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The Crux

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CONTENTS

Editorial

We have received communications to talk about Nipah Virus Infection and Viral Encephalitis in general from many of our readers.

Considering the gravity of the situation and current relevance we present to you Nipah Virus Infection right here under Editorial and Viral Encephalitis under **DISEASE DIAGNOSIS**.

INTERPRETATION AND TROUBLE SHOOTING have been clubbed together and are also related to diagnosis of viral infections, more so related to Cultures of Viruses in general. **"BOUQUET"** lurks somewhere in between.


Grey-headed flying foxes (*Pteropus poliocephalus*)

Nipah virus infection: Nipah virus (NiV) is a member of the family *Paramyxoviridae*, genus Henipavirus. NiV was initially isolated and identified in 1999 during an outbreak of encephalitis and respiratory illness among pig farmers and people with close contact with pigs in Malaysia and Singapore. Its name originated from Sungai Nipah, a village in the Malaysian Peninsula where pig farmers became ill with encephalitis. Given the relatedness of NiV to Hendra virus, bat species were quickly singled out for investigation and flying foxes of the genus *Pteropus* were subsequently identified as the reservoir for NiV. In the 1999 outbreak, Nipah virus caused a relatively mild disease in pigs, but nearly 300 human cases with over 100 deaths were reported. In order to stop the outbreak, more than a million pigs were euthanized, causing tremendous trade loss for Malaysia. Since this outbreak, no subsequent cases (in neither swine nor human) have been reported in either Malaysia or Singapore. In 2001, NiV was again identified as the causative agent in an outbreak of human disease occurring in Bangladesh. Genetic sequencing confirmed this virus as Nipah virus, but a strain different from the one identified in 1999. In the same year, another outbreak was identified retrospectively in Siliguri, India with reports of person-to-person transmission in hospital settings (nosocomial transmission). Unlike the Malaysian NiV outbreak, outbreaks occur almost annually in Bangladesh and have been reported. Transmission of Nipah virus to humans may occur after direct contact with infected bats, infected pigs, or from other NiV infected people. In Malaysia and Singapore, humans were apparently infected with Nipah virus only through close contact with infected pigs. The NiV strain identified in this outbreak appeared to have been transmitted initially from bats to pigs, with subsequent spread within pig populations. Incidental human infections resulted after exposure to infected pigs. No occurrence of person-to-person transmission was reported in this outbreak.

Conversely, person-to-person transmission of Nipah virus in Bangladesh and India is regularly reported. This is most commonly seen in the family and caregivers of Nipah virus-infected patients. Transmission also occurs from direct exposure to infected bats. A common example is consumption of raw date palm sap contaminated with infectious bat excretions.

Signs and Symptoms: ● Infection with Nipah virus is associated with encephalitis (inflammation of the brain). After exposure and an incubation period of 5 to 14 days,illness presents with 3-14 days of fever and headache, followed by drowsiness, disorientation and mental confusion. These signs and symptoms can progress to coma within 24-48 hours. Some patients have a respiratory illness during the early part of their infections, and half of the patients showing severe neurological signs showed also pulmonary signs. ● During the Nipah virus disease outbreak in 1998-99, 265 patients were infected with the virus. About 40% of those patients who entered hospitals with serious nervous disease died from the illness. ● Long-term sequelae following Nipah virus infection have been noted, including persistent convulsions and personality changes. ● Latent infections with subsequent reactivation of Nipah virus and death have also been reported months and even years after exposure.

Risk of Exposure: In the Malaysia and Singapore outbreak, Nipah virus infection was associated with close contact with Nipah virus-infected pigs.

In Bangladesh and India, where Nipah virus infection is more frequent, exposure has been linked to consumption of raw date palm sap and contact with bats. Importantly, human-to-human transmission has been documented and exposure to other Nipah virus infected individuals is also a risk factor.

Diagnosis: Laboratory diagnosis of a patient with a clinical history of NiV can be made during the acute and convalescent phases of the disease by using a combination of tests. Virus isolation attempts and real time polymerase chain reaction (RT-PCR) from throat and nasal swabs, cerebrospinal fluid, urine, and blood should be performed in the early stages of disease. Antibody detection by ELISA (IgG and IgM) can be used later on. In fatal cases, immunohistochemistry on tissues collected during autopsy may be the only way to confirm a diagnosis.

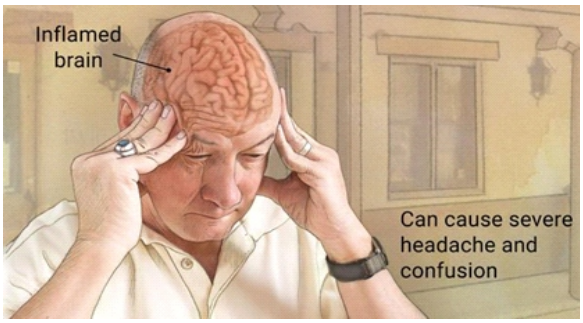
Treatment: Treatment is limited to supportive care. Because Nipah virus encephalitis can be transmitted person-to-person, standard infection control practices and proper barrier nursing techniques are important in preventing hospital-acquired infections (nosocomial transmission).

The drug ribavirin has been shown to be effective against the viruses in vitro, but human investigations to date have been inconclusive and the clinical usefulness of ribavirin remains uncertain. Passive immunization using a human monoclonal antibody targeting the Nipah G glycoprotein has been evaluated in the post-exposure therapy in the ferret model and found to be of benefit.

Prevention: Nipah virus infection can be prevented by avoiding exposure to sick pigs and bats in endemic areas and not drinking raw date palm sap. Additional efforts focused on surveillance and awareness will help prevent future outbreaks. Research is needed to better understand the ecology of bats and Nipah virus, investigating questions such as the seasonality of disease within reproductive cycles of bats. Surveillance tools should include reliable laboratory assays for early detection of disease in communities and livestock, and raising awareness of transmission and symptoms is important in reinforcing standard infection control practices to avoid human-to-human infections in hospital settings (nosocomial infection). A subunit vaccine, using the Hendra G protein, produces cross-protective antibodies against HENV and NIPV has been recently used in Australia to protect horses against Hendra virus. This vaccine offers great potential for henipavirus protection in humans as well.

DISEASE DIAGNOSIS

VIRAL ENCEPHALITIS



Background

Clinically relevant involvement of the central nervous system (CNS) by viruses is an uncommon event, considering the overwhelming number of individuals affected by the different human viral infections. Most commonly, clinically relevant viral encephalitis affects children, young adults, or elderly patients, but the spectrum of involvement depends on the specific viral agent, host immune status, and genetic and environmental factors. The term "acute viral encephalitis" (from Greek enkephalos + -itis, meaning brain inflammation) is used to describe restricted CNS involvement (ie, involvement of the brain, sparing the meninges); however, most CNS viral infections involve the meninges to a greater or lesser extent, leading to aseptic meningitis or causing mild meningoencephalitis rather than pure encephalitis. In addition to acute viral encephalitis, other less established and more unusual manifestations of viral infections include progressive neurologic disorders, such as postinfectious encephalomyelitis (such as may occur after measles or Nipah virus encephalitis) and conditions such as postpoliomyelitis syndrome, which has been considered by some to be as a persistent manifestation of poliovirus infection. More recently, provocative studies have found high antibody seroprevalence to viruses such as Ebola, Marburg, and Lyssa viruses in multiple African countries, indicating the presence of a high number of undiagnosed cases every year, including high neutralizing titers of antibodies to rabies virus in 11% of a small cohort of asymptomatic Peruvians living in the Amazon with prior exposure to bats. These studies raise the possibility that in some populations, those conditions may be more common than previously recognized. The emergence of new types of viral infections, such as the Toscana virus (in the Western European countries located on the northern border of the Mediterranean sea, Cyprus and Turkey) where seropositivity in the population is not matched by clinical symptoms (indicating that most infections are mild) also highlight the fact that we need to be alert about the possible threats from unknown pathogens, even in areas that are not necessarily tropical or surrounded by rain forests. An unusual CNS involvement leading to microcephaly due to infection of pregnant women by Zika virus has also been recently reported and highlights the constant need to look for new types of neurological manifestations of viral infections in humans.

Pathophysiology

The initial event in the replicative cycle of a virus is its interaction with receptors present on the surface of a cell. Knowledge of this interaction is important in understanding viral spread, tropism, and pathogenesis. The following cellular receptors have been described for these viruses (see Table 1, below):

- Measles virus - CD46
- Poliovirus - CD155
- Herpes simplex virus (HSV) - Heparan sulfate; Hve A, B, and C; tumor necrosis factor receptor superfamily 14 (TNFSF14); HVEM; Prr1 and Prr2; and nectin-1 and nectin-2
- Rabies virus - AChR, NCAM, and NGFR
- Human immunodeficiency virus-1 (HIV-1) - CD4, CCR5/3, and CXCR4
- JC virus - N-linked glycoprotein and alpha 2-6 sialic acid
- West Nile virus - Cholesterol-rich membrane microdomains

Table 1. Examples of Physiologic Roles of Known Viral Receptors

Virus	Receptor	Abbreviation/Synonym	Function
Measles virus	Membrane cofactor protein	CD46	Regulates complement and prevents activation of complement on autologous cells
Poliovirus	CD155	hPVR/CD155	Expressed on primary human monocytes; supports poliovirus replication in vivo
HSV	Heparan sulfate	None	Cell surface proteoglycans
	Herpesvirus entry mediator A	Hve A, HVEM	TNF receptor superfamily
	Herpesvirus entry mediator B	Hve B, Human nectin-2, or Prr2alpha-Hve B	Participate in organization of epithelial and endothelial junctions
	Herpesvirus entry mediator C	Hve C, nectin1delta, or Prr1-Hve C	Immunoglobulin superfamily
	TNFSF14	hTNFSF14/HVEM-L	TNF receptor superfamily

Rabies virus	Nicotinic AChR (a-bungarotoxin binding site) NCAM	AChR NCAM, CD56, D2CAM, Leu19, or NKH-1	Nicotinic AChR Cell adhesion glycoprotein of immunoglobulin superfamily
	NGFR	NGFR	NGFR
	p75 neurotrophin receptor (p75NTR)	p75NTR	
HIV-1	CD4	CD4	T lymphocyte protein with helper or inducer function in immune system
	CCR3	CCR3	Chemotactic activity
	CCR5	CCR5	Coreceptor for macrophage-tropic strain
	CCR6	CCR65	Chemotactic activity
	CXCR4	CXCR4	Coreceptor for CD4
JC virus	N-linked glycoprotein with alpha 2-6 sialic acid	N-linked glycoprotein	Unknown
Japanese B virus	Protein GRP78	---	ER-stress response protein

AChR—acetylcetylcholine receptor; CCR—chemokine receptor; HSV—herpes simplex virus; NCAM—neural cell adhesion molecule; NGFR—nerve growth factor receptor; TNF—tumor necrosis factor.

Despite viral tropism, the pattern of distribution of lesions in the brain is rarely specific enough to permit identification of the infecting virus.

