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Viruses of the Bunya- and Togaviridae families: potential as bioterrorism agents and means of control

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Abstract

When considering viruses of potential importance as tools for bioterrorism, several viruses in the Bunya- and Togaviridae families have been cited. Among those in the Bunyaviridae family are Rift Valley fever, Crimean-Congo hemorrhagic fever, hanta, and sandfly fever viruses, listed in order of priority. Those particularly considered in the Togaviridae family are Venezuelan, eastern and western equine encephalitis viruses. Factors affecting the selection of these viruses are the ability for them to induce a fatal or seriously incapacitating illness, their ease of cultivation in order to prepare large volumes, their relative infectivity in human patients, their ability to be transmitted by aerosol, and the lack of measures available for their control. Each factor is fully considered in this review. Vaccines for the control of infections induced by these viruses are in varying stages of development, with none universally accepted to date. Viruses in the Bunyaviridae family are generally sensitive to ribavirin, which has been recommended as an emergency therapy for infections by viruses in this family although has not yet been FDA-approved. Interferon and interferon inducers also significantly inhibit these virus infections in animal models. Against infections induced by viruses in the Togaviridae family, interferon-α would appear to currently be the most useful for therapy.

Keywords: Rift Valley fever virus; Sandfly fever virus; Crimean-Congo hemorrhagic fever virus; Hantaan virus; Hantavirus; Punta Toro virus; Venezuelan equine encephalitis virus; Antiviral

1. Introduction

Those organizing this volume on viral bioterrorism requested that we consider the viruses of potential bioterrorism importance in both the Bunyaviridae and Togaviridae families. Since these are quite unrelated groups of viruses, we will first consider all pertinent aspects of the Bunyaviridae viruses, including their significance, prevention and control, cell culture infectivity, and animal models useful for antiviral evaluation, then review the Togaviridae viruses in the same manner.

Rift Valley fever (RVF) and hantaviruses of the Bunyaviridae family have been considered of potential bioterrorism importance, and are categorized as Category A, or high priority, agents by the National Institute for Allergy and Infectious Diseases (http://www.niaid.nih.gov/dmid/biodefense/bandc_priority.htm). Crimean-Congo hemorrhagic fever (CCHF) virus is placed in Category C by the same agency. The Centers for Disease Control and Prevention, however, place a lower priority on these viruses (http://www.bt.cdc.gov/agent/agentlist.asp), with RVF and CCHF fitting the general definition of Category B agents while hantaviruses are listed among emerging pathogens in Category C. Sandfly fever virus (SFV) is not named in either list, but will be considered because the virus afflicted numerous servicemen in the Mediterranean during World War II. Oldfield et al. (1991) named all these members of the Bunyaviridae family as having potential impact on military operations, although in some cases due to natural transmission.

Among the viruses of the Togaviridae family, Venezuelan equine encephalitis (VEE) virus, eastern equine encephalitis (EEE) virus, and western equine encephalitis (WEE) virus are considered of particular significance as agents of bioterrorism importance. They are listed as Category C agents by the NIAID and as Category B agents by the CDC. There is a close resemblance between the three viruses in regards to disease induced, cultivation, transmission, and sensitivity to antiviral drugs (Griffin, 2001).

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2. Viruses of the Bunyaviridae family

2.1. Significance

2.1.1. Rift Valley fever (RVF)

This virus was originally isolated in 1930 in East Africa (Daubney et al., 1931). Since that time, severe epidemics of RVF have been reported throughout much of Africa and other areas of the Middle East. An outbreak occurred in Sudan in 1976 (Barnett and Suyemoto, 1961), presumably with the disease spreading to Egypt in 1977–1978 which resulted in an estimated 200,000 human cases and at least 600 deaths (Meegan, 1979). In the epidemic areas, the human infection rates were as high as 35% (Meegan et al., 1981). During a more recent outbreak of the disease in Kenya, it is estimated 27,500 infections occurred (Nichol, 2001). Outbreaks of the disease have also been reported in Mauritania, Senegal, Saudi Arabia, Yemen, India, and Sri Lanka (Zeller and Bouloy, 2000).

The RVF disease often resembles human influenza, with abrupt onset fever and associated symptoms lasting 2–5 days. Some cases may be more serious or fatal, resulting from liver necrosis with hemorrhagic phenomena, retinitis with visual impairment, and meningoencephalitis (Siam et al., 1980; Meegan and Shope, 1981).

