenopathy, pyrexia, oral petechiae, synovial effusion, muscle pain/stiffness, limb oedema, high serum fibrinogen and globulin levels and IgM titres in four horses which were diagnosed as RRV infected. The duration of disease is uncertain and disease can persist for weeks to months or recur in horses. Postmortem examination reports of horses with confirmed disease caused by RRV are not available. As the disease syndrome is not well characterized, the RRV pathogenicity in horses is not well understood. Disease descriptions are based on a small number of horses that established viremia parallel with the clinical signs or on larger number of seropositive horses. Horses experimentally infected with RRV also show minimal clinical symptoms (Studdert *et al*., 2003). Due to fewer disease reports, characteristic or diagnostic alterations in serum biochemistry or hematology are not exactly known to occur in affected horses. An area with year-round mosquito activity has high prevalence rate of seropositive horses (approximately 80% in Queensland) whereas with seasonal mosquito activity has lower (50% around Gippsland lakes in southern Australia) (Azuolas, 1998). RRV is minimally pathogenic in horses as reflected by the high rates of infection but the absence of similarly high rates of clinical disease. There is probability that seroconversion or virus isolation from horses with clinical

abnormalities is not causally related and may only be a chance affair (Hinchcliff, 2007). High rate of subclinical RRV infection of horses occur in endemic regions, which increases the risk of incorrect provenance of clinical abnormalities to infection by the virus. Thus, clinical abnormalities in a horse with Ross River viremia or serum antibodies may not be attributable to infection by RRV. Arthralgia is a common feature of the patients suffering from infection due to RRV with or without arthritis. Fatigue as well as fever, myalgia and maculopapular rashes are the constitutional symptoms in approximately 50% of the patients. The 20-60 years age group of patients is the worst sufferers but any age group may be affected by the disease (Harley *et al*., 2002; http://emedicine.medscape.com/article/233442).

In humans, it has been observed that RRV disease, although severe at onset, progressively resolve over 3-6 months and other causes primarily unrelated rheumatic conditions or depression have been attributed in patients experiencing long-term disease lasting for more than 12 months found responsible (Jeandel *et al*., 2004; Suhrbier and La Linn, 2004; Colin de Verdiere and Molina, 2007). RRV polyarthritis probably arises from inflammation associated with productive viral infections in macrophages of the synovia persisting despite neutralizing antibodies and antiviral cytokine responses. Downregulation of cytokine responses may facilitate persistence by virus-antibody complexes binding to Fc receptors and induction of interleukin-10. Escaping neutralizing antibodies by the virus remains unclear but may involve phagocytosis of apoptotic virus-infected cells and infection of the phagocyte via the phagosome (Suhrbier and La Linn, 2004; Rulli *et al*., 2007). Utilizing a mouse model of RRV disease, Morrison *et al.* (2006) observed that the primary targets of infection by RRV are bone and joint; muscle tissues (skeletal) of the hind limbs in both outbred CD-1 mice as well as adult C57BL/6J mice. Histological examinations showed severe inflammation of these tissues caused by RRV infection. The inflammatory infiltrate within the skeletal muscle tissue comprised of the macrophages, NK cells and $CD4^+$ and $CD8\ T^+$ lymphocytes. The researchers also found that adaptive immune response does not play a critical role in the development of disease. But in their further studies authors Morrison *et al.* (2007) demonstrated that the complement system enhances the severity of disease induced by RRV in mice. In the inflamed tissues and in the serum of RRV-infected wild-type mice products of activation of complement have been detected whereas mice deficient in C3 ($C3^{-/-}$), the central component of the complement system, developed disease signs of much less severity than did wild-type mice. Complement activation was also detected in synovial fluid from RRV-infected patients. They suggested that complement plays an essential role in the effector phase but not the phase of induction of arthritis and myositis induced by RRV. In yet another study using macrophage-depleted mice model, macrophage-derived pro-inflammatory factors were shown to be critical to the development of arthritis and myositis after infection with RRV. Histological analyses of muscle and ankle joint tissues revealed a substantial decrease in inflammatory infiltrates in infected "macrophage-depleted mice", where levels of the pro-inflammatory factors, tumor necrosis factor-alpha; interferon-gamma and macrophage chemo-attractant protein-1 were also dramatically reduced, compared with samples obtained from infected mice without depletion of macrophage. Detection of these factors has also been done in the synovial fluid of patients with polyarthritis induced by RRV. There is reduction in the severity of disease in mice as these factors get neutralized whereas nuclear factor kappaB has been blocked by treating with sulfasalazine ameliorated RRV inflammatory disease and tissue damage (Lidbury *et al*., 2008). There is resolution of majority of the symptoms within 3-6 months in most of the patients. There may be chronic course of the symptoms in certain patients with persistence of symptoms that are non-rheumatic in nature that includes fatigue as well as poor concentration. In certain instances a co-morbid condition may be responsible for prolonged illness and there is importance of investigation for certain other conditions that may either cause or contribute to the symptoms. Patients may experience a RRV disease course that is relapsing in nature (Mylonas *et al*., 2002).