Recent studies have reported a Mendelian predisposition to some forms of encephalitis (especially herpes simplex encephalitis) due to defects in the following pathways: TLR-3 interferon, autosomal recessive STAT-1 deficiency and X-linked NEMO deficiency, UNC-93B deficiency, and autosomal dominant TLR3 deficiency. The pathophysiology of viral encephalitis varies according to the viral family. Viruses enter the CNS through 2 distinct routes: (1) hematogenous dissemination and (2) retrograde neuronal dissemination. Hematogenous dissemination is the more common path. Humans are usually incidental terminal hosts of many viral encephalitides. Arbovirus encephalitides are zoonoses, with the virus surviving in infection cycles involving biting arthropods and various vertebrates, especially birds and rodents. The virus can be transmitted by an insect bite and then undergoes local replication in the skin. Transient viremia leads to seeding of the reticuloendothelial system and muscles. After continuous replication, secondary viremia leads to seeding of other sites, including the CNS. In fatal cases, little histopathologic change is noted outside the nervous system. St. Louis encephalitis is an exception, in that renal involvement is occasionally present. On gross examination, variable degrees of meningitis, cerebral edema, congestion, and hemorrhage are observed in the brain. Microscopic examination confirms a leptomeningitis with round-cell infiltration, small hemorrhages with perivascular cuffing, and nodules of leukocytes or microglial cells. Demyelination may follow the destruction of oligodendroglias, and involvement of ependymal cells may lead to hydranencephaly. Neuronal damage is seen as chromatolysis and neuronophagia. Areas of necrosis may be extensive, especially in eastern equine encephalitis (EEE) and Japanese encephalitis (JE). Recent experimental evidence has shown that arboviruses can induce apoptotic cell death in neurons in the brains of their hosts. Patients who survive the initial illness associated with viral encephalitis feature varying degrees of repair, which may include calcification in children.

Retrograde neural dissemination is the main route of spread for several important viral pathogens. Rabies virus usually spreads to the CNS through retrograde peripheral nerve dissemination. This virus tends to exhibit tropism for the temporal lobes, affecting the Ammon horns. One of the possible routes of CNS spread for herpes simplex virus (HSV) is through the olfactory tracts. Herpesviruses have tropism for the temporal cortex and pons, but the lesions may be widespread. Herpes simplex encephalitis (HSE) in infants is usually part of a widespread infection that produces focal necrotic lesions with typical intranuclear inclusions in many organs. In adults and in some children, lesions are confined to the brain. Necrotic foci may be macroscopically evident as softening. Inclusion bodies are found readily in the margins of areas of necrosis; focal perivascular infiltration and neuronal damage are evident. In addition to the direct effect of the viral pathogen, acute encephalopathy may be associated with viral infections and increased plasma concentrations of chemokine (CXC motif) ligand 8 (CXCL8; interleukin [IL]-8), chemokine (C-C motif) ligand 2 (CCL2; monocyte chemotactic protein-1 [MCP - 1]), IL-6, and CXCL10 (interferon gamma–induced protein 10 kd [IP-10], without viral neuroinvasion (hyperactivated cytokine response). Accordingly, it is important to differentiate encephalitis from encephalopathy as a disruption of brain function that is not related to a direct structural or inflammatory process. To illustrate the difficulty of making this distinction in daily clinical practice, until recently it was not clear whether encephalopathy after dengue fever infection was due to direct CNS invasion or to viremia. Studies have now documented the presence of IgM and IgG for dengue virus in the cerebrospinal fluid (CSF) of patients with dengue fever and neurologic manifestations.

Etiology

HSE is the most common form of encephalitis in the United States (see Herpes Simplex Encephalitis). Human herpesvirus (HHV)-6, the causative agent of exanthema subitum, has been associated with a wide spectrum of neurologic complications, including viral (focal)

encephalitis. Numerous other viruses are known to cause encephalitis (see Tables 2 and 3 below). The viruses most commonly associated with acute childhood encephalitis are mumps virus, measles virus, and varicella-zoster virus (VZV).

Table 2. Common Viral Encephalitides: Part 1

Virus (Family)	Viral Structure	Transmission	Mortality	Specific Clinical Patterns	Sequelae	Season
HSV (herpesvirus)	ds DNA	Unknown	70% if untreated	Rare forms: subacute, psychiatric, opercular, recurrent meningitis HSV-1: brainstem; HSV-2: myelitis	Common	All year
VZV (herpesvirus)	ds DNA	Direct contact (air), highly contagious	Variable; low in children	Rash, encephalitis in 0.1-0.2% of children with chickenpox; cerebellar ataxia (cerebellitis)	Adults worse; cerebellitis good	Late winter, spring
Influenza virus (orthomyxovirus)	ss RNA	Direct contact (air), highly contagious	Unknown	Reversible frontal syndrome in children; Guillain-Barré, myelitis	Parkinsonism (encephalitis lethargica)	Usually winter
Enteroviruses (picornavirus)	ss RNA	Fecal-oral route	Low; high for enterovirus 71	Herpangina; hand, foot, mouth disease; enterovirus 71 causes rhombencephalitis	Mild, except for enterovirus 71	Summer, fall; tropics: no season
Rabies virus (rhabdovirus)	ss RNA	Dogs, wild animals (eg, fox, wolf, skunk)	Virtually 100%	Paresthesias; confusion, spasms, hydrophobia; brainstem features	Mortality virtually 100%	All year

ds—double strand; HSV—herpes simplex virus; ss—single strand; VZV—varicella-zoster virus.

Table 3. Common Viral Encephalitides: Part 2

Virus (Family)	Viral Structure	Transmission	Mortality	Specific Clinical Patterns	Sequelae	Season
Lymphocytic choriomeningitis virus (arenavirus)	ss RNA	Rodents	Low (< 1%)	Progressive fever and myalgia; orchitis; aseptic meningitis; leukopenia, thrombocytopenia	Rare	More in winter
Lassa virus (arenavirus)	ss RNA	Rodents	15%	Multisystem disease; proteinuria	Deafness (one third)	All year
Mumps virus (paramyxovirus)	ss RNA	Direct contact (air), highly contagious	Low	Parotitis, pancreatitis, orchitis, aseptic meningitis	Frequent sequelae	Winter and spring
Measles virus (paramyxovirus)	ss RNA	Direct contact (air), highly contagious	10%	Characteristic rash; frequent EEG changes; myelitis	Frequent: mental retardation, seizures, SSPE	Winter and spring
Nipah virus (paramyxovirus)	ss RNA	Pigs; bats	40-75%	Brainstem or cerebellar signs; segmental myoclonus, dysautonomia	SSPE-like syndrome?	All year

ds—double strand; EEG—electroencephalographic; ss—single strand; SSPE—subacute sclerosing panencephalitis.

Arthropod-borne viruses (arboviruses) are important causes of encephalitis worldwide. More than 20 arboviruses that can cause encephalitis have been identified. These arboviruses are enveloped RNA viruses from different families: Togaviridae (genus Alphavirus), Flaviviridae (genus Flavivirus), Bunyaviridae (genus Bunyavirus), and Reoviridae (see Table 4).

Table 4. Common Arboviral Encephalitides

Virus (Family)	Vector	Reservoir	Mortality	Specific Clinical Patterns	Sequelae	Season
Eastern equine virus (alphavirus)	Aedes sollicitans	Birds	35%	Severe, rapid progression	Common, especially in children	June to October
Western equine virus (alphavirus)	Culex tarsalis	Birds	10%	Classic encephalitis	Moderate in infants; low in others	July to October
Venezuelan equine encephalitis virus (alphavirus)	Mosquito species	Horses, small mammals	~ 0.4 %	Low rate (4%) of CNS involvement	Mild	Rainy season
St Louis encephalitis virus (flavivirus)	Culex pipiens, C tarsalis	Birds	2% in young people; 20% in elderly people	SIADH	More in elderly people	August to October
Japanese encephalitis virus (flavivirus)	Culex taeniorhynchus	Birds	33% (50% in elderly people)	Extrapyramidal features	50% neuro psychiatric; parkinsonism	Summer
West Nile virus (flavivirus)	Culex,Aedes spp	Birds	In US: 12% (elderly people only)	Motor or brainstem involvement	Usually not prominent	Summer
Far East encephalitis virus (flavivirus)	Ixodes persulcatus (tick)	Small mammals, birds	20%	Epilepsia partialis continua	Frequent; residual weakness	Spring to early summer
Central European encephalitis virus (flavivirus)	Ixodes ricinus (tick)	Small mammals, birds	Less common than in Far East	Limb-girdle paralysis (spine/medulla)	Less common than in Far East	April to October
Powassan virus (flavivirus)	Ixodes cookei (tick)	Small mammals, birds	High	Severe encephalitis	Common (50%)	May to December
Dengue virus (flavivirus)	Aedes spp	Mosquitoes	Low, except hemorrhagic	Flulike syndrome; possible CNS involvement	Mild, except for hemorrhagic	Rainy season
La Crosse virus (bunyavirus)	Aedes triseriatus	Small mammals	Low (< 1%)	Mild, primarily in children	Mild; seizures	Summer
Colorado tick fever virus (orbivirus)	Dermacentor andersoni (tick)	Small mammals	Low		Mild	

CNS—central nervous system; SIADH—syndrome of inappropriate antidiuretic hormone secretion.

Important encephalitides caused by alphaviruses include EEE, western equine encephalitis (WEE), and Venezuelan equine encephalitis (VEE). EEE is endemic along the eastern and Gulf coasts of the United States, in the Caribbean region, and in South America. North American strains produce a fulminant disease (50-75% mortality) with a high incidence of neurologic sequelae. WEE is most common in the western and midwestern United States but has a lower mortality rate (10%) than EEE. VEE occurs in South America and Central America as well as in the southwestern United States, typically causing mild disease and, rarely, neurologic impairment. Flaviviruses are transmitted by ticks and mosquitoes and are found worldwide. The most common form of flavivirus is the Japanese B encephalitis virus. This flavivirus is one of the most important causes of viral encephalitis worldwide, with 50,000 new cases and 15,000 deaths annually. It has been found in China, Southeast Asia, the Indian subcontinent, the Philippines, New Guinea, Guam, and Australia. West Nile virus is a flavivirus similar to the