A number of laboratory workers have become infected with RVF virus, indicating it can be readily transmitted by aerosol (Smithburn et al., 1949). A group of WHO consultants have estimated that if 50 kg of RVF virus were released from an aircraft along a 2 km line upwind of a population center of 500,000 persons, 400 would die and 35,000 would be incapacitated (Christopher et al., 1997). The virus is included by a Working Group for Civilian Biodefense among those considered likely as a biological weapon, citing also the concern that if used in such a manner, susceptible domestic livestock could become infected resulting in the establishment of the disease in the environment (Borio et al., 2002).

2.1.2. Crimean-Congo hemorrhagic fever (CCHF)

Viruses included in the CCHF group are CCHF and Hazura viruses, which are members of the *Nairovirus* genus. CCHF is becoming recognized as an important zoonotic disease of humans in the Middle East as well as in eastern Europe and Asia (Nichol, 2001). The infection caused by CCHF virus was first reported in World War II among Soviet military personnel in Crimea (Hoogstraal, 1979), and subsequent outbreaks have been reported in Bulgaria, Pakistan, Iraq, sub-Saharan Africa, the former Yugoslavia, the former Soviet Union, Greece, the Arabian peninsula, Dubai, Kuwait, and northwest China (Burney et al., 1980; Suleiman et al., 1980; Al-Makib et al., 1984; Nichol, 2001).

The disease in man is characterized by sudden onset with a long-lasting (7–9 days) fever with rigors and chills which subside and then remanifests itself. Intense myalgia, nausea and vomiting frequently also occur; patients may also develop diarrhea, facial hyperemia, hepatomegaly, and petechial rash. CCHF disease is often lethal, with fatality rates of 13–50% reported (Swanepoel et al., 1989).

Humans may acquire the infection through tick bite, contact with blood on tissues from infected livestock, and infections of medical personnel treating or performing surgery on CCHF patients have been reported (Nichol, 2001). In the latter situation, the virus has been frequently associated with small hospital-centered outbreaks (Franz et al., 1997). It is known that in early 1990, Iraq studied the CCHF virus as a potential biological weapon, but concluded that it was unsuitable because it required vectors for dispersal (Zilinskas, 1997). In contrast to this, however, a recent review has indicated that the CCHF virus could possibly be disseminated by aerosolization and used as a weapon (Bronze et al., 2002).

2.1.3. Hantavirus infections

Two disease manifestations are known to be associated with the viruses of the *Hantavirus* genus. Hemorrhagic fever with renal syndrome (HFRS) is the result of infection with the Hantaan, Seoul, Puumala, and Doprava viruses (Lee et al., 1982; Niklasson and LeDuc, 1987; Avsic-Zupanc et al., 1992). Hantavirus pulmonary syndrome (HPS) can be caused by Sin Nombre virus and a number of other recently isolated hantaviruses shown in Table 1 (Nichol, 2001). More than 3000 cases of HFRS occurred among troops during the Korean War, and a mortality rate of 5–10% was reported (Smadel, 1951). This disease has been confirmed in China, Korea, the eastern former Soviet Union, Japan, Scandinavia, and the Balkan region (Casals et al., 1970; Lee et al., 1982; LeDuc, 1987; Avsic-Zupanc et al., 1992). To date, hantaviruses do not replicate well in vitro and are subsequently not considered to be significant threats as biological warfare agents (Franz et al., 1997), although Bronze et al. (2002) have speculated that because the Sin Nombre virus is infectious in aerosolized particles it is a likely candidate for bioterrorism. The Andes virus has been shown to readily transmit from person to person by aerosol in a hospital setting (Chaparro et al., 1998), suggesting it could also be a bioterrorism threat.

Cases of HPS have occurred in most of the United States and Canada (Monroe et al., 1999) and have also been identified in South and Central America, with a higher prevalence of hantavirus-specific antibodies in the general population in these latter areas than seen in the US (Nichol, 2001).

