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BICHAT GUIDELINES* FOR THE CLINICAL MANAGEMENT OF BIOTERRORISM-RELATED VIRAL ENCEPHALITIS

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Most of the viruses involved in causing encephalitis are arthropod-borne viruses, with the exception of arenaviruses that are rodent-borne. Even if little information is available, there are indications that, most of these encephalitis-associated viruses could be used by aerosolisation during a bioterrorist attack. Viral transfer from blood to the CNS through the olfactory tract has been suggested. Another possible route of contamination is by vector-borne transmission such as infected mosquitoes or ticks. Alphaviruses are the most likely candidates for weaponisation. The clinical course of the diseases caused by these viruses is usually not specific, but differentiation is possible by using an adequate diagnostic tool. There is no effective drug therapy for the treatment of these diseases and treatment is mainly supportive, but vaccines protecting against some of these viruses do exist.

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Introduction

Most of the viruses that are candidates for bioterrorist agents and which cause encephalitis are arthropod-borne viruses, with the exception of arenaviruses that are rodent-borne (TABLE 1). All known arthropod-borne viruses that can cause encephalitis are also implicated in zoonoses and are maintained in complex life cycles involving a non-human primary vertebrate host and a primary arthropod vector. Humans and domestic animals can develop clinical illness but usually are 'dead-end' hosts because they do not develop significant viraemia to contribute to the transmission cycle. Many arboviruses that cause encephalitis have a variety of different vertebrate hosts and some are known to be transmitted by more than one vector. Their scientific histories, geographic distributions, virology, epidemiology, vectors, vertebrate hosts, transmission, pathogenesis, clinical features, control, treatment and laboratory diagnosis are quite different and therefore require separate descriptions.

The clinical course of the diseases caused by these viruses is usually not specific, but differentiation is possible by using good and adequate laboratory methods of diagnosis. For many of the diseases, the majority of human infections are asymptomatic or may result in a non-specific flu-like syndrome (TABLE 2). Onset may be insidious or sudden with fever, headache, myalgia, malaise and occasionally prostration. Infection may, however, lead to encephalitis with a fatal outcome or permanent neurologic sequelae. Experimental studies have shown that invasion of the central nervous system (CNS), generally follows initial virus replication in various peripheral sites and a period of viraemia. There is no effective drug therapy for the treatment of these diseases and treatment is mainly supportive, but vaccines protecting against some of these viruses do exist.

Encephalitis viruses and bioterrorism

Even if data are very rare, many authors think that most of these encephalitis-associated viruses could be used by aerosolisation during a bioterrorist attack (TABLES 3 and 4). Viral transfer from blood to the CNS through the olfactory tract has been suggested. Another possible route of contamination is the use of vector-borne transmission such as infected mosquitoes or ticks. Weaponisation of alphaviruses, especially the equine encephalitis viruses, would be the easiest. We have chosen to describe the viruses that could be implicated in biological weapons by family.

Togaviridae

Eastern equine encephalitis (EEE), western equine encephalitis (WEE) and Venezuelan equine encephalitis (VEE) are alphaviruses that can cause encephalitis in both humans and equines. All these viruses are transmitted to humans by mosquito bites. Of importance, all these viruses are transmittable by aerosol, as already reported in laboratory accidents. These highly infectious particles have caused more laboratory-acquired disease than any other arbovirus [1]. These viruses would lend themselves well for weaponisation for the following reasons: they replicate to very high titres, they can be manipulated easily in unsophisticated systems, as they are relatively stable and highly infectious for humans by aerosolisation; strains exist that produce incapacitating or lethal infections in humans; multiple serotypes exist, making the production of adequate appropriate vaccine difficult [1]. The infectious dose for inhalation is extremely low for some viruses (1 plaque forming unit (pfu) for VEE, per historical military data), but is unknown for all individual viruses. Some authors also suggest that these viruses could be easily modified by genetic manipulation [1]. Although these viruses cause similar clinical syndromes, the consequences and evolution of associated individual diseases differ. Person-to-person transmission has never been reported, despite the theoretical possibility (e.g. VEE from nasopharynx).