Japanese B virus. Its life cycle occurs between birds and mosquitoes. Culex mosquitoes, Anopheles mosquitoes, and Aedes mosquitoes are the primary vectors to humans. West Nile virus is endemic in Africa, the Middle East, Russia, India, Indonesia, and parts of Europe. It was detected for the first time in the Western hemisphere during an outbreak of encephalitis in the summer of 1999 in New York City. Dengue fever is the most important arboviral infection of humans, with 100 million cases per year. It can now be seen in any country between the tropics of Capricorn and Cancer (placing an estimated 2.5 billion people at risk). Until recently, dengue fever was considered to be uncommonly associated with neurologic manifestations (except when dengue hemorrhagic fever is present). However, this view has changed; in endemic areas, dengue fever may be one of the most common forms of viral encephalitis. It is important to emphasize that the geographic distribution of dengue virus has increased. In 2009, locally acquired disease was diagnosed in New York City and subsequently in Key West in Florida (no locally acquired disease had been reported in the US since 1945). Before the 1999 outbreak of West Nile encephalitis (WNE), St Louis encephalitis was the most common disease caused by a flavivirus in the United States. Outbreaks of St Louis encephalitis occur from August to October throughout the country. Individual susceptibility to the St Louis virus increases with age, and encephalitis can be accompanied by hyponatremia due to the syndrome of inappropriate antidiuretic hormone secretion (SIADH). Mortality is age related, ranging from 2-20%, and sequelae are present in 20% of survivors. Other important flaviviral diseases include Far East tick-borne encephalitis (former eastern Russia), Central European tick-borne encephalitis (Central Europe), and Powassan encephalitis (Canada and northern United States). Bunyaviruses are the largest group of arboviruses and include the viruses that cause La Crosse encephalitis, Jamestown Canyon encephalitis, and California encephalitis (CE). La Crosse virus is the most common cause of arboviral encephalitis in the United States and produces seizures and focal neurologic signs, manifested primarily in children, with a mortality of less than 1% and rare sequelae. Toscana virus was identified in Central Italy in 1971. It has a larger geographic distribution over the northern border of the Mediterranean Sea, including Cyprus and Turkey. Most affected individuals are not symptomatic or mildly symptomatic, although severe cases of meningitis and meningo-encephalitis have been reported. Orbivirus is transmitted by the tick *Dermacentor andersoni* and is seen in the Rocky Mountains of the United States. Retroviruses are also a cause of encephalitis. Human T-cell lymphotrophic virus type 1 (HTLV-I) is associated predominantly with spastic paraparesis, not with causing encephalitis. Certain forms of encephalitis are observed almost exclusively in patients with HIV. Among those, cytomegalovirus (CMV) ventriculoencephalitis has emerged as a unique entity in patients with advanced HIV infection. Measles and mumps viruses (paramyxoviruses) can also cause neurologic disease. Measles typically does not cause encephalitis in the acute phase, but 1 in 1000 cases can give rise to postinfectious autoimmune syndrome (ie, SSPE). Nipah virus (Paramyxoviridae family) was first detected after an outbreak of encephalitis in pig farmers in Malaysia. Nipah virus is a zoonosis and infects pigs. Subsequent outbreaks occurred in several countries in South Asia, including Bangladesh (2001 and 2003). Arenaviruses usually infect rodents. Thus, lymphocytic choriomeningitis most commonly occurs during the winter, when mice are indoors and humans have contact with their excreta. Meningitis or meningoencephalitis follows a 5- to 10-day incubation period. Recovery can be prolonged but is usually complete. Lassa fever is a West African disease that starts with gastrointestinal (GI) and respiratory complaints and progresses to hemorrhagic shock. Unilateral or bilateral deafness may follow the period of encephalitis. Mortality is in the range of 8-52%. Enteroviruses are picornaviruses. The Picornaviridae family includes coxsackievirus A and B, poliovirus, echovirus, enterovirus (EV) 68 and 71, and hepatitis A virus (HAV). Enteroviruses are transmitted by the fecal-oral route, and CNS spread is through the hematogenous route. Infection is most common in summer and early fall. In 2014, the United States experienced a nationwide outbreak of EV-D68 associated with severe respiratory illness. From mid-August 2014 to January 15, 2015, CDC or state public health laboratories confirmed a total of 1,153 people in 49 states and the District of Columbia with respiratory illness caused by EV-D68. Almost all of the confirmed cases were among children, many whom had asthma or a history of wheezing. One study reports that EV-D68 inhibits innate antiviral immunity by downregulation of interferon regulatory factor 7 (IRF7). Outbreaks of enterovirus 71 have occurred in Japan, Malaysia, and Taiwan. Enterovirus 71 is typically associated with hand-foot-and-mouth disease, but up to 30% may develop neurological manifestations. In 2012, a severe encephalitis outbreak in Cambodia, with a 69% mortality rate in children, was secondary to enterovirus 71 serotype C4. The disease mainly affected children aged 3 years and younger. Rabies is an important pathogen in developing countries, where endemic canine infection still exists. In Europe and the United States, rabies is present in wild animals (eg, skunks, foxes, raccoons, bats); however, it is controlled in domestic animals with vaccination. Rabies usually incubates for 20-60 days but can incubate for years. In a 2007 outbreak of Chikungunya virus infection in Italy, 1 elderly patient developed encephalitis and died. This reinforces the risk of new outbreaks of newer forms of encephalitis in Europe and other parts of the world. Recent outbreaks of Zika virus in Brazil led to the development of CNS malformations in newborns of infected pregnant women, especially due to microcephaly.

Frequency

International

The annual incidence of viral encephalitis is most likely underestimated, especially in developing countries, because of problems with pathogen detection. In a study from Finland, the incidence of viral encephalitis in adults was 1.4 cases per 100,000 persons per year.

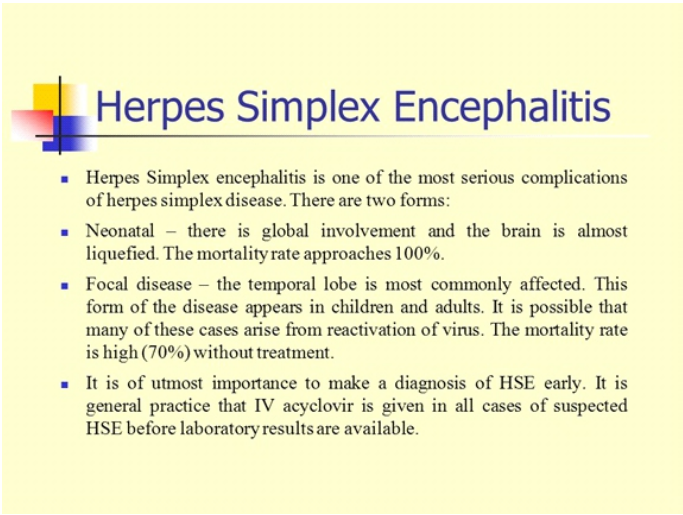
Epidemiology

International statistics

The annual incidence of viral encephalitis is most likely underestimated, especially in developing countries, because of problems with pathogen detection. JE affects at least 50,000 individuals per year. In a study from Finland, the incidence of viral encephalitis in adults was 1.4 cases per 100,000 persons per year. HSV was the organism most frequently identified as the cause (16%), followed by VZV (5%), mumps virus (4%), and influenza A virus (4%).

Age-, sex-, and race-related demographics

Children and young adults are typically the groups that are most often affected. However, severity is usually more pronounced in infants and elderly patients. The clinical course in children may be considerably different from that seen in adults. HSE may be associated with a relapse in 25% of the cases, which may present as a movement disorder, most often choreoathetosis. Mumps meningoencephalitis affects men more often than women. Men working in areas infested by infected mosquitoes have a higher incidence of arboviral infections. No racial predilection exists, although different genetic factors may predispose individuals to more severe forms of CNS involvement.



Prognosis

The mortality depends largely on the etiologic agent of the encephalitis. The severity of sequelae apparently varies according to the causative virus as well. The average lifetime cost of the sequelae of encephalitis approaches US$3 million. HSE carries a mortality of 70% in untreated patients, with severe sequelae among survivors. After WEE, sequelae are uncommon in adults but are frequent in children. Recurring convulsions with motor or behavioral changes affect more than half of children who are infected when younger than 1 month. With EEE, most adults older than 40 years who survive (10% mortality) do so unscathed; children younger than 5 years have crippling sequelae consisting of mental retardation, convulsions, and paralysis. Permanent sequelae after St. Louis encephalitis are uncommon, except for elderly individuals; the mortality rate is 2% in young adults and 20% in elderly patients. A large number of cases of newborns with microcephaly has been reported in pregnant women infected by Zika virus. La Crosse virus causes a relatively mild encephalitis with a low fatality rate. Mortality is low in VEE, CE, and encephalitis due to Colorado tick fever virus. Neurologic sequelae in these conditions are not frequent and are usually mild. JE has a mortality of almost 50% in patients older than 50 years and a mortality rate of less than 20% in children. The Far East form of tick-borne encephalitis is more severe than the Central European form of tick-borne encephalitis, with mortalities as high as 20% and frequent sequelae. Epilepsia partialis continua may develop during the convalescent period or later. Residual weakness may also be present. The 20-year risk of developing an unprovoked seizure is 22% for patients with viral encephalitis associated with early seizures and 10% for viral encephalitis without early seizures. Of patients with CNS infection, 18-80% develop epilepsy, which is usually refractory to medical treatment. A considerable number of such patients develop unilateral mesial temporal lobe epilepsy and can have a good outcome after surgery.

Patient Education

Education helps in the early diagnosis of encephalitis, especially in areas of endemic disease. Control of the mosquito vector has been effective in several recent epidemics. The belief that HSV-2 lesions initially appear 2 weeks after primary infection can lead to false accusations of infidelity. The physician should emphasize that the initial outbreak of lesions may occur at any time, possibly years, after infection. For patient education resources, see the Brain and Nervous System Center, the Bacterial and Viral Infections Center, and the Bites and Stings Center, as well as Meningitis in Adults, Mumps, and Encephalitis.

Clinical presentation

History

Viral encephalitis is usually marked by acute onset of a febrile illness. Patients with viral encephalitis generally experience signs and symptoms of leptomeningeal irritation (eg, headache, fever, neck stiffness). Patients with viral encephalitis also develop focal neurological signs; seizures; and alteration of consciousness, starting with lethargy and progressing to confusion, stupor, and coma. Behavioral and speech disturbances are common. Abnormal movements can be seen but are rare. Involvement of the hypothalamic/pituitary axis can lead to hyperthermia or poikilothermia.