The symptomology of HFRS in patients will vary according to the infective virus; the infections induced by Hantaan or Dobrava virus are usually the most severe, characterized by influenza-like symptoms, flushing of the face and neck and conjunctival and pharyngeal congestion followed later by thrombocytopenia and petechial hemorrhage associated with some degree of shock. Diminished urine production may occur associated with often fatal renal failure seen in 5–15% of the patients (Smadel, 1951). The disease induced by the Seoul virus is usually less severe (mortality rate of 1–2%), although some liver involvement may occur (Kim et al., 1995); Puumala-associated HFRS is the mildest form

Table 1
Viruses of the Bunyaviridae family considered of bioterrorism importance

Virus	Genus	Geographic distribution	Disease induced
Crimean-Congo hemorrhagic fever	*Nairovirus*	Africa, Asia, Europe	Crimean-Congo hemorrhagic fever
Hazara	*Nairovirus*	Africa, Asia, Europe	Crimean-Congo hemorrhagic fever
Rift Valley fever	*Phlebovirus*	Africa, Middle East, Southern Asia	Rift Valley fever
Sandfly fever Naples	*Phlebovirus*	Africa, Asia, Europe	Sandfly fever
Sandfly fever Sicilian	*Phlebovirus*	Africa, Asia, Europe	Sandfly fever
Doprava	*Hantavirus*	Europe	Hemorrhagic fever with renal syndrome
Hantaan	*Hantavirus*	Asia	Hemorrhagic fever with renal syndrome
Puumala	*Hantavirus*	Asia, Europe	Hemorrhagic fever with renal syndrome
Seoul	*Hantavirus*	Asia	Hemorrhagic fever with renal syndrome
Bayou	*Hantavirus*	North America	Hantavirus pulmonary syndrome
Black Creek Canal	*Hantavirus*	North America	Hantavirus pulmonary syndrome
Sin Nombre	*Hantavirus*	North America	Hantavirus pulmonary syndrome
Andes	*Hantavirus*	South America	Hantavirus pulmonary syndrome
Araraquara	*Hantavirus*	South America	Hantavirus pulmonary syndrome
Castelo dos Sonlios	*Hantavirus*	South America	Hantavirus pulmonary syndrome
Choclo	*Hantavirus*	South America	Hantavirus pulmonary syndrome
Juquitiba	*Hantavirus*	South America	Hantavirus pulmonary syndrome
Laguna nigra	*Hantavirus*	South America	Hantavirus pulmonary syndrome

of the disease, with a mortality rate of less than 1% (Nichol, 2001).

The clinical symptoms of HPS include rapid onset of influenza-like illness with fever, myalgia, malaise and headache of about 4 days duration often accompanied by gastrointestinal distress. An increased permeability of microvascular endothelial cells surrounding the lungs occurs which is associated with fluid leakage into the lungs, rapid deterioration of the patient and death occurring in approximately 50% of the patients (Duchin et al., 1994). The HPS symptomology will differ somewhat, depending upon the infecting virus (Nichol, 2001).

2.1.4. Sandfly fever

During World War II, approximately 19,000 members of the Allied armed forces in the Middle Eastern area were afflicted with sandfly fever (also called *pappataci*, *Phlebotomus*, and 3-day fever) infections, with most requiring hospitalization (Hertig and Sabin, 1964). From 3 to 10% of all troops were afflicted with the disease at that time, with some units reporting attack rates of over 50% (Oldfield et al., 1991). These rates were especially high in the Persian Gulf command, reaching a peak of 235 cases/1000 men (Hertig and Sabin, 1964).

The significance of sandfly fever is enhanced because of the short incubation period (2–6 days) and rapid onset, with the disease manifested by intense symptoms of fever, severe frontal headache with retro-orbital pain associated with severe myalgias, and often nausea, vomiting, abdominal pain and diarrhea which present 2–4 days (Sabin et al., 1944).

Importantly, hospital personnel caring for patients with sandfly fever have become infected, suggesting the virus can be spread by aerosol or direct contact (Hertig and Sabin, 1964). No information is available regarding any attempted weaponization of this virus, however.

2.2. Prevention and control

Both a live attenuated virus vaccine and a formalin-inactivated virus vaccine are available for use in livestock to prevent transmission of RVF (El-Karamany et al., 1981). Formalin-activated RVF virus vaccines have also been developed for human use (Randall et al., 1964; Meadors et al., 1986), although multiple doses are needed for adequate antibody response (Frank-Peterside, 2000). Experimental vaccines have had limited use for CCHF, but their efficacy has not been established (Vasilenko et al., 1975). Several inactivated Hantaan and Seoul virus vaccines for HFRS have been used, with apparent protection seen (Hooper and Li, 2001). A number of recombinant DNA HFRS vaccines are also under development (Hooper and Li, 2001).