Eastern equine encephalitis

EEE virus was first identified in the 1930s and currently occurs in focal locations along the eastern seaboard, the Gulf Coast and some inland midwestern locations of the United States (US). While small outbreaks of human disease have occurred in the US, equine epizootics are a common occurrence during the summer and autumn. The natural reservoir of the virus are swamp-living birds. The disease is transmitted between birds by

the mosquito *Culiseta melaneura* and to horses and humans by species of the genus *Aedes*. Human cases are usually preceded by an outbreak in horses.
The incubation period varies from 4 to 15 days after the bite of an infected mosquito. Symptoms range from mild flu-like illness to encephalitis, coma and death. The symptoms begin with sudden onset of high fever, chills, vomiting, general muscle pains and headache of increasing severity. Fever can persist up to 11 days before the onset of neurological disease. Generalised facial or periorbital oedema is frequent in children. Patients progress to more severe symptoms and signs such as confusion, somnolence, delirium, stupor, disorientation, dysphasia, paresis, ataxia, myoclonus and cranial nerve palsies, seizures and coma, and finally death. It has been estimated that 1 out of 23 infected patients developed neurological symptoms such as encephalitis [2]. Despite the development of a rapid and neutralising humoral response, the virus is not eradicated from the CNS and progressive neuronal destruction and inflammation continues [1]. EEE is the most severe of the arboviral encephalitides, with case fatality rates of 50% to 70%, mostly in young children and the elderly. It is also associated with frequent neurological sequelae in survivors, requiring permanent institutional care (seizures, spastic paralysis and cranial neuropathies). However, mortality and morbidity rates due to aerosolisation of EEE virus are unknown.
Laboratory findings show increased ASAT, lymphopenia and an early leukopenia followed by a leukocytosis. A lymphocytic pleocytosis is often found in cerebrospinal fluid (CSF). Virus isolation (throat, serum, CSF), serologic tests and PCR can assist definitive diagnosis.
There is no specific treatment. Antisera are available, but are of limited use when encephalitis is already present. There is no licensed vaccine for humans. An investigational vaccine for humans exists but is poorly immunogenic. Multiple immunisations need to be given to achieve significant protection.

Western equine encephalitis

WEE was first isolated in California in 1930 from the brain of a horse with encephalitis, and remains an important cause of encephalitis in horses and humans in North America, mainly in western parts of the US and Canada. Seroprevalence rates among people living in or near endemic WEE areas is about 100% [1]. This indicates that most of the human cases are asymptomatic. Human cases are usually reported in June or July. As mentioned above, most of these mosquito-borne infections are asymptomatic or present as mild, non-specific illness. The incubation period varies from 5 to 10 days. WEE is less neuroinvasive than EEE, but signs and symptoms are identical to those noted in cases of EEE.
Patients with clinically apparent illness usually have sudden onset of fever, malaise and headache, followed by nausea, vomiting and anorexia. Then the symptoms intensify and are followed by altered mental status, somnolence, weakness, meningismus and delirium that can progress into coma. Children, especially those under 1 year old, are affected more severely than adults and may be left with permanent sequelae, seen in 5% to 30% of young patients. The ratio of encephalitis cases per infection has been estimated at 1/1 150 in adults, 1/58 in children and 1/1 in infants [3]. The mortality rate is about 3% overall. Total recovery can take months to years. Laboratory findings are the same than those noted with EEE. Virus isolation or serologic tests are definitively diagnostic. As for EEE, there is no specific treatment. An investigational vaccine exists but is poorly immunogenic. There is no licensed vaccine available for humans.