Symptoms associated with specific viral infections

Specific clues taken from the patient's history depend on the viral etiology. Clinical findings reflect disease progression according to viral tropism for different central nervous system (CNS) cell types. Atypical presentations include a reversible frontal lobe and limbic syndrome without disturbances of consciousness or motor function. These presentations have been described in children with influenza virus infection. Herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) encephalitis have subacute forms, presenting with a psychiatric syndrome and an anterior opercular syndrome, known as benign recurrent meningitis. HSV-1 encephalitis may produce a brainstem encephalitis, and HSV-2 encephalitis may also produce a myelitis. West Nile encephalitis (WNE) is usually asymptomatic in areas of endemic disease. In symptomatic individuals, an influenzalike illness occurs after incubation of 3-15 days; CNS involvement occurs in less than 15% of cases. Severe neurologic infection is more common when the virus is introduced in an area of nonendemic disease. In 1999, during the New York City outbreak of West Nile virus infection, 62 patients developed encephalitis, and 7 died (a case fatality rate of 12%, with all deaths occurring in older patients). Axonal neuropathy, demyelinating polyneuropathy similar to that seen in Guillain-Barré syndrome, encephalitis with and without muscle weakness, and aseptic meningitis were described. Delayed weakness or recurrent clinical weakness after West Nile virus infection has been described. Japanese encephalitis (JE) typically affects children and young adults. Older adults are affected in epidemics. The clinical presentation includes a nonspecific prodrome and frequent seizures. Dengue fever classically presents with a severe influenzalike illness or dengue hemorrhagic fever. Less commonly, dengue fever can lead to encephalitis or encephalopathy, transverse myelitis, and mononeuropathy or polyneuropathy similar to that in Guillain-Barré syndrome. The hemorrhagic form may also cause hepatic failure leading to a Reye syndrome–like illness. Enteroviral encephalitis is usually associated with a good prognosis. However, enterovirus 71 has a high mortality and can present with herpangina or enteroviral hand, foot, and mouth disease. Complications include myocarditis and acute flaccid paralysis. Enterovirus 71 can cause a chronic meningoencephalitis in patients who are immunocompromised. Mumps encephalitis typically starts 3-10 days after parotitis and usually resolves without sequelae, except for occasional hydrocephalus due to ependymal cell involvement. Measles does not usually cause acute encephalitis. Rabies virus usually incubates for 20-60 days but is capable of incubating for years. Infection does not occur in all humans bitten by an infected animal but is uniformly fatal when clinical disease develops. After a prodrome of fever, headache, malaise, seizures, and

behavioral abnormalities, hydrophobia and aerophobia supervene. Coma and death occur in 1 to several weeks. Once symptoms start, treatment is ineffective. In southern Vietnam, a viral encephalitis that was caused by avian influenza A (H5N1) and did not involve the respiratory tract was diagnosed in 2 siblings: a 4-year-old boy, who presented with severe diarrhea, seizures, coma, and death, and his sister. The boy's cerebrospinal fluid (CSF) revealed only high protein levels, but H5N1 was isolated from CSF, fecal, throat, and serum specimens. Newborns from mothers infected by Zika virus early during pregnancy exhibit higher risk of developping microcephaly and other several forms of CNS disease.

Physical Examination

Findings from physical examination are not usually diagnostic. Focal neurologic deficits (eg, opisthotonos, pareses, tremors, ataxia, hypotonia, diplopia), accentuated reflexes, and extensor plantar responses may be observed. Abnormal movements and, rarely, tremor may be seen. Increased intracranial pressure (ICP) can also lead to papilledema and cranial nerve VI palsy.

Findings associated with specific viral infections

A minority of patients with arbovirus infections develop acute encephalitis (or encephalomyelitis), meningitis, or a combination of both. Focal signs are only occasionally prominent in arboviral encephalitis. Patients may also have evidence of spinal cord involvement. JE can cause marked extrapyramidal manifestations, such as dull masklike face with wide staring eyes, tremor, choreoathetosis, head nodding, and rigidity. Flaccid paralysis, especially involving the lower extremities, has been described as being due to damage to the anterior horn cells. Parkinsonism can be a sequela of JE, and von Economo encephalitis (encephalitis lethargica) is considered to be a sequela of influenza encephalitis. Enterovirus 71 can cause rhombencephalitis with myoclonus, tremor, ataxia, cranial nerve involvement, neurogenic pulmonary edema, and coma. Nipah virus, in addition to the classical encephalitis presentation, produces cerebellar and brainstem signs, as well as segmental myoclonus, significant hypertension, and tachycardia. Encephalitis delayed 4 months after exposure to the virus has been described, suggesting similarities to the subacute sclerosing panencephalitis (SSPE) phenotype. Microcephaly may be seen in newborns from mothers infected by Zika virus early during pregnancy.

Complications

Secondary bacterial infections of the respiratory and urinary tracts are major complications of encephalitis. Complications depend on the severity of the encephalitis and generally decline in importance as the acute illness passes. With recovery from acute viral encephalitis, evidence of neuronal injury and death becomes apparent as residual neurologic defects, impairment of intelligence, and psychiatric disturbances. The severity of these sequelae varies according to the causative virus. Sequelae occur in 30-40% of patients aged 5-40 years and include extrapyramidal features (especially dystonia and occasionally parkinsonism), weakness, and seizure disorders. Sequelae are reported in only 3-10% of cases of JE in Japan. Yet 25-30% of young adult males serving in the armed forces of the United States during World War II had sequelae (including neuroses) 6 months after infection. In addition, 10 of 25 individuals who had JE in Guam in 1948 had neurological or intellectual defects 10 years later. Hyponatremia due to the syndrome of inappropriate antidiuretic hormone secretion (SIADH) may be frequent in St Louis encephalitis. Dehydration, respiratory complications, nosocomial infections, and decubitus ulcers may also occur.

Diagnostic Considerations

See the following for complete information on these topics:

- Herpes Simplex Encephalitis
- California Encephalitis
- Eastern Equine Encephalitis
- Japanese Encephalitis
- St Louis Encephalitis
- Venezuelan Encephalitis
- Western Equine Encephalitis
- West Nile Encephalitis

Other conditions to be considered include the following:

- Myoclonus
- Partial seizures with secondary generalization
- Seizure, partial (focal)
- Benign epilepsy syndromes
- Pseudomigraine with CSF pleocytosis

Differential Diagnoses

- Acute Disseminated Encephalomyelitis
- Acute Subdural Hematoma in the ED
- Aseptic Meningitis
- Basilar Artery Thrombosis
- Benign Neonatal Convulsions
- Cardioembolic Stroke
- Cavernous Sinus Syndromes
- Central Nervous System Complications in HIV
- Cerebral Venous Thrombosis
- Confusional States and Acute Memory Disorders
- Epileptic and Epileptiform Encephalopathies
- Frontal Lobe Syndromes
- Haemophilus Meningitis
- Intracranial Hemorrhage
- Leptomeningeal Carcinomatosis
- Meningococcal Meningitis
- Neonatal Meningitis
- Neonatal Seizures
- Neurocysticercosis
- Neurological Sequelae of Infectious Endocarditis
- Neurosarcoidosis
- Neurosyphilis
- Paraneoplastic Encephalomyelitis
- Pediatric Febrile Seizures
- Pediatric Status Epilepticus
- Staphylococcal Meningitis
- Subdural Empyema
- Thrombotic Thrombocytopenic Purpura (TTP)
- Tuberculous Meningitis
- Viral Meningitis

WORKUP

Approach Considerations

Usually, general laboratory studies are not helpful, except for identifying a viral infectious process (eg, a lymphocytic predominance in the complete blood count [CBC], rather than the polymorphonuclear

predominance indicative of bacterial infection). The diagnostic evaluation should include a CBC, tests of renal and hepatic function, coagulation studies, and chest radiography. During epidemics, viral encephalitis is diagnosed readily on clinical grounds. However, sporadic cases of viral encephalitis are often difficult to distinguish from other febrile illnesses (eg, gastroenteritis with dehydration and convulsions) or from intoxication. Although specific treatment for most causes of viral encephalitis is still not available, establishing the final diagnosis is important to avoid unnecessary treatments with potential side effects. In most instances, the currently available specific laboratory tests only help provide a retrospective diagnosis. Serologic tests depend on the occurrence of a rise in antibody titer. However, the early detection of specific immunoglobulin M (IgM) antibody may assist early diagnosis. Analysis of cerebrospinal fluid (CSF), including polymerase chain reaction (PCR) testing, plays an important role. Reliance on magnetic resonance imaging (MRI) findings to make the diagnosis of encephalitis or to distinguish among the different viral etiologies is usually not advisable.

Blood and Skin Cultures

All patients with encephalitis should have blood cultures to rule out bacterial and fungal infections. Specific clinical findings should also guide the evaluation of other sites for culture (scraping of vesicles, sputum, nasopharynx, and stool). For most arboviral infections, the viremia is usually of low magnitude and short duration, so blood viral cultures are low yield tests most of the time. Skin biopsies may be useful for diagnosis conditions such as Rocky Mountain spotted fever, and full-thickness skin biopsy from the neck with staining of sensory axons may be useful for the diagnosis of rabies. Viral cultures from throat, stool samples, and antigen detection for herpes and respiratory viruses are recommended during the first week.

Serologic Tests

Some causes of encephalitis can be diagnosed by detecting serum IgM antibodies (varicella and arboviruses). Currently, IgM and immunoglobulin G (IgG) capture enzyme linked immunosorbent assays (ELISAs) are the most useful and most widely used tests for the diagnosis of arboviral encephalitis. However, there is significant cross-reactivity among flaviviruses (Japanese encephalitis virus, St Louis encephalitis virus, and West Nile virus). Anti-West Nile virus IgM is detectable in CS) and serum 10 days after infection onset. A PCR-based test for rapid detection of West Nile virus has been developed in California. A diagnosis of Japanese encephalitis (JE) can be confirmed serologically with demonstration of IgM in the CSF (sensitivity and specificity >95%). The PCR test may detect the virus within 2 days, but its reliability is uncertain. ELISAs for detection of dengue virus IgM and IgG are available for serum and CSF. Antibodies to Borrelia burgdorferi and serologic testing for Rickettsia, Ehrlichia, and Anaplasma species should be checked in all patients coming from endemic areas. Blood from the acute phase should be saved for future comparisons with the titers from the convalescent phase. Despite all major efforts, in a recent study from Spain, a significant number of cases of aseptic CNS infection (42.9% meningitis, 59.3% meningoencephalitis, 72.4% encephalitis) may still have no etiological diagnosis.

Analysis of Cerebrospinal Fluid

Lumbar puncture

Lumbar puncture should be performed immediately once a space-occupying lesion is ruled out. CSF examination is critical to establish the diagnosis and reveals, acutely, a typical viral profile: mildly to moderately elevated protein (60-80 mg/dL), normal glucose, and a moderate pleocytosis (up to 1000 leukocytes/μL). Mononuclear cells usually predominate, though early in fulminant encephalitis, polymorphonuclear leukocytes predominate. Persistent neutrophilic pleocytosis can occur in patients with West Nile encephalitis (WNE). Viral cultures are rarely helpful for acute management. Findings from CSF cultures for enteroviruses, mumps, and certain arboviruses may be positive. Low CSF glucose is unusual with viral encephalitis and suggests infection by bacteria, fungal agents, or tuberculosis. Herpes simplex encephalitis (HSE), as well as other forms of hemorrhagic encephalitis, may be associated with increased red blood cells (RBCs) and xanthochromia in the CSF. The fluid should be sent for PCR evaluation to detect herpes simplex virus (HSV) DNA; PCR is highly specific and remains positive for as long as 5 days after initiation of treatment (see below). Intrathecal antibodies can also be quantified. Eosinophils can be present in infections with helminths, Treponema pallidum, Mycoplasma pneumoniae, Rickettsia rickettsii, Coccidioides immitis, and Toxoplasma gondii. They can be mistaken for neutrophils if cell count is done in automated cell counters or can be easily destroyed or distorted during processing. Up to 10% of the patients with viral encephalitis may have completely normal CSF studies. CSF findings in patients with acute disseminated encephalomyelitis (ADEM) are similar to those in patients with viral encephalitis, but pleocytosis is less marked or absent, and markers of intrathecal immunoglobulin synthesis may be present (less than in multiple sclerosis).