A number of studies with the nucleoside analog ribavirin have indicated this drug has potential to treat patients infected with these viruses. The infection was prevented in humans who were experimentally challenged with the sandfly fever virus and treated orally with 400 mg of the drug three times daily for 8 days (Huggins, 1989). Ribavirin was efficacious in preventing RVF infections in mice, hamsters, and rhesus monkeys (Peters et al., 1986), but no human RVF trials with the drug have been reported. A Working Group for Civilian Biodefense has recommended intravenous ribavirin be administered in the case of a contained casualty situation with RVF infections, and in the case of mass casualties, an oral regimen of ribavirin is recommended (Borio et al., 2002). The CCHF virus has been shown to be highly sensitive to ribavirin in vitro (Watts et al., 1989) and the drug significantly inhibited CCHF virus infection in mice (Tignor and Hanham, 1993). Limited human cases of CCHF have also reportedly responded well to oral ribavirin (Fisher-Hoch et al., 1995). The Hantaan virus has been found to be sensitive to the drug in vitro and treatment of

HFRS in human patients was successful in a controlled trial (Huggins et al., 1991). Against HPS infections in human patients, ribavirin therapy was not successful (Anonymous, 1993). It is probable in the HPS infections that therapy was initiated too late in the course of the disease.

Although no other antiviral drug is known to have been evaluated against these virus infections in clinical trials, a number of nucleoside analogs have been found highly inhibitory in animal models. These are shown in Table 2. In addition, the virus infections appear highly sensitive to the effects of interferon (Sidwell et al., 1994), interleukin 2 (Mead et al., 1991), and interferon inducers, the latter included in Table 2. It is noteworthy that many of the immunomodulators shown in Table 2 were highly efficacious when treatment was begun as late as 48 h after virus inoculation of the mice (Sidwell et al., 1994).

2.3. Cell culture infectivity

The sandfly (Naples, and Sicilian) viruses, RVF virus, and the related Punta Toro virus will cause discernible cytopathic effect (CPE) in a wide variety of cell lines, including Vero, LLC-MK2, BHK-21, and mouse macrophage (BW-JM) cells (Stephen et al., 1980; Nichol, 2001). The CCHF viruses also are readily propagated in vitro, susceptible cells including Vero, LLC-MK2, BHK-21 and SW-13

Table 2
Compounds considered significantly inhibitory in animal models to viruses of the Bunyaviridae family

Virus	Animal model	Compound	Reference
Crimean-Congo hemorrhagic fever	Mouse	Ribavirin	Huggins et al. (1984) and Tignor and Hanham (1993)
Hantaan	Mouse	Ribavirin	Huggins (1989), Huggins et al. (1986) and Murphy et al. (2000)
Punta Toro	Mouse	Ribavirin	Stephen et al. (1980), Huggins et al. (1984) and Sidwell et al. (1988a)
Punta Toro	Mouse	Ribamidine	Sidwell et al. (1988b, 1994) and Huffman et al. (1989)
Punta Toro	Mouse	Ribavirin 2′,3′,5′-triacetate	Huffman et al. (1989) and Sidwell et al. (1994)
Punta Toro	Mouse	3-Deazaguanine	Huffman et al. (1989) and Sidwell et al. (1994)
Punta Toro	Mouse	Selenazofurin	Smee et al. (1990a) and Sidwell et al. (1994)
Punta Toro	Mouse	Tiazofurin	Smee et al. (1990a) and Sidwell et al. (1994)
Punta Toro	Mouse	Tiazofurin-5′-monophosphate	Sidwell et al. (1994)
Punta Toro	Mouse	Tiazofurin-2′,3′,5′-triacetate	Sidwell et al. (1994)
Punta Toro	Mouse	7-Thia-8-oxoguanosine	Smee et al. (1991a,b, 1992) and Sidwell et al. (1994)
Punta Toro	Mouse	Poly(ICLC)	Sidwell et al. (1992, 1994)
Punta Toro	Mouse	Pyrazofurin	Sidwell et al. (1994)
Punta Toro	Mouse	3-Deazaguanosine	Sidwell et al. (1994)
Punta Toro	Mouse	Ampligen (polyI·polyC$_{12}$,U)	Sidwell et al. (1992, 1994)
Punta Toro	Mouse	MVE-1 (maleic anhydride divinyl ether copolymer)	Sidwell et al. (1992, 1994)
Punta Toro	Mouse	MVE-2 (1:2 divinyl ether maleic anhydride cyclic polymer)	Sidwell et al. (1992, 1994)
Punta Toro	Mouse	AM-3 (inmunoferon, a glucomannan polysaccharide from *Candida utilis*)	Sidwell et al. (1992, 1994)
Punta Toro	Mouse	AM-5 (a glucomannan polysaccharide from *Candida utilis*)	Sidwell et al. (1992, 1994)
Punta Toro	Mouse	Mannozym (glucomannan polysaccharide from *Sacchromyces cerevisiae*)	Sidwell et al. (1992, 1994)
Punta Toro	Mouse	Bropirimine (5-bromo-2,3-dihydro-2-imino-6-phenyl-4(1*H*) pyrimidinone)	Sidwell et al. (1990, 1992, 1994)
Punta Toro	Mouse	3,6-Bis(2-*p*-peridinoethoxy) acridine trihydrochloride	Sidwell et al. (1994)
Punta Toro	Mouse	Phenyleneamine	Sidwell et al. (1994)
Punta Toro	Mouse	Human recombinant interferon-α-ND	Sidwell et al. (1994)
Rift Valley fever	Mouse, hamster, Rhesus monkey	Ribavirin	Stephen et al. (1980) and Peters et al. (1986)
Rift Valley fever	Mouse, monkey	Ribavirin	Huggins et al. (1984)
Rift Valley fever	Mouse	Ribavirin	Canonico et al. (1982) and Peters et al. (1986)
Rift Valley fever	Monkey	Recombinant-leukocyte interferon-αA, human interferon-α	Morrill et al. (1989)
Rift Valley fever	Mouse	Poly(ICLC)	Peters et al. (1986), Kende (1985) and Kende et al. (1987)