Venezuelan equine encephalitis

VEE is an important veterinary and public health problem in Central and South America. Occasionally, large regional epizootics and epidemics can occur resulting in thousands of equine and human infections. Historical military data report that the virus was studied extensively for weaponisation by the US, United Kingdom (UK) and the Soviet Union. It can be aerosolised in a form which is quite effective; infecting 100% of exposed persons to cause clinical features similar to the natural infection.
In nature mosquitoes can transmit many different strains of VEE to humans and cause disease. A large epizootic that began in South America in 1969 reached Texas in 1971: 200 000 horses died and several thousand human infections were reported. A more recent VEE epidemic occurred in autumn 1995 in Venezuela and Colombia with an estimated 90 000 human infections. The human infection is less severe than with EEE and WEE viruses, and fatalities are rare.
The incubation period varies from 28 hours to 6 days [1]. Patients usually complain of a constant and often debilitating headache, which may last up to six months. They may also develop high fever, photophobia, myalgia, sore throat, erythematous pharynx, conjunctival injection, vomiting, diarrhoea and malaise mimicking an influenza-like illness. Encephalitis is usually confined to children: it has been estimated that less than 0.5% of adults and 4% of children develop encephalitis [4]. Lethargy, somnolence, confusion, seizures, ataxia, paralysis and coma are possible. Neurological recovery is usually complete and takes 1-2 weeks. Laboratory findings are the same as those noted with EEE and WEE. During the first 3 days the VEE virus can be isolated either from the serum or the nasopharynx. Treatment is only symptomatic. Specific diagnosis can be accomplished with different serologic tests.
Effective VEE virus vaccines are available for equines. A live-attenuated (TC-83 and C-84) and a whole inactivated vaccine for humans (C-84) exist, but they are both associated with numerous side effects [1]. Only the live-attenuated vaccine could be useful in the case of a deliberate release of aerosolised VEE viruses (1).

Flaviviridae

The recent outbreaks of West Nile encephalitis in the US and Israel are drawing attention to the potential threat posed by arbovirus encephalitis due to small enveloped RNA viruses, members of the family Flaviviridae, genus flaviviruses [5].
Many viruses are included in this family and are associated with encephalitis. The Japanese encephalitis (JE) virus remains the most widely spread virus of this family, associated with numerous cases of encephalitis annually. Knowledge concerning the weaponisation of these viruses is only hypothetical, but nevertheless, theoretically possible. No specific antiviral therapy is available for treatment or prophylaxis of infections with members of the Flaviviridae, although a licensed human vaccine exists against JE [6].

Japanese encephalitis (JE)

JE virus is widespread throughout Asia, mostly in agricultural areas. It is the most important cause of arboviral encephalitis in the world, with over 50 000 cases reported annually and an attributed 15 000 deaths [5].
In recent years JE virus has expanded its geographic distribution with outbreaks recognised in the regions of the Pacific. Epidemics occur in the summer and autumn in temperate regions, but the infection is enzootic and occurs throughout the year in many tropical areas of Asia. The virus is maintained in a cycle involving mosquitoes and water birds that live in rural and

pig farming regions. Domestic pigs are the main amplifying hosts of JE virus while it is transmitted to humans by *Culex* mosquitoes.
In humans, the incubation period varies from 5 to 14 days. The majority of infections are asymptomatic or associated with mild symptoms. Onset of more severe illness is usually abrupt, with fever, chills, headache, nausea and vomiting. Usually the illness resolves in 5 to 7 days in the absence of CNS involvement. In case of acute encephalitis, confusion, agitation, paralysis, seizures, coma and death can occur. The mortality in most outbreaks is less than 10%, but is higher in children (up to 30%). Neurologic and psychiatric sequelae in patients who recover are reported in up to 30% of cases [5]. There is no specific treatment for this disease.
A formalin-inactivated vaccine prepared in mice is used widely in Japan, China, India, Korea, Taiwan and Thailand, and has proven effective. In Europe, this vaccine is available in some countries for individuals who might be travelling to rural areas in endemic countries for 4 weeks or more.