Polymerase chain reaction

PCR testing should be performed to detect viral nucleic acid in CSF. In undiagnosed cases, PCR should be repeated after 3-7 days, and blood tests should be performed after 2-4 weeks to show possible seroconversion or diagnostic increase in antibody levels. PCR is especially useful for infections caused by herpesviruses and enteroviruses. In infants and neonates, the sensitivity and specificity for CSF PCR for HSV are more variable. In adults, the test may initially yield negative results, especially if the white blood cell (WBC) count in the CSF is lower than 10/μL. Results may turn positive 1-3 days after initiation of treatment. PCR can also detect varicella-zoster virus (VZV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), JC virus, and West Nile virus (positive in < 60% serologically confirmed cases). Molecular testing of the saliva may establish the diagnosis of rabies. PCR testing may also be important for the diagnosis of nonviral encephalitis (as in ehrlichiosis and Bartonella henselae infection).

Computed Tomography and Positron Emission Tomography

In HSV encephalitis, computed tomography (CT) scanning may show low-density lesions in the temporal lobes, which may not be present until 3-4 days after onset. Edema and hemorrhages may be found, and, after 1 week, contrast enhancement may be observed. CT findings are usually not helpful in differentiating the different viral encephalitides. However, given its low cost and its ready availability in most institutions, CT scanning may be a good choice for evaluating acute disease progression and following up on complications. It scan can readily reveal important complications (eg, hemorrhage, hydrocephalus, and herniation) and can help guide neurosurgical interventions. Positron emission tomography (PET) scanning may be useful for the evaluation of possible paraneoplastic disorders.

Magnetic Resonance Imaging

Relying on MRI findings to make the diagnosis of encephalitis or to

distinguish among the different viral etiologies is usually not advisable. MRI is more sensitive and specific than CT for identifying viral encephalitides, especially in the early phase. Diffusion-weighted imaging may be useful for the early diagnosis of HSV, enterovirus 71, and West Nile virus infections. In HSE, MRI typically shows temporal lobe lesions, which may be hemorrhagic and unilateral or bilateral. Inferomedial temporal lobe and cingulate gyrus are the areas most commonly detected by MRI. In children and infants, a more widespread pattern may be observed. MRI may help in differentiating Japanese encephalitis (JE) from Nipah virus encephalitis. JE is characterized by gray matter involvement, whereas Nipah virus encephalitis is associated with multiple, small, white matter lesions. With flavivirus encephalitis and eastern equine encephalitis (EEE), MRI may show mixed intense or hypointense lesions in the thalamus, basal ganglia, and midbrain, being hyperintense on fluid attenuated inversion recovery (FLAIR) and T2. The rhombencephalitis caused by enterovirus 71 can be visualized by T2-weighted MRI, which shows hyperintense signals in the brainstem. A peculiar MRI pattern on diffusion-weighted imaging and magnetic resonance spectroscopy has been described in an acute and rapid form of subacute sclerosing panencephalitis (SSPE).

Electroencephalography

In HSE, electroencephalography (EEG) shows abnormalities in four fifths of biopsy-proven cases. Focal temporal changes, diffuse slowing, and periodic complexes and periodic lateralizing epileptiform discharges (PLEDs) are commonly described. Frontal slowing and occasional frontal spikes have been described in encephalitis associated with influenza virus. JE is commonly associated with 3 EEG patterns: (1) diffuse continuous delta activity, (2) diffuse delta activity with spikes, and (3) alpha coma pattern. In 1 study, the EEG pattern did not correlate with the Glasgow Coma Scale score and outcome. In St Louis encephalitis, EEG is characterized by diffuse delta activity, and spike and waves are not prominent in the acute stage.

Brain Biopsy

Brain biopsies can yield definitive diagnosis of encephalitis, but at present they are rarely performed. A biopsy may be considered when a lumbar puncture is precluded or when the diagnosis is uncertain (eg, to rule out other conditions, such as vasculitis) and the patient's condition is deteriorating despite treatment with acyclovir. If considered, it should be performed earlier in the course, rather than later, so that a potentially treatable condition can be identified.

Histologic Findings

In acute viral encephalitis, capillary and endothelial inflammation of cortical vessels is a pathologic hallmark occurring in the gray matter or at the junction of the gray matter and white matter. Lymphocytic infiltration of the gray matter and neuronophagia may also occur. Astrocytosis and gliosis become prominent with disease progression. Some histopathologic features, such as Cowdry type A inclusion bodies in HSV infection and Negri bodies in rabies, are unique to viral infections. Arboviruses cause little histopathologic change outside the nervous system, with the possible exception of renal involvement in St Louis encephalitis. Gross examination reveals varying degrees of meningitis, cerebral edema, congestion, and hemorrhage in the brain. Microscopic examination confirms a leptomeningitis with round-cell infiltration, small hemorrhages with perivascular cuffing, and nodules of leukocytes or microglial cells. Demyelination may follow the destruction of oligodendroglias, and involvement of ependymal cells may lead to hydranencephaly. Neuronal damage is seen as chromatolysis and neuronophagia. Areas of necrosis may be extensive, especially in EEE, JE, and the Far East form of tick-borne encephalitis. In patients who survive the initial illness, varying degrees of repair are observed, which may include calcification. The pattern of distribution of lesions in the brain is rarely sufficiently specific to enable identification of the infecting virus. Generally, in EEE, the lesions are concentrated in the cortex; in western equine encephalitis (WEE), they are concentrated in the basal nuclei; and in St Louis encephalitis, they are concentrated in the substantia nigra, thalamus, pons, cerebellum, cortex, bulb, and anterior horn cells. HSE in infants is usually part of a widespread infection that produces focal necrotic lesions with typical intranuclear inclusions in many organs. In adults and in some children, lesions are confined to the brain. Necrotic foci may be macroscopically evident as softening. Hemorrhage and Cowdry type A inclusions bodies are found readily in the margins of areas of necrosis. Herpesviruses have tropism for the temporal cortex and pons, but the lesions may be widespread. Rabies virus tends to exhibit a tropism for the temporal lobes, affecting the Ammon horns. Autopsy studies in individuals with West Nile virus have shown particular brainstem involvement, especially the medulla, with endoneural mononuclear inflammation of cranial nerve roots.

TREATMENT AND MANAGEMENT

Approach Considerations

Medical care should be devoted to appropriate management of the airway, bladder function, fluid and electrolyte balance, nutrition, prevention of bedsores, secondary pulmonary infection, and hyperpyrexia. A multidisciplinary approach must be instituted as early as possible to start physical and cognitive rehabilitation and to minimize cognitive problems and long-term sequelae. Care in an intensive care unit (ICU) setting may be required, especially if seizure activity or increased intracranial pressure (ICP) is present. Delayed diagnosis of herpes simplex encephalitis (HSE) increases morbidity and mortality rates; failure to diagnose and treat early could result in litigation. With the wide availability of effective therapy, initiating antiviral treatment before a definitive diagnosis of HSE encephalitis (ie, during the workup) is now common practice. The use of corticosteroids as an adjunctive therapy for viral encephalitis is controversial and currently being evaluated in a large clinical trial. Recent promising therapies with recombinant fully humanized antibody against Nipah and Hendra virus have been tested in experimental animals and in a compassionate basis in humans.

See the following for complete information on these topics:

- Herpes Simplex Encephalitis
- California Encephalitis
- Eastern Equine Encephalitis
- Japanese Encephalitis
- St Louis Encephalitis
- Venezuelan Encephalitis
- Western Equine Encephalitis
- West Nile Encephalitis

Antiviral Therapy

Pharmacotherapy for HSE consists of acyclovir and vidarabine. Outcome is improved with either agent, but acyclovir is more effective and less toxic. Even if the final diagnosis of HSE has not been established, intravenous (IV) acyclovir should be initiated immediately. Acyclovir is also the drug of choice for varicella-zoster virus (VZV) encephalitis, although ganciclovir is also considered an alternative option. Ganciclovir has been used for cytomegalovirus (CMV) encephalitis, but with therapeutic failures; consequently, the optimal therapy for CMV encephalitis is unknown. Ganciclovir combined with foscarnet has been used in the treatment of patients infected with HIV. No specific treatment is available for the arbovirus encephalitides. Ribavirin seems to be effective for Lassa fever; its efficacy in other viral infections is being evaluated. Intraventricular ribavirin has been associated with clinical improvement in 4 patients with subacute sclerosing panencephalitis (SSPE) and apparently reduced mortality in an open-label trial in patients with Nipah virus encephalitis. Results from a small series suggested that interferon alfa-2b reduced the severity and duration of the complications of St Louis encephalitis virus meningoencephalitis. Because specific therapy for encephalitis is limited and because potentially serious sequelae (or death) may result from HSE, early treatment with acyclovir should be started as soon as possible in all patients with suspected viral encephalitis, pending the results of diagnostic studies. Once an etiologic agent of the encephalitis is eventually identified, therapy should be targeted to that agent (if available). Other antibacterial treatments (eg, for bacterial meningitis) should be administered on the basis of epidemiological and clinical factors or given until the diagnosis of bacterial meningitis is excluded. Doxycycline should be added if there is suspicion of rickettsial or ehrlichial infection during the appropriate season.

Management of Increased Intracranial Pressure

Increased ICP should be managed in the ICU setting with head elevation, gentle diuresis, mannitol, and hyperventilation. Surgical decompression may be necessary if there is impending uncal herniation or increased ICP that is refractory to medical management. Controlled studies are lacking, but there is some evidence that patients with life-threatening cerebral edema may benefit from craniectomy or other approaches to lower the increased ICP in neurocritical care units.

Management of Seizures

Encephalitis causes a wide range of behavioral manifestations with limbic and frontal syndromes that can be difficult to distinguish from partial seizures. Seizure activity can be closely observed using electroencephalography (EEG), and the threshold for administering temporary anticonvulsant therapy should be low. Phenytoin and valproic acid can be administered intravenously. Phenytoin and carbamazepine can be administered when oral or intragastric drug administration is possible. Benzodiazepines are also important when used to abort status epilepticus. If seizure activity persists after the acute phase, patients may need long-term anticonvulsant therapy. Accordingly, additional therapy may be necessary for extrapyramidal, motor, and behavioral complications.

Prevention

Surveillance is important to predict outbreaks of arboviral infections. Mosquitoes can be sampled to estimate infection rates in mosquito pools. Protective clothing and repellents are useful in the prevention of arthropod bites. Avoidance of outdoor activities is also useful. Prompt removal of ticks may decrease the risk of transmission of a tick-borne virus. Effective preventive measures include removing water-holding containers and discarded tires. Insecticides may be useful in the emergency control of infected mosquitoes. Control of the mosquito vector has been used with apparently good results in several recent epidemics. Vaccines are available for eastern equine encephalitis (EEE), western equine encephalitis (WEE), and Venezuelan equine encephalitis (VEE) in horses. A live attenuated vaccine (TC-83) has been used to protect laboratory and field workers from the virus that causes VEE. Vaccines have also been developed for Japanese B virus encephalitis (JE) and tick-borne encephalitis. Killed virus vaccines have been produced experimentally for several arboviruses. A live-attenuated Japanese B vaccine (SA 14-14-2) has been used widely in Asia. Since 1989, 120 million children have been immunized, and a recent report has demonstrated the efficacy of a single dose in preventing Japanese encephalitis (JE) when administered only days or weeks before exposure to infection. The only internationally licensed JE virus vaccine is a formalin-inactivated vaccine. Limited use (eg, in exposed laboratory workers) has been made of vaccines for VEE and tick-borne viral encephalitis. Passive immunization of laboratory workers exposed to a known virus in a laboratory accident has been accomplished with immune (human) serum or gamma globulin. Despite control efforts and disease surveillance, the 1999 outbreak of West Nile virus in New York, with subsequent spread to other states in the United States, showed that different viruses may be spread in the Western hemisphere because of increased international travel and trade. Massive culling of pigs in Malaysia decreased the incidence of Nipah virus infection.