(Watts et al., 1989; Nichol, 2001). By contrast, the hantaviruses are more difficult to propagate in vitro, growing slowly with little CPE production. Vero cells (E6 clone) are recommended (Nichol, 2001).

2.4. Animal models useful for antiviral evaluation

Rift Valley fever virus has been shown to be highly infectious to mice, with subcutaneous or intraperitoneal injection inducing fulminant hepatitis and a late-developing encephalitis (Stephen et al., 1980). Hamsters can be similarly infected (Huggins et al., 1984). Rhesus monkeys infected with RVF virus do not die of the infection or show clinical signs of disease, but develop relatively high viremias (Stephen et al., 1980; Huggins et al., 1984). All of the animal models for RVF have been successfully used in antiviral experiments by these same investigators.

The infectivity of the CCHF virus has been studied in 11 species of African rodents and in laboratory rabbits, guinea pigs and hamsters (Shepherd et al., 1989), but no clinical signs of infection were seen, although viremia was occasionally detected. Infant mice infected intraperitoneally with CCHF virus displayed a viremia with high titers of virus in the liver, followed later by lower titers in the brain and heart (Tignor and Hanham, 1993). Using the latter model, ribavirin therapy was shown to reduce the virus titers.

The Hantaan virus is infectious to suckling laboratory mice when inoculated intraperitoneally or intracerebrally, with high viremia and death occurring (Kim and McKee, 1985). The infection in mice appeared age-dependent. The suckling mouse model has been used for antiviral evaluations (Huggins et al., 1986). Severe combined immunodeficient (SCID) mice were also found susceptible to infection with either Hantaan or Seoul viruses, the animals dying of a wasting disease 32–35 days after virus inoculation (Yoshimatsu et al., 1997). Adult Syrian hamsters have been shown to develop rapid respiratory distress with pathological findings of pulmonary edema and pleural effusion following exposure to the Andes strain of HPS virus (Hooper et al., 2001). Cynomolgus macaques infected with the Puumala virus showed typical signs of HFRS including lethargy, anorexia, proteinuria, and/or hematuria (Groen et al., 1995; Klingstrom et al., 2002). Viral RNA was detected in the plasma from as early as day 3 to as late as day 28 post-virus inoculation (Klingstrom et al., 2002).

The only animal model reported for sandfly fever viruses is the cynomolgus monkey (*Macaca fascicularis*), and in this animal, no clinical signs of disease were seen (McClain et al., 1997). The most notable event reported was a decrease in lymphocytes following intramuscular challenge with the Sicilian virus.

