Gender Differences in Emerging Infectious Diseases

SHARON LEWIN

Fellow, Royal College of Physicians, Canada; Attending Physician, St. Luke's–Roosevelt Hospital Center, Division of Infectious Disease, New York, NY; Attending Physician, New York Presbyterian Hospital Center, Division of Internal Medicine, New York, NY; Assistant Clinical Professor of Medicine, Columbia University School of Medicine, New York, NY, USA

INTRODUCTION

An urgent worldwide threat is posed by the introduction and spread of novel infectious diseases. The reasons for emerging infectious diseases are numerous and complex. An emerging infectious disease is defined as any of the following:[1]

- a newly recognized illness
- a known pathogen now affecting new populations
- a known pathogen previously responsible for limited, sporadic disease now infecting large numbers of animals or people
- a known pathogen now causing disease in new geographic areas
- a known pathogen now resistant to previously effective treatment.

Among the most significant explanations for these emerging diseases are changes in environment and ecology caused by natural phenomena such as droughts, hurricanes, and floods; and human-made phenomena such as agricultural development, urbanization, and denuding of forests. Nipah virus infection in Bangladesh, discussed in this chapter, is largely attributable to migration of flying foxes whose natural habitat has been deforested.

World-wide conflict, including wars, ethnic cleansing, and genocide, have led to displacement of large populations into overcrowded settlements where safe water is not available and sanitation is poor. For example, unsanitary conditions led to a huge increase in the rat population in post-war Kosovo, resulting in a tularemia outbreak with 327 confirmed cases in 8 months.[2] Regional conflict leads to breakdown in infection control, inadequate surveillance, impeded access to populations, and spread of infectious diseases through movement of refugees and aid workers.[3]

Climate change increases the risk of infectious diseases by many mechanisms. Mosquito populations will increase where they already exist. Mosquitoes and other arthropod vectors will migrate to new habitats where warmer climate is conducive to their survival. For vector-borne diseases to occur, only a host reservoir and a specific vector are necessary. If humans are the reservoir and the vector has been able to adapt to new locales, a previous zoonosis or confined disease has the potential to become a global disease. The emergence of West Nile virus infection in North America is the best example of vectors and/or amplifying hosts migrating to a new location.

Insect vectors can also overcome geographic barriers via global shipping of goods and human air travel. Introduction of foreign plants, animals, and invertebrates is being increasingly noted in temperate climates.[4] Hantavirus infection in the Four Corners region of the United States was traced to imported prairie dogs from Africa.

Increased precipitation, a result of climate change, leads to more agricultural run-off, allowing pathogens to enter drinking water systems. In developing countries where poverty and inadequate infrastructure are the norm, public health monitoring systems must be supported and improved so that new or more severe risks to health can be identified and curtailed.

As new infectious diseases are recognized, critical issues arise regarding pregnant women and their unborn children. Physiologic changes during pregnancy and gestational age both alter decision-making regarding vaccinations and medications.

Because the infectious diseases discussed in this chapter are emerging pathogens or known pathogens with new epidemiology, much is unknown and unstudied regarding gender differences in disease severity, risk to the pregnant woman or impact on the fetus.

Questions regarding pregnancy's effect on the clinical course of these new diseases, implications for prophylaxis, and treatment of exposed pregnant women, and transmission of disease during pregnancy, labor, delivery, and breastfeeding are, as yet, unanswered in many emerging infectious diseases.[5] Wherever there is information, it will be discussed.

Health disparities exist for women around the globe. Poverty, malnutrition, and educational inequities fuel the spread of disease. Poor women are much more vulnerable to disease than their male counterparts. Disparate factors ranging from immune alterations in pregnancy, economic factors, and complex cultural expectations are partial explanations. The majority of the 1.2 billion people living in extreme poverty are women (70%). Unemployment is higher among women in most developing countries, and even when employed, women's salaries are lower. The World Health Organization (WHO) reports that less is spent on healthcare for women and girls worldwide than for men and boys. Access to doctors, clinics, and hospitals is hampered by the fact that women remain in rural areas while men travel to work in urban areas more accessible to medical care. Lack of employment and high illiteracy rates among women in developing nations create huge obstacles to healthcare.

More than one-third of 15- to 19-year-old girls in parts of Africa and Asia are married. Once married, the husband's family is unlikely to support continued education of their daughter-in-law.[6] Education must be provided to improve women's socioeconomic status.

Black women have higher infant death rates than mixed-race and white women. Although greater than eight years of education lowers infant death rates significantly, the three groups still stratify similarly based on race.[7]

Unfortunately, violence against women, despite awareness in developed nations, has not been stanched at home or abroad. Unprotected sex and rape occur with much greater regularity and aggression in areas where there is armed conflict and civil unrest. Measurable increases of HIV and other sexually transmitted diseases occur but so do the intangibles of fear, humiliation, and social stigma.

Pregnant women may fare poorly even in developed countries because they are often denied medications and vaccines because of unknown effects on the fetus. Healthcare workers and public health officials must be knowledgeable about benefits and risks of drugs and immunizations in pregnancy, so they assist their patients in making informed decisions.[8] Pregnant patients and their physicians overestimate the risk to the fetus of medication.[9] Misconceptions abound even about vaccines and medications proven to be safe and beneficial in pregnancy, often resulting in healthcare workers either not offering their pregnant patients appropriate prophylaxis or treatment, or pregnant patients declining interventions that are likely to protect or benefit themselves and their fetuses.