West Nile virus encephalitis (WNV)

WNV was first isolated in the blood of a febrile woman in the West Nile district of Uganda in 1937 [7]. Its usual distribution includes Africa, the Middle East, western Asia and southern Europe. The first recorded epidemics occurred in Israel during 1951-1954 and in 1957. Epidemics have been reported in Europe in the Rhône delta of France in 1962 and in Romania in 1996, but the largest recorded epidemic occurred in South Africa in 1974.
The recent outbreaks of WNV in the US raise important issues about the spread, control and pathogenesis of this virus. Many theories have been reported on how this virus reached the US: imported exotic birds, container ships, aeroplane-borne mosquitoes, European or African refugees, biological terrorism. Infected birds migrating from Israel seems the most likely theory of all [7-12]. WNV is transmitted principally by *Culex* species mosquitoes, but can also be transmitted by *Aedes*, *Anopheles* and other species. Most patients infected with WNV will either have no symptoms or only mild flu-like illness lasting 3-4 days. Some will experience fatal encephalitis or meningoencephalitis [13]. Usually, the disease is symptomatic in elderly patients with pre-existing illnesses such as hypertension, diabetes mellitus, ischaemic heart disease, renal failure, obstructive lung disease, malignancy, history of organ transplantation and in general immunocompromising conditions such as chemotherapy [14]. The incubation period varies from 3 to 14 days. Symptoms may include fever, chills, fatigue, altered mental status, headache, weakness, rash, nausea, vomiting, myalgia, photophobia, abdominal pain, diarrhoea and cough [14,15]. Neurological symptoms include meningitis, encephalitis or meningoencephalitis [14,15].
Laboratory findings are non-specific and include mild hyponatraemia that may be due to inappropriate secretion of antidiuretic hormone, mild elevated liver enzymes, and leukocyte count within normal values. A lymphocytosis is often found in the CSF, together with a mild increase of protein and normal glucose [14,15]. CT scan and MRI of the brain are non-specific [14]. The incidence of severe neuroinvasive disease and the mortality rate increases with age [13]. The mortality rate in patients with encephalitis or meningoencephalitis is about 12%-14% [13,14]. More than 50% of patients who survive have neurological sequelae [15].
Diagnosis can be made by using reverse transcriptase PCR (RT-PCR), immunohistochemical and serological testing or ELISA in serum, CSF and brain tissue specimens [11].
In case of encephalitis, treatment is only symptomatic. Ribavirin is not effective and in fact, it has been associated with poor outcomes (death) in patients with encephalitis [14]. There is no vaccine against WNV but research is ongoing and scientists hope to have a new vaccine available in a few years.

St Louis encephalitis (SLE)

SLE is the most common mosquito-transmitted human pathogen in the US. Since 1964, there have been 4437 confirmed cases of SLE with an average of 193 cases per year (range 4 – 1967). During late summer or early autumn, SLE virus is maintained in a mosquito-bird-mosquito cycle, with periodic amplification by peridomestic birds and *Culex* mosquitoes.
In humans the incubation period varies from 5 to 15 days. Less than 1% of SLE viral infections are symptomatic, with a severity ranging from a simple febrile headache to meningoencephalitis. The onset of the illness is marked by fever and headache, followed in some cases by encephalitis or meningoencephalitis [16]. In patients who develop such neurological disorders, case-fatality ranges from 3% to 30 %. The disease is usually milder in children than in adults, but for children who do become ill, there is a high rate of encephalitis. The elderly are at highest risk of severe disease and death. Diagnosis is confirmed by using serologic tests or RT-PCR. There is no specific treatment and no vaccine available for SLE.

Tickborne complex encephalitis (TBE)- Russian spring-summer encephalitis (RSSE) and Central European encephalitis (CEE)