A problem in dealing with most of the viruses in the Bunyaviridae family is the concern for safety, since, with the exception of the sandfly fever virus, the viruses described above are rated as biosafety level (BSL) three or four agents (Richmond and McKinney, 1999). To circumvent this, much work has been done with the Punta Toro (PTV), a BSL-2-rated *Phlebovirus* isolated from a patient in Panama and initially described by Pifat and Smith (1987) to induce a nonencephalitic, lethal infection in mice that is characterized particularly by fulminant hepatocellular necrosis following peripheral inoculation of the virus. This murine infection appears similar in many respects to the disease induced by other phleboviruses in both humans and livestock (Pifat and Smith, 1987; Sidwell et al., 1988a). Extensive studies have been undertaken utilizing the PTV, initially through the support of the US Army Medical Research and Development Command, to develop drugs that would have potential for treating infections by viruses of the Bunyaviridae family. As a first phase of this development, test substances were evaluated for in vitro efficacy against the PTV using LLC-MK2 cells. Compounds found to have in vitro efficacy, together with those known to have immunomodulatory activity, were then evaluated in mice infected with the Adames (hepatotropic) strain of PTV. Materials considered to have sufficient inhibitory activity in the murine PTV infection were subsequently evaluated under the appropriate biosafety level conditions against experimentally induced infections of Rift Valley fever, sandfly fever, and CCHF viruses at the US Army Medical Research Institute for Infectious Diseases. The overall results of the PTV studies have been reviewed (Sidwell et al., 1994, 1995). Based on results of the comparison studies run with these other viruses, as summarized in Table 2, it was felt that in vitro and murine hepatotropic PTV infections can be used to predict efficacy against other viruses in the Bunyaviridae family.

There is an additional PTV infection model in mice, using the neurotropic Balliet strain of the virus inoculated intraperitoneally or intracerebrally. In this infection, the mice die of encephalitis (Sidwell et al., 1988a). This infection has been much more difficult to treat effectively, although a number of compounds, particularly analogues of ribavirin, were synthesized especially for use against such infections (Deyrup et al., 1991; Bhagrath et al., 1991; Brewster et al., 1992).

The PTV will also induce infections in hamsters (Anderson et al., 1990). Subcutaneous injection of the Adames strain of the virus induced an acute fatal disease characterized by severe necrosis of the liver, spleen, and small intestine, and high virus titers in these tissues. The Balliet strain of PTV, similarly administered, induced only a mild hepatocellular infection with moderate virus titers in the liver. No antiviral studies have been reported using the hamster PTV model.

3. Viruses of the Togaviridae family

3.1. Significance

The alphaviruses EEE, WEE, and VEE all induce encephalitis in humans and are also responsible for encephalitic

disease in equines. All are transmitted by mosquitoes, but aerosol transmission may also occur, and it is the ability of these viruses to remain highly infectious in an aerosol state which particularly leads to their consideration as biological weapons (Huxsoll et al., 1987). In addition, these alphaviruses can be produced in large quantity in inexpensive and simplified systems and are relatively stable when stored and manipulated (Franz et al., 1997). Of special significance is the report that the US military weaponized and stockpiled VEE virus in the 1960s as an incapacitating agent; the stored virus was later destroyed (Christopher et al., 1997). Overall, VEE virus is more strongly considered as a biowarfare candidate because of its lower human infectious dose (Bronze et al., 2002). The primary differentiation in the significance of these viruses is in the locality in which they commonly occur in nature.

The EEE virus is enzootic in North America along the eastern seaboard and the Gulf Coast to Texas, and is also found in the Caribbean, in Central America, the north and east coast of South America, in the Amazon Basin, and occasionally in other inland areas of the United States (Causey et al., 1961; Morris, 1988). Periodic outbreaks of the human disease, which are often fatal (up to 70% in children) are usually associated with proximity to equine cases (Fothergill et al., 1999). The EEE virus has been the cause of four laboratory associated cases of disease (Richmond and McKinney, 1999).

WEE virus is primarily found in the western US and Canada as well as in South America (Hammon et al., 1942; Griffin, 2001). The fatality rate of WEE virus infections in humans is approximately 10% (Griffin, 2001), with the disease severity being greatest among infants and young children (Earnest et al., 1971). Seven laboratory infections with two deaths have been reportedly caused by WEE virus (Richmond and McKinney, 1999).

VEE virus epizootics/epidemics were initially reported in Venezuela, Colombia, Peru, and Ecuador, spreading then into Central America (Sidwell et al., 1967), and then into the southern United States (Kinney et al., 1992). A major outbreak of VEE occurred in Venezuela and Colombia in 1995, causing disease in over 75,000 people with 300 deaths reported (Weaver et al., 1996). Significantly, VEE virus has caused at least 150 human laboratory infections, indicating the ease of aerosol transmission of the virus and resulting in a BSL-3 categorization of the agent (Casals et al., 1943; Richmond and McKinney, 1999).