Consultations and Additional Care

Encephalitis is a neurological emergency. Consultation with a neurologist is recommended. Consultation with a neurosurgeon is helpful if a brain biopsy is considered. Consultation with an infectious disease specialist is also appropriate. Given the high likelihood of long-term need for cognitive rehabilitation and physical rehabilitation, especially in moderately severe and severe forms of encephalitis, establishing a multidisciplinary approach early in the disease course is appropriate. A multidisciplinary approach includes consultations with physical, occupational, and speech therapists. No dietary restrictions are necessary. The infectious process, especially with the presence of fever, increases nutritional requirements. Early assessment by a speech therapist and a dietitian helps prevent further body wasting and detects early behavioral manifestations that prevent adequate nutritional intake, such as placidity, apraxia, dysphagia, or agitation.

MEDICATION

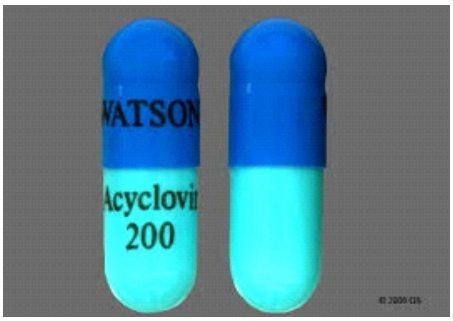
Medication Summary

Pharmacotherapy for herpes simplex encephalitis (HSE) consists of acyclovir and vidarabine. Outcome is improved with either agent, but acyclovir is more effective and less toxic. Even if the final diagnosis of HSE has not been established, intravenous (IV) acyclovir should be initiated immediately. Acyclovir is also the drug of choice for varicella-zoster virus (VZV) encephalitis, although ganciclovir is also considered an alternative option. Ganciclovir has been used for cytomegalovirus (CMV) encephalitis, but with therapeutic failures; optimal therapy for CMV encephalitis is unknown. Ganciclovir combined with foscarnet has been used in the treatment of patients infected with HIV. No specific treatment is available for the arbovirus encephalitides. The efficacy of ribavirin in other viral infections is being evaluated.

Antiviral Agents

Class Summary

Antiviral agents shorten the clinical course, prevent complications,



prevent development of latency and subsequent recurrences, decrease transmission, and eliminate established latency.

Acyclovir (Zovirax)

Acyclovir has demonstrated inhibitory activity against both herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) and is taken up selectively by infected cells. Before the use of acyclovir, mortality from HSE was 60-70%; since acyclovir, it has been approximately 30%. Acyclovir may also be effective for VZV encephalitis.

Ribavirin (Virazole, Ribasphere)

Ribavirin is a synthetic guanosine analogue (1-beta-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide) that inhibits viral replication by inhibiting DNA and RNA synthesis. It is phosphorylated in vivo, and the active form may interfere with viral genomic synthesis. Clinical experience in the treatment of arenavirus infections is primarily with Lassa fever, but anecdotal experience in South American arenaviruses also exists. Ribavirin is used clinically in combination with interferon for hepatitis C, in aerosol form for respiratory syncytial virus (RSV), and as potential prophylaxis and/or treatment of Congo-Crimean hemorrhagic fever, hantavirus infections, and arenavirus hemorrhagic fevers. In vitro evidence exists for activity against West Nile virus. The IV form of the drug is not readily available, and the manufacturer should be contacted if the need arises.

Ganciclovir (Cytovene, Vitrasert)

Ganciclovir is a synthetic guanine derivative that is active against CMV. It is an acyclic nucleoside analogue of 2'-deoxyguanosine that inhibits viral replication in vitro and in vivo by competing with deoxyguanosine triphosphate for viral DNA polymerase, inhibiting DNA synthesis. Ganciclovir triphosphate levels are up to 100-fold greater in CMV-infected cells than in uninfected cells, possibly because of preferential phosphorylation in infected cells.

Foscarnet (Foscavir)

Foscarnet is an organic analogue of inorganic pyrophosphate that inhibits viral replication in vitro. It exerts its antiviral activity by selective inhibition at pyrophosphate-binding sites on virus-specific DNA polymerases at concentrations that do not affect cellular DNA polymerases, inhibiting DNA synthesis. Viral resistance should be considered in patients with poor clinical response or persistent viral excretion. Patients who show excellent tolerance of foscarnet may benefit from initiation of a maintenance dosage (ie, 120 mg/kg/d) earlier in their treatment. Individualize the dosing according to the patient's renal function status. Foscarnet has been demonstrated to be effective against CMV encephalitis.

Anticonvulsant Agents

Class Summary

These agents prevent seizure recurrence and terminate clinical and electrical seizure activity.

Classification of Anticonvulsants

Action on Ion Channels	Enhance GABA Transmission	Inhibit glutamate Transmission
Na^+:	Benzodiazepines	Felbamate
Phenytoin,	Barbiturates	Topiramate
Carbamazepine,	Valproic acid	
Lamotrigine	Gabapentin	
Topiramate	Vigabatrin	
Valproic acid	Topiramate	
Ca^{++}:	Felbamate	
Ethosuximide		
Valproic acid		

Phenytoin (Dilantin, Phenytek)

Phenytoin may act in the motor cortex, where it may inhibit the spread of seizure activity. The activity of brain stem centers responsible for the tonic phase of grand mal seizures may also be inhibited. Individualize the dose. Administer a larger dose before retiring if the dose cannot be divided equally. The rate of infusion must not exceed 50 mg per minute to avoid hypotension and arrhythmia.

Diazepam (Valium)

Diazepam depresses all levels of the CNS (eg, limbic, reticular formation), possibly by increasing the activity of gamma-aminobutyric acid (GABA). Alternatively, lorazepam can be used when indicated.

Carbamazepine (Tegretol, Carbatrol, Epitol, Equetro)

Carbamazepine is effective in treatment of complex partial seizures; it appears to act by reducing polysynaptic responses and blocking posttetanic potentiation. Once a response is attained, attempt to reduce the dose to the minimum effective level or to discontinue the drug at least once every 3 months.

Valproic acid (Depakote, Depakene, Depacon, Stavzor)

Valproic acid is chemically unrelated to other drugs that treat seizure disorders. Although its mechanism of action is not established, its activity may be related to increased brain levels of gamma-aminobutyric acid (GABA) or enhanced GABA action. It also may potentiate postsynaptic GABA responses, affect potassium channels, or have a direct membrane-stabilizing effect.

Osmotic Diuretics

Class Summary

Mannitol is recommended by some experts to help reduce intracranial pressure. Mannitol induces diuresis, which increases serum osmotic concentration. In the brain, this causes water to flow from brain cells into vascular space, thereby decreasing intracranial pressure.

Mannitol (Osmitrol, Resectisol)

Mannitol may be used to decrease intracranial pressure. It may reduce subarachnoid space pressure by creating an osmotic gradient between CSF in the arachnoid space and plasma. This agent is not for long-term use. Initially assess the patient for adequate renal function by administering a test dose of 200 mg/kg intravenously over 3-5 min. It should produce a urine flow of at least 30-50 mL per hour of urine over 2-3 hours. In children, assess for adequate renal function by administering a test dose of 200 mg/kg intravenously over 3-5 min. It should produce a urine flow of at least 1 mL/kg/h over 1-3 hours.

INTERPRETATION & TROUBLESHOOTING

Isolation, Culture, and Identification of Viruses

Isolation of Viruses

Unlike bacteria, many of which can be grown on an artificial nutrient medium, viruses require a living host cell for replication. Infected host cells (eukaryotic or prokaryotic) can be cultured and grown, and then the growth medium can be harvested as a source of virus. Virions in the liquid medium can be separated from the host cells by either centrifugation or filtration. Filters can physically remove anything present in the solution that is larger than the virions; the viruses can then be collected in the filtrate (see Figure 1).

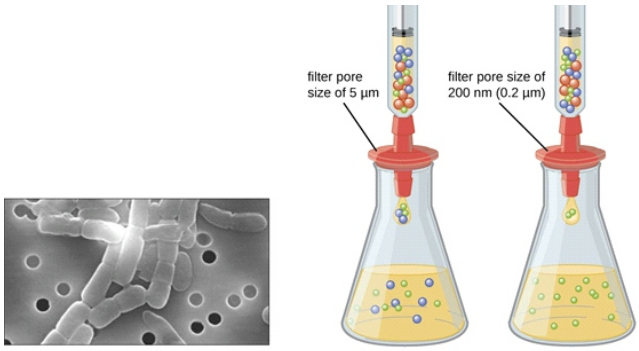


Figure 1. Membrane filters can be used to remove cells or viruses from a solution. (a) This scanning electron micrograph shows rod-shaped bacterial cells captured on the surface of a membrane filter. Note differences in the comparative size of the membrane pores and bacteria. Viruses will pass through this filter. (b) The size of the pores in the filter determines what is captured on the surface of the filter (animal [red] and bacteria [blue]) and removed from liquid passing through. Note the viruses (green) pass through the finer filter.

Cultivation of Viruses

Viruses can be grown in vivo (within a whole living organism, plant, or animal) or in vitro (outside a living organism in cells in an artificial environment, such as a test tube, cell culture flask, or agar plate). Bacteriophages can be grown in the presence of a dense layer of bacteria (also called a bacterial lawn) grown in a 0.7 % soft agar in a Petri dish or flat (horizontal) flask (see Figure 2). The agar concentration is decreased from the 1.5% usually used in culturing bacteria. The soft 0.7% agar allows the bacteriophages to easily diffuse through the medium. For lytic bacteriophages, lysing of the bacterial hosts can then be readily observed when a clear zone called a plaque is detected (see Figure 2). As the phage kills the bacteria, many plaques are observed among the cloudy bacterial lawn.