Diagnosis: The diagnosis of RRV differentially from other diseases is done with a broad sense including a spectrum of infectious as well as non-infectious causes of polyarthopathy. The disease must be differentiated from Burma forest virus as well as B19 strain of Parvovirus otherwise known as erythema infectiosium. Among the diseases that are non-infectious in nature but must be differentiated from RRV are: Rheumatoid arthritis; Still's disease in adults; Reiter's syndrome and Henoch Schonlein purpura. The diagnosis of RRV should be taken into consideration if the patient has a high erythrocyte sedimentation rate (ESR); anaemia; persistence of reduction in movement of joints; and radiological changes (Harley *et al*., 2001; Smith, 2001).

Diagnosis of RRV infection is based on virus isolation from samples (blood or serum) collected during the acute phase of the disease or by detecting specific viral antibodies in serum. RRV can be isolated from infected horses during the short time period when there is

an overlap of clinical signs, positive IgM serology and viremia (Azuolas *et al*., 2003). Virus can be isolated in mice or tissue cultures. The detection of a RRV serum IgM titre, either alone or in combination with an IgG titre, is indicative of a recent infection, whereas presence of IgG antibodies is indicative of more distant infection. Generally, IgM response occurs within 7 to 10 days post infection and peaks within 2 to 3 weeks, before rapidly declining, as later IgG becomes the dominant antibody (Azuolas *et al*., 2003; El-Hage *et al*., 2008). Both virus exposure and presumably infection can be confirmed by seroconversion. The virus isolation has been made from horses with IgM antibodies to RRV but not from horses with IgG antibodies because of the chronological pattern of antibody appearance in the blood. Polymerase chain reaction (PCR) techniques have been demonstrated to identify RRV (Studdert *et al*., 2003). The molecular tool of reverse transcription-polymerase chain reaction (RT-PCR) has also been developed for RRV which can be detected in blood and synovial fluid. Specific, sensitive and rapid diagnostic tests using RT-PCR have been developed and validated for the detection of RRV, Kunjin virus (KV) and Murray Valley encephalitis virus (MVEV) infections in horses. The primer sets used for the RT-PCR assay were based on nucleotide sequence encoding the envelope glycoprotein E2 of RRV and on the nonstructural protein 5 (NS5) of KV and MVEV, which detected RRV in sera from 8 horses showing clinical signs consistent with RRV infection. The RRV RT-PCR was analytically found to be sensitive enough to detect as little as 50 $TCID_{50}$ of RRV per mL of serum. Not only sera samples, the RRV primers were also able to detect virus in three independent mosquito pools known to contain RRV by virus isolation in cell culture (Studdert *et al*., 2003). Recently, an epitope-blocking enzyme-linked immunosorbent assay (ELISA) has been developed and described to be a very sensitive and rapid detection method for antibodies to RRV in human sera and other known vertebrate host species (Stocks *et al*., 1997; Oliveira *et al*., 2006).

Treatment: Treatment for RRV infection is only supportive as that for Getah virus infection. It is prudent to minimize the exposure of horses to infected mosquitoes. There is no vaccine to prevent infection or disease of horses by RRV. However, in humans, nonsteroidal anti-inflammatory drugs (NSAIDs) have been reported to give immediate symptomatic relief with no evidence of long-term sequelae or relapse (Fraser and Marshall, 1989; Suhrbier and La Linn, 2004). Condon and Rouse (1995) found that of 255 patients, 36.4% felt that the "best and most effective relief," is provided by NSAIDS while 16.4% felt that aspirin or paracetamol was the most 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.