TBE is a viral infection of the CNS transmitted by bites of certain vector ticks or occasionally by consuming non-pasteurised dairy products from viraemic infected cows, goats, or sheep [17,18]. It is caused by two closely related flaviviruses that are biologically distinct. The eastern subtype causes RSSE and is transmitted by *Ixodes persulcatus.* The western subtype is transmitted by *Ixodes ricinus* and is associated with CEE. TBE is common in Austria, Estonia, Latvia, the Czech Republic, Slovakia, Germany, Hungary, Poland, Switzerland, Russia, Ukraine, Belarus and Slovenia. It occurs with lower frequency in Bulgaria, Romania, Denmark, France, the Aland archipelago and neighbouring Finnish and Swedish coastlines. Occasionally cases occur in other parts of Sweden too. RSSE occurs in China, Korea, Japan and eastern areas of Russia. Risk of acquiring the disease is greatest from April to August, when *Ixodes ricinus* is most active. Signs and symptoms of RSSE and CEE are practically the same, but the severity of the syndrome is greater with RSSE.
The incubation period varies from 7 to 14 days. Infection usually presents as a mild, influenza-type illness or as aseptic meningitis. TBE is typically a biphasic disease [19]. Fever, myalgia and severe headache lasting up to a week, mark the onset. After a mean 1-3 days asymptomatic phase (up to 3 weeks), a third to a quarter of the symptomatic patients may develop different signs of CNS involvement: rigidity, transient paralysis of the limbs, shoulders or less commonly the respiratory musculature, leading to meningoencephalitis, meningitis with or without myelitis. Recovery from a severe case of the disease is usually slow and 20% of patients may have residual paralysis, permanent paresis, coordination disturbances, headaches, hearing defects or minor neuropsychiatric sequelae. RSSE is the more severe infection, having a mortality rate of up to 25%, whereas mortality in CEE is near 4%.
Leukopenia, thrombocytopenia, elevation of the erythrocyte sedimentation rate (ESR), increased C-reactive protein (CRP) and pleocytosis in the CSF are frequent [19]. Diagnosis is confirmed by serology or RT-PCR [20].

Australian encephalitis: Murray Valley encephalitis (MVE) and Kunjin viruses

MVE and Kunjin viruses are potentially fatal mosquito-borne diseases endemic in Papua New Guinea and in the northern half of Australia. Most of the cases are reported between February and April. Most of the infections (99%) are asymptomatic [21]. Most clinical cases occur in children or people who have recently arrived in an endemic area. Humans are normally infected through mosquito bites. Three cases of laboratory-acquired infections with MVE virus have been reported after exposure to aerosols of infectious solutions. The incubation period is usually 5-15 days. The onset of the disease is abrupt with high fever and headache. This is followed in some patients by nuchal rigidity and neurological signs including stupor, coma, spastic quadriplegia, seizures and paralysis. The mortality rate in patients with neurologic symptoms is very high, up to 60% and neurologic sequelae are reported in up to 40% of the survivors, including paraplegia, gait impairment and cognitive defects [21]. Diagnosis is confirmed by serology. There is no specific treatment or vaccine.

Powassan encephalitis (POW)

POW virus is a tickborne arbovirus occurring in the US and Canada [22]. Transmission by consumption of raw milk from infected animals is also suspected to occur. Two laboratory-acquired infections after exposure to aerosols of infectious solutions have been reported. Recently a Powassan-like virus was isolated from the deer tick *Ixodes scapularis*. Its relationship to POW and its ability to cause human disease has not been fully investigated.

POW a rare cause of acute viral encephalitis and fewer than 50 cases have been reported to date in the literature [22,23]. Like most of the other arthropod-borne viruses associated with encephalitis, POW virus usually causes no symptoms at all or only mild clinical illness. The incubation is 7 to 34 days [23]. The onset of the disease is abrupt, with fever, headache, sore throat, nausea, vomiting, stiff neck and lethargy. As the disease progresses, neurological signs occur with breathing distress, tremor, seizures, coma, spastic paralysis, aseptic meningitis and, in some cases, death. Hemiplegia is frequent [23]. The case mortality rate is 10-15% [22]. POW encephalitis is associated with significant long-term neurological sequelae in 50% of cases [22-24]. Diagnosis is confirmed by serology (ELISA) and RT-PCR [24]. There is no specific treatment and no vaccine.

Rocio virus

This virus was first identified as an agent causing an epidemic of human encephalitis in Sao Paulo, Brazil in 1978: about 1000 patients were infected [25]. Mosquitoes transmit the disease. Wild birds are the natural host and reservoir of the virus. The disease is poorly characterised. The onset of the disease includes fever, headache, vomiting and conjunctivitis. It progresses to neurological symptoms and muscle weakness. About one-third of cases enter a coma; one third go on to die and about 20% have neurological sequelae. Diagnosis is made by serology.