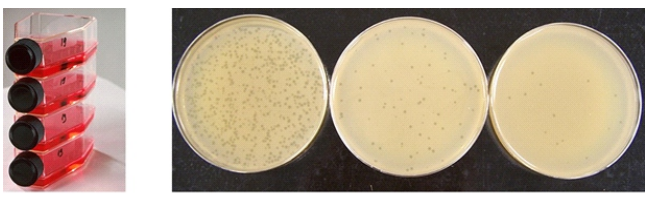


Figure 2. (a) Flasks like this may be used to culture human or animal cells for viral culturing. (b) These plates contain bacteriophage T4 grown on an Escherichia coli lawn. Clear plaques are visible where host bacterial cells have been lysed. Viral titers increase on the plates to the left. (credit a: modification of work by National Institutes of Health; credit b: modification of work by American Society for Microbiology)

Animal viruses require cells within a host animal or tissue-culture cells derived from an animal. Animal virus cultivation is important for 1) identification and diagnosis of pathogenic viruses in clinical specimens, 2) production of vaccines, and 3) basic research studies. In vivo host sources can be a developing embryo in an embryonated bird's egg (e.g., chicken, turkey) or a whole animal. For example, most of the influenza vaccine manufactured for annual flu vaccination programs is cultured in hens' eggs. The embryo or host animal serves as an incubator for viral replication (see Figure 3). Location within the embryo or host animal is important. Many viruses have a tissue tropism, and must therefore be introduced into a specific site for growth. Within an embryo, target sites include the amniotic cavity, the chorioallantoic membrane, or the yolk sac. Viral infection may damage tissue membranes, producing lesions called pox; disrupt embryonic development; or cause the death of the embryo.

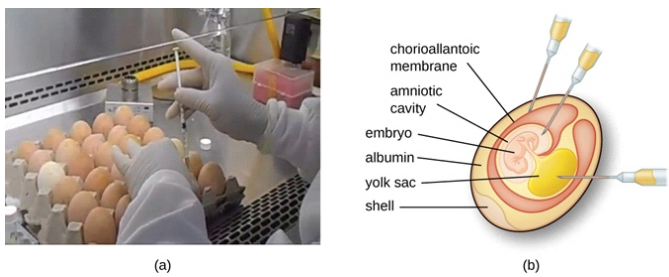


Figure 3. (a) The cells within chicken eggs are used to culture different types of viruses. (b) Viruses can be replicated in various locations within the egg, including the chorioallantoic membrane, the amniotic cavity, and the yolk sac. (credit a: modification of work by "Chung Hoang"/YouTube)

For in vitro studies, various types of cells can be used to support the growth of viruses. A primary cell culture is freshly prepared from animal organs or tissues. Cells are extracted from tissues by mechanical scraping or mincing to release cells or by an enzymatic method using trypsin or collagenase to break up tissue and release single cells into suspension. Because of anchorage-dependence requirements, primary cell cultures require a liquid culture medium in a Petri dish or tissue-culture flask so cells have a solid surface such as glass or plastic for attachment and growth. Primary cultures usually have a limited life span. When cells in a primary culture undergo mitosis and a sufficient density of cells is produced, cells come in contact with other cells. When this cell-to-cell-contact occurs, mitosis is triggered to stop. This is called contact inhibition and it prevents the density of the cells from becoming too high. To prevent contact inhibition, cells from the primary cell culture must be transferred to another vessel with fresh growth medium. This is called a secondary cell culture. Periodically, cell density must be reduced by pouring off some cells and adding fresh medium to provide space and nutrients to maintain cell growth. In contrast to primary cell cultures, continuous cell lines, usually derived from transformed cells or tumors, are often able to be subcultured many times or even grown indefinitely (in which case they are called immortal). Continuous cell lines may not

exhibit anchorage dependency (they will grow in suspension) and may have lost their contact inhibition. As a result, continuous cell lines can grow in piles or lumps resembling small tumor growths (see Figure 4).

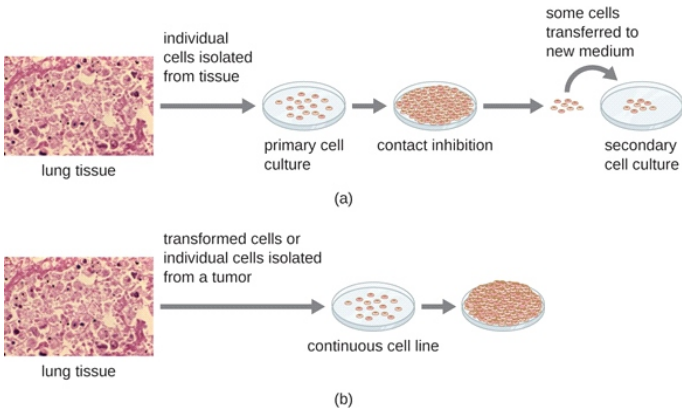


Figure 4. Cells for culture are prepared by separating them from their tissue matrix. (a) Primary cell cultures grow attached to the surface of the culture container. Contact inhibition slows the growth of the cells once they become too dense and begin touching each other. At this point, growth can only be sustained by making a secondary culture. (b) Continuous cell cultures are not affected by contact inhibition. They continue to grow regardless of cell density. (credit "micrographs": modification of work by Centers for Disease Control and Prevention)

An example of an immortal cell line is the HeLa cell line, which was originally cultivated from tumor cells obtained from Henrietta Lacks, a patient who died of cervical cancer in 1951. HeLa cells were the first continuous tissue-culture cell line and were used to establish tissue culture as an important technology for research in cell biology, virology, and medicine. Prior to the discovery of HeLa cells, scientists were not able to establish tissue cultures with any reliability or stability. More than six decades later, this cell line is still alive and being used for medical research. See "The Immortal Cell Line of Henrietta Lacks" below to read more about this important cell line and the controversial means by which it was obtained.

The Immortal Cell Line of Henrietta Lacks

In January 1951, Henrietta Lacks, a 30-year-old African American woman from Baltimore, was diagnosed with cervical cancer at John Hopkins Hospital. We now know her cancer was caused by the human papillomavirus (HPV). Cytopathic effects of the virus altered the characteristics of her cells in a process called transformation, which gives the cells the ability to divide continuously. This ability, of course, resulted in a cancerous tumor that eventually killed Mrs. Lacks in October at age 31. Before her death, samples of her cancerous cells were taken without her knowledge or permission. The samples eventually ended up in the possession of Dr. George Gey, a biomedical researcher at Johns Hopkins University. Gey was able to grow some of the cells from Lacks's sample, creating what is known today as the immortal HeLa cell line. These cells have the ability to live and grow indefinitely and, even today, are still widely used in many areas of research.

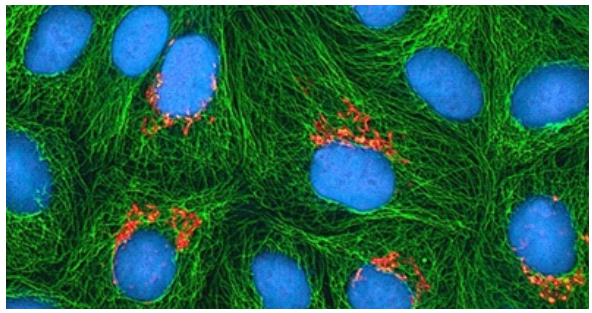


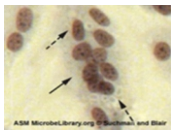
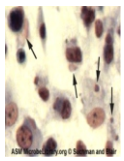
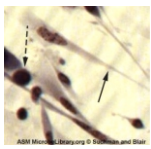
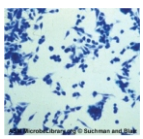
Figure 5. A multiphoton fluorescence image of HeLa cells in culture. Various fluorescent stains have been used to show the DNA (cyan), microtubules (green), and Golgi apparatus (orange). (credit: modification of work by National Institutes of Health)

According to Lacks's husband, neither Henrietta nor the family gave the hospital permission to collect her tissue specimen. Indeed, the family was not aware until 20 years after Lacks's death that her cells were still alive and actively being used for commercial and research purposes. Yet HeLa cells have been pivotal in numerous research discoveries related to polio, cancer, and AIDS, among other diseases. The cells have also been commercialized, although they have never themselves been patented. Despite this, Henrietta Lacks's estate has never benefited from the use of the cells, although, in 2013, the Lacks family was given control over the publication of the genetic sequence of her cells. This case raises several bioethical issues surrounding patients' informed consent and the right to know. At the time Lacks's tissues were taken, there were no laws or guidelines about informed consent. Does that mean she was treated fairly at the time? Certainly by today's standards, the answer would be no. Harvesting tissue or organs from a dying patient without consent is not only considered unethical but illegal, regardless of whether such an act could save other patients' lives. Is it ethical, then, for scientists to continue to use Lacks's tissues for research, even though they were obtained illegally by today's standards? Ethical or not, Lacks's cells are widely used today for so many applications that it is impossible to list them all. Is this a case in which the ends justify the means? Would Lacks be pleased to know about her contribution to science and the millions of people who have benefitted? Would she want her family to be compensated for the commercial products that have been developed using her cells? Or would she feel violated and exploited by the researchers who took part of her body without her consent? Because she was never asked, we will never know.

Detection of a Virus

Regardless of the method of cultivation, once a virus has been introduced into a whole host organism, embryo, or tissue-culture cell, a sample can be prepared from the infected host, embryo, or cell line for further analysis under a brightfield, electron, or fluorescent microscope. Cytopathic effects (CPEs) are distinct observable cell abnormalities due to viral infection. CPEs can include loss of adherence to the surface of the container, changes in cell shape from flat to round, shrinkage of the nucleus, vacuoles in the cytoplasm, fusion of cytoplasmic membranes and the formation of multinucleated syncytia, inclusion bodies in the nucleus or cytoplasm, and complete cell lysis (see Table 1). Further pathological changes include viral disruption of the host genome and altering normal cells into transformed cells, which are the types of cells associated with carcinomas and sarcomas. The type or severity of the CPE depends on the type of virus involved. Table 1 lists CPEs for specific viruses.

Table 1. Cytopathic Effects of Specific Viruses

Virus	Cytopathic Effect	Example
Paramyxovirus	Syncytium and faint basophilic cytoplasmic inclusion bodies	
Poxyvirus	Pink eosinophilic cytoplasmic inclusion bodies (arrows) and cell swelling	
Herpesvirus	Cytoplasmic stranding (arrows) and nuclear inclusion bodies (dashed arrow)	
Adenovirus	Cell enlargement, rounding, and distinctive grape-like clusters	

Hemagglutination Assay

A serological assay is used to detect the presence of certain types of viruses in patient serum. Serum is the straw-colored liquid fraction of blood plasma from which clotting factors have been removed. Serum can be used in a direct assay called a hemagglutination assay to detect specific types of viruses in the patient's sample. Hemagglutination is the agglutination (clumping) together of erythrocytes (red blood cells). Many viruses produce surface proteins or spikes called hemagglutinins that can bind to receptors on the membranes of erythrocytes and cause the cells to agglutinate. Hemagglutination is observable without using the microscope, but this method does not always differentiate between infectious and noninfectious viral particles, since both can agglutinate erythrocytes. To identify a specific pathogenic virus using hemagglutination, we must use an indirect approach. Proteins called antibodies, generated by the patient's immune system to fight a specific virus, can be used to bind to components such as hemagglutinins that are uniquely associated with specific types of viruses. The binding of the antibodies with the hemagglutinins found on the virus subsequently prevent erythrocytes from directly interacting with the virus. So when erythrocytes are added to the antibody-coated viruses, there is no appearance of agglutination; agglutination has been inhibited. We call these types of indirect assays for virus-specific antibodies hemagglutination inhibition (HAI) assays. HAI can be used to detect the presence of antibodies specific to many types of viruses that may be causing or have caused an infection in a patient even months or years after infection (see Figure 6). This assay is described in greater detail in Agglutination Assays.