The clinical manifestation of EEE may be relatively mild, consisting of fever, chills, malaise, and myalgias of 1–2 weeks duration, but can proceed to severe fulminant encephalitis manifested as fever, headache, vomiting, seizure, and coma followed typically by death in 2–10 days (Morris, 1988). The disease induced by WEE virus is similar to EEE and may also include photophobia, rigidity of the neck, altered mental status and paralysis (Medovy, 1943; Finley et al., 1955). Survivors may suffer permanent brain damage, with possible continued progression of the disease. The VEE disease in humans may range from mild disease manifestations, in cases induced by enzootic strains of the virus, to fatal encephalitis with symptomology as described above (Weaver et al., 1996). Seizure disorders and other neurological deficits are often seen in children who recover from VEE virus-induced encephalitis (Leon, 1975). If infection occurs during pregnancy, spontaneous abortions, stillbirths, and fetal abnormalities often occur (Weaver et al., 1996).

3.2. Cell culture infectivity

These Togaviridae viruses grow readily in both primary and continuous lines of primate, mouse, hamster, guinea pig, chick embryo and duck embryo cells, causing cytopathic effect and plaques (Mussgay et al., 1975). Large-plaque strains of WEE virus usually have an increased virulence compared to small-plaque strains (Jahrling, 1976).

3.3. Prevention and control

These alphaviruses are capable of being genetically manipulated using recombinant DNA technology, which provides an opportunity to develop more effective and safer vaccines (Davis et al., 1991). Currently a formalin-inactivated vaccine for EEE is available for investigational use in humans and is recommended to protect laboratory workers (Richmond and McKinney, 1999). A similar formalin-inactivated vaccine for WEE is used for protection of horses and is also available for laboratory workers (Randall et al., 1947). A formalin-inactivated vaccine for VEE, designated C-84, has been prepared from an attenuated virus (TC-83); the vaccine has been used for laboratory workers, but the live TC-83 virus is preferred (Jahrling and Stephenson, 1984). The latter vaccine is reactogenic, often associated with development of fever, malaise, and headache as well as pharyngeal viral shedding (Pittman et al., 1996). Work is underway to develop a more effective vaccine for VEE (Bennett et al., 1998).

No antiviral drugs are known to have been used in the clinic for treatment of infections induced by viruses in the Togaviridae family. A number of compounds have been reported to have at least moderate disease-inhibitory effects against encephalitis infections in mice. These are summarized in Table 3. The observation that recombinant interferon appears to have a therapeutic effect against such virus infections (Pinto et al., 1988, 1990; Lukaszewski and Brooks, 2000) is significant, since this material has use in the clinic against other diseases.

Treatment with virus-neutralizing antisera has reportedly been ineffective if the brain infection has been established (Franz et al., 1997).

3.4. Animal models

The EEE virus has a strong neurovirulence in monkeys, hamsters, guinea pigs and mice (Wyckoff and Tesar, 1939;

Table 3
Compounds considered significantly inhibitory in animal models to viruses of the Togaviridae family

Virus	Animal model	Compound	Reference
Semliki Forest	Mouse	2-Amino-5-halo-6-aryl-4(3*H*)-pyrimidinones	Skulnick et al. (1985)
Semliki Forest	Mouse	Ribavirin-5′-sulfamate	Smee et al. (1988)
Semliki Forest	Mouse	7-Thia-8-oxoguanosine	Smee et al. (1990b)
Semliki Forest	Mouse	2-Amino-5-bromo-6-methyl-4(3*H*)-pyrimidinone	Pinto et al. (1990)
Semliki Forest	Mouse	Ampligen	Pinto et al. (1990)
Semliki Forest	Mouse	Poly ICLC	Pinto et al. (1990)
Semliki Forest	Mouse	MVE-2	Pinto et al. (1990)
Semliki Forest	Mouse	Recombinant interferons	Pinto et al. (1990) and Morahan et al. (1991)
Semliki Forest	Mouse	7,8-Didehydro-7-methyl-8-thioxoguanosine	Henry et al. (1990)
Semliki Forest	Mouse	7-Deazaguanosine	Smee et al. (1991c)
Semliki Forest	Mouse	Melatonin	Ben-Nathan et al. (1995)
Semliki Forest	Mouse	8-Chloro-7-deazaguanosine	Smee et al. (1995)
Venezuelan equine encephalitis	Mouse	Recombinant interferon	Pinto et al. (1988)
Venezuelan equine encephalitis	Mouse	Pegylated interferon-α	Lukaszewski and Brooks (2000)
Venezuelan equine encephalitis	Mouse	CL246,738	Pinto et al. (1988)
Venezuelan equine encephalitis	Mouse	Ampligen	Pinto et al. (1988)
Venezuelan equine encephalitis	Mouse	MVE-2	Pinto et al. (1988)