Louping ill virus

This virus has been identified in Scotland. It is transmitted by the sheep tick *Ixodes ricinus*. Fewer than 50 cases of this encephalitis have been reported in humans [26]. Laboratory infections with this virus have been reported after aerosolisation of infected solutions [26,27]. Clinical features include an influenza-like illness, biphasic encephalitis, a poliomyelitis-like illness and a haemorrhagic fever [26].

Bunyaviridae

La Crosse encephalitis (LAC)

LAC is the most common arboviral infection in children in the US, mostly in children under 16 years of age, with approximately 75 cases reported annually [28,29]. Jamestown Canyon, Trivittatus and Cache Valley viruses are related to LAC, but rarely cause encephalitis.

LAC virus is a zoonotic pathogen circulated between the eastern tree hole mosquito *Aedes triseriatus*, and vertebrate amplifier hosts (small animals) in hardwood forests [28]. The virus is maintained over the winter by transovarial transmission in mosquito eggs. An infected female mosquito lays eggs that carry the virus and the adult mosquitoes coming from those eggs can transmit the virus to chipmunks and to humans.

After an incubation period of 3-7 days, LAC encephalitis initially presents, as the other arthropod-borne viruses, as a non-specific summertime illness with fever, chills, headache, nausea, vomiting and abdominal pain lasting 1-4 days. The presence of rash is rare. Severe disease (encephalitis, meningitis, meningoencephalitis) occurs most commonly in children aged 6 months to 15 years and is characterised by somnolence, obtundation, seizures, aphasia, poor coordination, coma, and focal motor paralysis [30]. The total duration of the illness rarely exceeds 10-14 days. Neurological sequelae, present in more than 10% of cases, include sixth-nerve palsy, hemiparesis, speech problems, aphasia, decreased short-term memory and balance problems. Case-fatality rate in patients with encephalitis is less than 1%. Complete blood cell count is usually within normal values. Hyponatraemia may be present [30]. CSF examination shows normal glucose level, mild elevated protein level and a polymorphonucleosis followed by lymphocytosis. CT scan or MRI of the brain are not specific. Specific serologic tests or PCR [31,32] confirm the diagnosis. There is no specific therapy and no vaccine available.

Toscana (TOS)

The TOS virus is widely distributed in Italy but the syndrome occurs mainly in the Siena province. This virus is transmitted by sandflies to humans. The clinical picture is usually similar to that observed in other viral infections described before. Clinical features range from aseptic meningitis to meningoencephalitis, occurring throughout the summertime. The rate of encephalitis is low, and usually the outcome is benign [33-35].

Rift Valley fever

Rift Valley fever is usually found in regions of eastern and southern Africa but the virus also exists in most countries of the sub-Saharan Africa and in Madagascar, and has recently moved into the Arabian peninsula. It is a zoonosis that can affect domestic animals (cattle, buffalo, sheep, goats, camels) and occasionally humans [36]. Mosquitoes (usually of the genus *Aedes*) transmit the virus, but many other mosquito species may transmit the disease. Humans can also become infected after handling blood or body fluids of infected animals. Infection through aerosolisation has been reported in laboratory workers working with viral cultures or other laboratory samples containing the virus.

The incubation period varies from 2 to 6 days. The initial clinical manifestations are a biphasic fever, the first bout lasting about 4 days. After one or two days without fever, the second fever spike occurs lasting for 2 to 4 days. Usually, the illness is mild and associated with fever and liver abnormalities. But in severe cases, haemorrhage, encephalitis and retinitis can occur [37]. Less than 1% of patients develop a haemorrhagic fever syndrome 2 to 4 days after the onset of the illness. Clinical features are the same as observed in other VHF syndromes (epistaxis, haematemesis, melaena, and gastrointestinal haemorrhage). Patients usually recover within 2 days to 1 week after the onset of illness. Retinitis and meningoencephalitis are

usually observed 1 to 3 weeks after the onset of the disease. In 1%-10% of cases patients may suffer vision loss when the lesions are in the macula. The mortality rate for patients with Rift Valley fever is near 1%, mainly noted in patients that develop the haemorrhagic syndrome [37].
Thrombocytopenia and leukopenia, proteinuria, elevation of liver enzymes and jaundice are frequent [38]. Methods of diagnosis include viral isolation, antigen detection by antigen capture ELISA, IgM antibody detection by antibody-capture ELISA, and RT-PCR [38]. There is no specific treatment. Only a single antiviral drug, ribavirin, is recommended for the treatment [39-41]. No vaccine is licensed, although there is an investigational one available in the US for use by laboratory personnel and the US army.