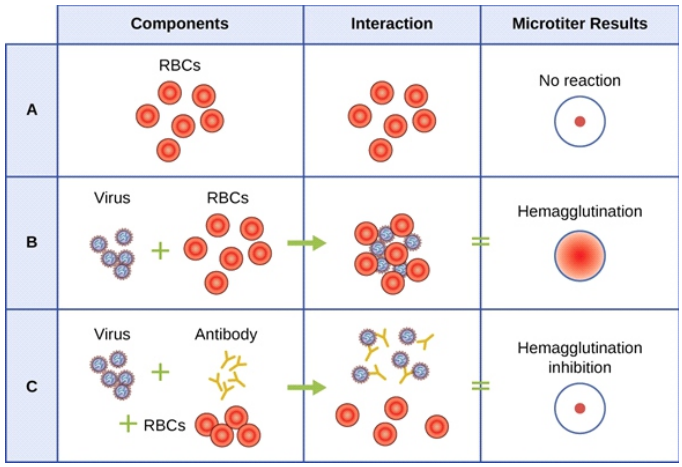


Figure 6. This chart shows the possible outcomes of a hemagglutination test. Row A: Erythrocytes do not bind together and will sink to the bottom of the well plate; this becomes visible as a red dot in the center of the well. Row B: Many viruses have hemagglutinins that causes agglutination of erythrocytes; the resulting hemagglutination forms a lattice structure that results in red color throughout the well. Row C: Virus-specific antibody, the viruses, and the erythrocytes are added to the well plate. The virus-specific antibodies inhibit agglutination, as can be seen as a red dot in the bottom of the well. (credit: modification of work by Centers for Disease Control and Prevention)

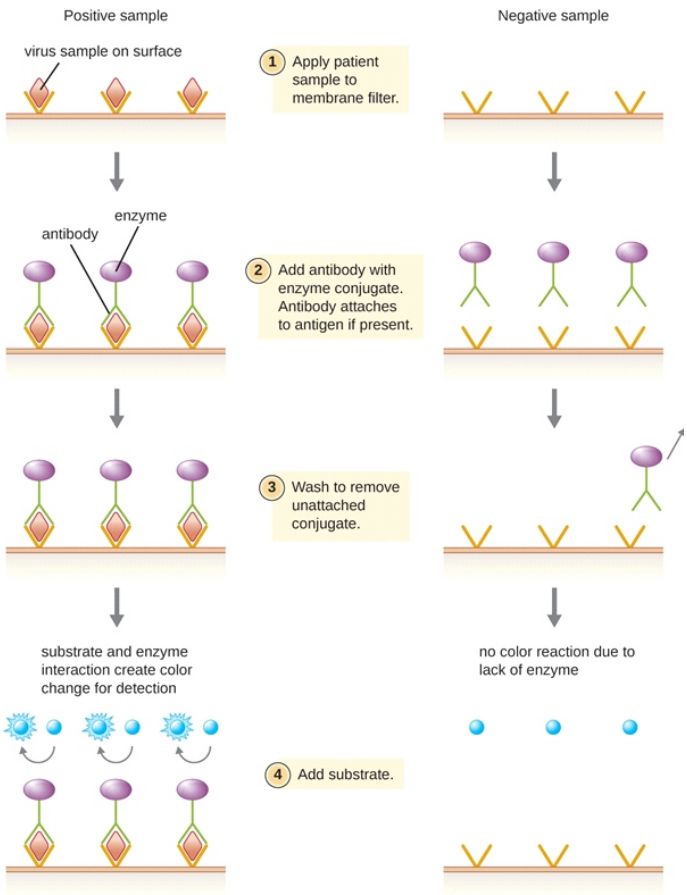
Nucleic Acid Amplification Test

Nucleic acid amplification tests (NAAT) are used in molecular biology to detect unique nucleic acid sequences of viruses in patient samples. Polymerase chain reaction (PCR) is an NAAT used to detect the presence of viral DNA in a patient's tissue or body fluid sample. PCR is a technique that amplifies (i.e., synthesizes many copies) of a viral DNA segment of interest. Using PCR, short nucleotide sequences called primers bind to specific sequences of viral DNA, enabling identification of the virus. Reverse transcriptase-PCR (RT-PCR) is an NAAT used to detect the presence of RNA viruses. RT-PCR differs from PCR in that the enzyme reverse transcriptase (RT) is used to make a cDNA from the small amount of viral RNA in the specimen. The cDNA can then be amplified by PCR. Both PCR and RT-PCR are used to detect and confirm the presence of the viral nucleic acid in patient specimens.

Enzyme Immunoassay

Enzyme immunoassays (EIAs) rely on the ability of antibodies to detect and attach to specific biomolecules called antigens. The detecting antibody attaches to the target antigen with a high degree of specificity in what might be a complex mixture of biomolecules. Also included in this type of assay is a colorless enzyme attached to the detecting antibody. The enzyme acts as a tag on the detecting antibody and can interact with a colorless substrate, leading to the production of a colored end product. EIAs often rely on layers of antibodies to capture and react with antigens, all of which are attached to a membrane filter (see Figure 7). EIAs for viral antigens are often used as preliminary screening tests. If the results are positive, further confirmation will require tests with even greater sensitivity, such as a western blot or an NAAT.

Figure 7. Click to view larger image. Similar to rapid, over-the-counter pregnancy tests, EIAs for viral antigens require a few drops of diluted patient serum or plasma applied to a membrane filter. The membrane filter has been previously modified and embedded with antibody to viral antigen and internal controls. Antibody conjugate is added to the filter, with the targeted antibody attached to the antigen (in the case of a positive test). Excess conjugate is washed off the filter. Substrate is



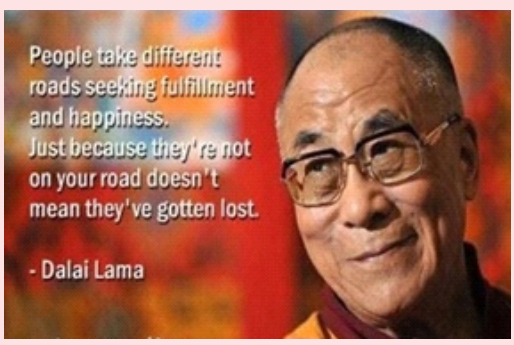
added to activate the enzyme-mediated reaction to reveal the color change of a positive test. (credit: modification of work by "Cavitri"/Wikimedia Commons)

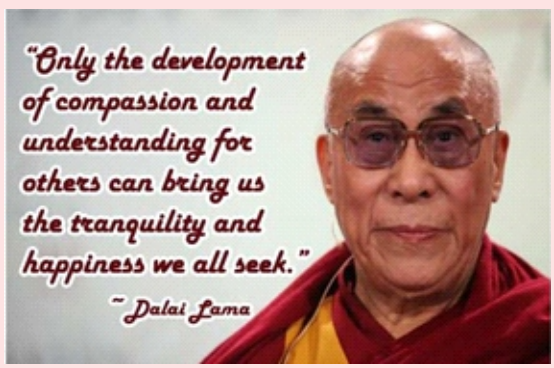
Key Concepts and Summary

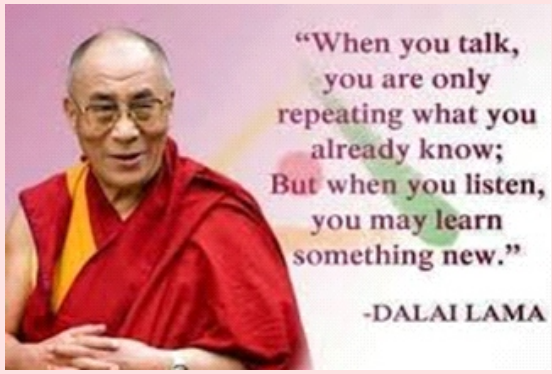
- Viral cultivation requires the presence of some form of host cell (whole organism, embryo, or cell culture).
- Viruses can be isolated from samples by filtration.
- Viral filtrate is a rich source of released virions.
- Bacteriophages are detected by presence of clear plaques on bacterial lawn.
- Animal and plant viruses are detected by cytopathic effects, molecular techniques (PCR, RT-PCR), enzyme immunoassays, and serological assays (hemagglutination assay, hemagglutination inhibition assay).

BOUQUET

Wisdom Whispers

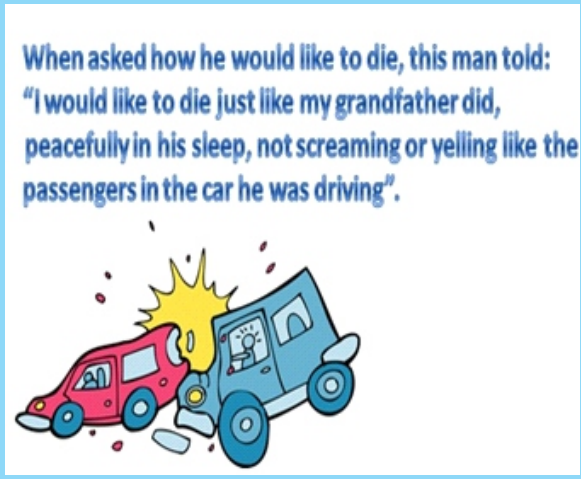






BOUQUET

In Lighter Vein



Brain Teasers

1. **Pipettes may be made of:**
   A. Glass
   B. Metal
   C. Plastic
   D. Any of the above materials.

2. **What pipetting techniques/methods are usually employed?**
   A. Forward
   B. Reverse
   C. Repetitive
   D. All of the above.

3. **What is usual normal reference range given for TSH in µIU/ml?**
   A. 0.3–6.2
   B. 0.8–2.0
   C. 1.4–4.2
   D. 10–20.

4. **Which of the following are the tests used for diagnosing syphilis?**
   A. VDRL test
   B. TRUST antigen
   C. Latex agglutination test
   D. All of the above.

Answers: 1: D, 2: D, 3: A, 4: D

TULIP NEWS

Know the Unknown

- Rickettsia and Brucellosis infections are more prevalent in India.
- One of the important causes of Pyrexia of Unknown Origin (fever of unknown origin).
- Differential diagnosis is important to prevent fatal complications.

Tulip presents simple slide test for the screening of Rickettsia, Brucellosis and Salmonella infections in PUO cases.

PROGEN

Proteus antigen suspensions for the detection of antibodies to rickettsial infection by **Weil-Felix Test**

Progen OXK	Scrub typhus
Progen OX2	Spotted fever
Progen OX19	Typhus fever & RMSP

• Suitable for slide & tube test • Available in 5ml packs

TYDAL

Widal antigen set with innovative VISIMAX STAINING SYSTEM

- Enhanced readability of result
- Suitable for slide & Tube test
- Available in 4x5 ml & 2x2x5 ml packs sizes

BRUCEL - A

Standardized, specific, smooth B. abortus stained antigen suspension for detection of antibodies to B. abortus.

- Suitable for slide & tube test
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Screen and differentiate PUO cases with Tydal, Progen and Brucel antigens.

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