Morgan, 1941; Liu et al., 1970; Dremov et al., 1978). The infection in mice is age-dependent, with newborns sensitive to either peripheral or intracerebral injection of the virus, whereas young adults succumb only to intracerebral injection (Morgan, 1941; Liu et al., 1970; Murphy and Whitfield, 1970). The EEE virus infection in hamsters causes hepatitis and lymphatic organ infection as well as encephalitis (Dremov et al., 1978).

The WEE virus has been reported to be infective for mice, guinea pigs, and hamsters (Hitchcock and Porterfield, 1961; Aguilar, 1970; Zlotnick et al., 1972; Monath et al., 1978; Bianchi et al., 1997). Newborn mice die with skeletal muscle, cartilage and bone marrow involvement within 2 days following peripheral injection of WEE virus, whereas weanlings display infection in the brain, lung, heart, and brown fat (Aguilar, 1970). Hamsters infected with relatively avirulent WEE virus strains develop progressive neuropathologic changes (Zlotnick et al., 1972). North American strains of WEE virus appear to be more virulent for laboratory animals than South American strains (Bianchi et al., 1997; Hardy et al., 1997).

Extensive work has been done with VEE virus in laboratory animals. Rhesus macaques, hamsters, guinea pigs, rabbits, and mice are all susceptible to experimental infection. Infection of monkeys with epizootic strains induces a biphasic febrile response, with generally mild symptoms including anorexia, irritability, diarrhea, and tremors (Gleiser et al., 1962; Monath et al., 1974). Histopathologic examination indicates mild hepatitis, myocarditis, and encephalitis (Victor et al., 1956; Gleiser et al., 1962). Hamsters, guinea pigs and rabbits inoculated subcutaneously develop fatal infections involving bone marrow, lymph nodes, spleen, liver, intestinal wall, pancreas, and brain (Victor et al., 1956; Gleiser et al., 1962; Jahrling and Scherer, 1973a,b; Gorelkin and Jahrling, 1975). The VEE infection in mice will kill the animals in 6–7 days, due to encephalomyelitis and myeloid and lymphoid necrosis (Victor et al., 1956; Gleiser et al., 1962). Intranasal exposure or peripheral injection of mice with VEE still leads to a fatal encephalitis (Charles et al., 1995; Jackson and Rossiter, 1997; Steele et al., 1998).

Despite the availability of the animal models described above for EEE, WEE, and VEE viruses, much of the in vivo antiviral studies reported to date have utilized the infection in mice induced by the related Semliki Forest virus as summarized in Table 3. This virus can induce encephalitis in mice, rats, hamsters, guinea pigs, and rabbits, with the severity and type of disease being dependent on the age of the animal, the route of virus inoculation and the virulence of the virus strain used (Smithburn and Haddow, 1944; Zlotnick and Harris, 1970; Bradish et al., 1971; Atkins et al., 1990).

4. Future directions

It is apparent that certain viruses in the Bunyaviridae and Togaviridae families are likely agents for use by bioterrorists. They induce significant infection, making them useful as incapacitating agents; with the possible exception of the hantaviruses, they are relatively easy to cultivate so large stocks can be prepared; they are amenable to aerosolization which would be the preferred route of their dispersal; and none are fully controllable yet by vaccine or antiviral therapy. Certain vaccines have shown some promise for the majority of the viruses considered, but further studies are needed, particularly using recombinant technologies, to develop more acceptable vaccines. Although ribavirin and certain of its analogs, as well as interferon and interferon inducers, hold some promise for therapy of viruses in the Bunyaviridae family, and interferon may prove useful for treatment of Togavirus infections, much additional work is

needed to identify and develop additional therapies for these virus infections. Animal models for most of these virus infections have been developed and should prove useful in these needed additional studies.

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