Arenaviridae

Lymphocytic choriomeningitis (LCM)

This is a rodent-borne viral infectious disease that has been reported in Europe, the Americas, Australia and Japan. Humans become infected by inhalation of aerosolised particles from infected rodents, by ingestion of food contaminated with the virus, by contamination of mucus membranes with infected body fluids or by directly exposing cuts or abrasions to virus-infected products. Many cases of laboratory infections have been reported. Vertical transmission from an infected mother to fetus is also possible and perinatal exposure is associated with spontaneous abortion, congenital hydrocephalus, chorioretinitis and mental retardation [42].
The incubation period is between 8 and 13 days [43]. Most of the patients are asymptomatic or experience non-specific signs. The onset is marked by a biphasic fever, malaise, anorexia, myalgia, retro-orbital headache, abdominal pain, nausea and vomiting, sore throat, cough, joint pain usually of the hands, chest pain, testicular and parotid pain, lymphadenopathy, alopecia and maculopapular rash. This may last up to a week. Following a few days of remission, the second phase of the disease is defined by the occurrence of neurologic symptoms suggesting encephalitis, meningoencephalitis or meningitis. Acute hydrocephalus, myelitis and myocarditis have been reported. Leukopenia, thrombocytopenia and mild elevated liver enzymes are common during the first stage of the disease [43]. During the second phase, increased protein levels, pleocytosis and decreased glucose level are usually found in CSF.
In patients with neurological disorders mortality is less than 1%. Patients with encephalitis are at high risk of neurological sequelae. Diagnosis is confirmed with specific serology and PCR or by isolating the virus from the CSF. There is no specific treatment, although ribavirin is effective in vitro.

Machupo and Junin viruses

These two viruses are usually associated with a syndrome of severe haemorrhagic fever. These viruses are present in South America, specifically in a limited agricultural area of the pampas in Argentina (Junin) and in the remote savannas of the Beni province of Bolivia (Machupo) [20]. Each virus is associated with either one or a few closely related species of rodents. These rodents are chronically infected with these viruses, yet they do not develop disease. Human infection is usually caused by exposure to the urine or body secretions of infected rodents, either through breathing contaminated dust or consuming contaminated food or by direct contact of broken skin with rodent excrement. Transmission to humans is usually due to aerosolisation of dried excreta, especially urine that has been deposited in the environment [19]. These haemorrhagic fevers have occasionally been transmitted person-to-person, for example in healthcare settings and in intimate contacts. In these cases, direct contact with infected body secretions is believed to be a more important route of transmission than aerosol exposure [20]. Following an incubation period of 10 to 14 days (range: 5-18 days), most patients develop either no symptoms or a mild flu-like illness. Usually the onset of the disease is insidious with fever and general malaise over a 2 to 4-day period. In more severe cases, early clinical signs include weakness, retro-orbital pain, joint and lumbar pain, myalgia, headache, pharyngitis, cough and conjunctival injection [19,20]. In the most severe form of the disease patients can exhibit prostration, abdominal pain, facial and neck oedema, haemorrhages (conjunctival haemorrhages, mucosal bleeding, melaena, haematochezia, haematuria, vaginal bleeding, haematemesis), encephalitis, capillary leak syndrome and shock. Bleeding diathesis and neurologic signs are also common. Neurologic signs may include delirium, confusion, encephalitis, convulsions and coma. Conjunctival injection, facial flushing, petechial and/or vesicular palatal enanthem and skin petechiae, generalised lymphadenopathy and orthostatic hypotension are common. Lymphopenia, leukopenia and thrombocytopenia are characteristic. Junin and Machupo fevers are associated with high case fatality rates, ranging from 10-16% [18].