Prevention and control: Attempts to prepare formaldehyde inactivated RRV vaccine failed at preclinical trial levels (Kistner *et al*., 2007). Recent insights into the RRV-host relationship in association with pathology and molecular biology of infection have generated a number of potential avenues for improvement in treatment. Although proposal has been given for development of vaccine, the small size of market and potential for antibody-dependent enhancement (ADE) of disease has decreased the attraction of this approach. In RRV-ADE insights into the basis of molecular mechanism recently and the ability of the virus to manipulate host inflammatory and immune responses create potential new opportunities for invention therapeutically. Dysregulation induced by these viruses must be overcome by such interventions of protective host responses to promote clearance of virus and/or ameliorate inflammatory immunopathology (Rulli *et al*., 2005, 2007; Lidbury *et al*., 2008). A Vero cell culture-derived, whole-virus inactivated RRV vaccine was found to be highly protective in animal models of viremia and disease (Holzer *et al*., 2011). This vaccine was further tested in humans and was found to be safe and induced protective antibodies in them. This vaccine did not cause any antibody-dependent enhancement (ADE) of disease (Aichinger *et al*., 2011). The development of protective antibody responses against RRV is influenced by Toll-like receptor 7 (TLR7)-dependent signalling (Neighbours *et al*., 2012).

The best way to prevent against the disease is to take precautionary measures against mosquito bites. During the time of heavy infestations of mosquitoes it is better to avoid remaining outside. Such are the early evenings during the months of warm weather which must be avoided. Insect repellants must be used along with wearing of protective clothings which are light coloured. The living as well as sleeping areas must be screened. Regular checking of the home is necessary to prevent the spread of mosquito breeding areas potentially. Any type of water containers which remain uncovered must be emptied on regular basis (Heymann, 2004). For studying the virus spread it is significant to collect suspected region of acquisition for all types of cases. The national dataset has got one of the limitation that there is no any routine collection of suspected region of acquisition for all kinds of cases and thus there is requirement of using the residential place as a proxy for the particular region of

acquisition. It is now mandatory to collect suspected region of acquisition in all states as well as territories and to record the information at the national level. Precise data collection will help the researchers to understand the RRV disease geographical distribution in a more better way (Selden and Cameron, 1996; Mackenzie, 1999; Kelly-Hope *et al*., 2004; Ratnayake, 2005).

In the era of One Health, One Medicine, One World notion, issue of climate changes and global warming and ever increasing mosquitoes/vector populations, quick, confirmatory and advanced diagnostics supported with early warning and surveillance/monitoring systems need to be fully applied for detecting RRV infections in animals and humans (Studdert *et al*., 2003; Schmitt and Henderson, 2005; Oliveira *et al*., 2006; Woodruff *et al*., 2006; Belak, 2007; Tong *et al*., 2008; McIver *et al*., 2010; Deb and Chakraborty, 2012; Deb *et al*., 2013; Dhama *et al*., 2012, 2013a, b, c; 2014; Suhrbier *et al*., 2012). Continuous efforts need to be made for developing effective, safer and novel vaccines (Meeusen *et al*., 2007; Dhama *et al*., 2008, 2013d; Jones *et al*., 2010; Aichinger *et al*., 2011; Holzer *et al*., 2011; Aaskov *et al*., 2012) and exploring alternative treatment modalities against this virus (Dhama *et al*., 2013e, f, g; Mahima *et al*., 2012; Tiwari *et al*., 2014). Due attention need to be given to follow appropriate and timely prevention and control measures including of strict biosecurity plans for tackling RRV infection and the disease it causes, which would help safeguard health of animals and humans.

CONCLUSION AND FUTURE PERSPECTIVES

RRV is an important member of the family Alphavirus causing polyarthralgia in human and several different symptoms in horses starting from pyrexia till arthritis. As the disease is mosquito-borne special study is required regarding the ecology as well as climate and environment governing the breeding of mosquitoes. Epidemiologists as well as environmentalists have given special attention to the pattern of the distribution of the disease caused by RRV with special reference to the mosquito breeding. In recent times much attention has been paid towards the antiviral immunity especially regarding the cells of the immune system involved in the disease process. Special experiments have been carried out in mice from time to time to understand the immune response to the viral antigen. With the advent of special diagnostic assays like RRV RT-PCR as well as special type of ELISA viz., epitope-blocking ELISA it has become easier for the diagnosticians to better diagnose the disease with rapidity as well as accuracy. Treatment for RRV infection is only supportive as that for Getah virus infection. Physical interventions may benefit certain patients. There have been a number of potential avenues for improving treatment due to recent insights into the RRV-host relationship and this is because of elaborate study regarding the pathology as well as molecular biology of infection. The small market size has decreased the vaccinal approach for preventing the disease. Precise data collection will help the researchers to understand the RRV disease and its geographical distribution in an efficient manner as well as comprehend the disease dynamics procedures in an interesting way.

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