Paramyxoviridae

Hendra virus

This virus (formerly equine morbilli virus) is usually found in the horse population in Australia. The natural reservoir is thought to be bats of the genus *Pteropus*. The usual route of transmission of the disease to humans is by direct exposure to tissues or secretions from infected horses. Only three human cases of Hendra virus disease have been reported, including one patient with encephalitis [44]. The patient presented the first signs of encephalitis three months after having contact with an infected horse, and died.

Nipah virus

This virus is mainly found in Malaysia in the same bat reservoir as Hendra virus. It causes mild disease in pigs [45] and the transmission of the disease to humans occurs through close contact with them. The incubation period varies from 5 to 14 days. The onset of the illness includes 3-14 days of fever and headache and may be followed by encephalitis. The case fatality rate is near 40%. Survivors of acute encephalitis may have recurrent neurological disease (relapsing encephalitis), attributed to a persistent Nipah virus infection in the CNS [44,46]. The diagnosis is performed with serologic tests and PCR. There is no specific treatment or vaccine.

Even if data are very scarce, except for the more common encephalitis syndromes, most of these encephalitis-associated viruses could be used in an aerosolised form during a bioterrorist attack, as proven by the numerous laboratory associated cases of the diseases. A clinically compatible case, confirmed by laboratory results, in a patient without history of travelling to endemic areas or working with the agents, could indicate an intentional release.

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* **BICHAT**, the European Commission's Task Force on Biological and Chemical Agent Threats, has developed this set of guidelines that may be the basis of national authorities' guidance, and may also be used directly by clinicians, general practitioners and specialists when confronted with patients infected by agents that may be due to deliberate release of biological agents. Ref. Bossi P, Van Loock F, Tegnell A, Gouvras G. Bichat clinical guidelines for bioterrorist agents. *Euro Surveill*. 2004; 9(12)

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Editorial note: *These clinical guidelines were reviewed by the Task Force and by two experts designated by each Member State of the European Union. This review was completed at the end of February 2003. The revised guidelines were submitted to the Health Security Committee which approved them in April 2003 and agreed their publication in a widely disseminated journal so as to allow access to as large an audience as possible. The editorial process of Eurosurveillance also introduced modifications that improved the contents of these guidelines.*

TABLE 1

Encephalitis viruses that could be used in a bioterrorist attack

Family	Genus	Species
Togaviridae	Alphavirus	Eastern equine Western equine Venezuelan equine
Flaviviridae	Flavivirus	St Louis Australian encephalitis (Murray valley and Kunjin) West Nile Japanese Dengue Tickborne complex encephalitis = (Central European and Russian spring-summer) Powassan Rocio Louping ill
Bunyaviridae	Bunyavirus	La Crosse Rift Valley Toscana
Arenaviridae	Arenavirus	Lymphocytic Choriomeningitis Machupo Junin
Toroviridae		Hendra virus
Others		Herpesvirus simiae (B virus)

TABLE 2

Summary of clinical and biological description of viral encephalitis

Clinical description
· The majority of human infections are asymptomatic or may result in a non-specific flu-like syndrome. · Onset may be insidious or sudden with: fever, headache, myalgia, malaise and occasionally prostration. · Infection may lead to encephalitis, with a fatal outcome or permanent neurological sequelae. · Only a small proportion of infected persons progress to frank encephalitis.

TABLE 3

Case definitions of suspected and confirmed cases

Case definition
• **Possible:** NA • **Probable:** A clinically compatible case with an epidemiological link • **Confirmed:** A clinically compatible case that is laboratory-confirmed

NA: Not applicable
Source: [46,47]

TABLE 4

Case definition of a deliberate release of viral encephalitis

Suspicion of deliberate release
An unusual cluster of clinical encephalitis cases in an area and/or a period of the year: o where the virus is not endemic o when it is not the natural time of the year encephalitis is noted o without a travel history to endemic areas o without a history of laboratory exposure o where the cases are linked in time and place, especially ***geographically related groups*** of illness following a wind direction pattern

Source: [46,47]