Data from pregnant women are often not collected in surveillance of a recognized infectious disease outbreak. When a second outbreak occurs, there will be little information about the natural history of that particular infection in pregnant women. Obstetrician-gynecologists are often the only physicians a woman may see and, therefore, are in a unique position to detect unusual patterns of illness or novel diseases.[10]

Pregnant women may have more severe illness in some emerging infectious diseases such as SARS (severe acute respiratory syndrome) and the hemorrhagic fevers. An attempt to avoid radiographs and scans in pregnant patients may lead to diagnostic delays. Ciprofloxaxin is normally contraindicated in pregnancy because of studies suggesting joint and cartilage toxicity in juvenile animals from fluoroquinolones. Despite this data, during the anthrax attacks in 2001, the CDC recommended a 60 day course of ciprofloxacin prophylaxis for pregnant women who had a high-risk exposure.[11]

The Second International Conference on Women and Infectious Disease was held in Atlanta in 2006. The conference underlined the need for accumulating gender-based information on infectious diseases. Monitoring pregnant women during outbreaks must become an integral part of public health investigations. If general guidelines exist for an emerging infectious disease outbreak, pre-event recommendations for prophylaxis and treatment of pregnant women must also be specifically provided, rather than cobbling together guidelines for these vulnerable women and their fetuses during an emergency.

Dozens of new diseases, new syndromes, well-known infectious agents which have become resistant to treatment, and known diseases with a recently identified organism contribute to a vast number of emerging infectious diseases at home and abroad. Space and time do not permit a discussion of all of them.

Five emerging viral infections, three bacterial infections, and one prion disease will be discussed in this chapter.

EMERGING VIRAL INFECTIONS

Nipah Virus

Nipah virus (NiV), along with Hendra virus (HeV), belongs to the family Paramyxoviridae. Over the past decade, both have recently emerged in humans and livestock in Australia and South-East Asia as contagious, virulent viruses capable of causing illness and death. Due to little immunological cross-reactivity with other paramyxoviruses, HeV and NiV have been classified into a new genus within the family Paramyxoviridae named Henipavirus.[12]

HeV and NiV are designated as biosafety level (BSL) 4 agents and are potential bioterrorist agents because there is no licensed vaccine or antiviral therapy. The emergence of

henipaviruses has been linked to increased contact between bats and humans, paralleling the emergence of other zoonotic viruses such as SARS, coronavirus, Australian bat lyssavirus, Manangle virus, and probably Ebola and Marburg. Loss of habitat and food availability has driven bats toward human-populated areas, and encroachment by human agriculture into bat habitats create exposure to these emerging pathogens.

Hendra virus was discovered in Australia in 1994 when a pregnant mare named Drama Series fell ill and died. A prominent horse trainer who nursed the mare became ill within one week and died from respiratory and renal failure. The source of the virus was thought to be the mare's frothy nasal discharge. Altogether, 19 of 23 horses housed with the index case were stricken and 12 of those died. This outbreak suggested both high attack and high mortality rates.

Nipah virus, named after the village Sungai Nipah in Malaysia, was first identified in 1999 when 265 people became ill and 105 of them died. Ninety percent of the human cases were pig farmers or had contact with pigs. Pigs tested positive for the virus which was highly contagious among them, spread by coughing. Over a million pigs were destroyed on the Malay peninsula to try to contain the outbreak. Eleven cases in Singapore (with one death) occurred in abattoir workers exposed to pigs from those Malaysian farms.[13]

The primary reservoir for NiV is the Pteropid fruit bat, also called the flying fox.[14] At the index farm, fruit orchards were close to confined swine herds, allowing bat urine, feces, and fruit partially eaten by the bats to contaminate pig feed.

Since the original reports, there have been at least eight more outbreaks of Nipah virus in India and Bangladesh. Case fatality rates have ranged from 33% to 92%. In Siliguri, India in January 2001 75% of the infected patients were either hospital staff or visitors of a sick patient, suggesting person-to-person transmission.

In an outbreak in April 2004 in Bangladesh, epidemiologic evidence once again strongly suggested that person-to-person transmission occurred. There was no apparent intermediate animal host. Most of the cases were relatives of a local religious leader. Twenty-seven of 36 persons died. Sharing eating utensils with the sick, sleeping in their beds, close contact at the time of death, and a ritual of special cleansing of the orifices of the dead bodies for burial may have all contributed to person-to-person spread.[15]

In January 2005, also in Bangladesh, an outbreak of Nipah virus infection in 12 people, 11 of whom died, was traced to drinking raw date-palm juice. Fruit bats, a nuisance to date-palm juice collectors, drink date-palm juice directly from the cut in the tree or the clay pot used to collect the sweet sap overnight. The juice is gathered in the morning and sold fresh as it ferments quickly and loses its sweet taste. Because palm juice is consumed within a few hours of harvest, Nipah virus, introduced into the juice by the fruit bats, might be able to survive in sufficient numbers for transmission.[16]

CLINICAL ILLNESS

The illness begins with fever, myalgia, and headache after an incubation period ranging from 7 to 40 days. Cough and dyspnea are common. In 60% of patients the disease progresses rapidly, with drowsiness, disorientation, and confusion with ensuing coma in 5–7 days. Neurologic findings include seizures, myoclonus, cerebellar dysfunction, and areflexia. Survivors of NiV may have persistent fatigue and neurologic impairment, such as convulsions and personality changes.[17]

LABORATORY FINDINGS

Laboratory abnormalities are non-specific. Moderate thrombocytopenia and elevated liver enzymes can occur. In patients with neurologic involvement, cerebrospinal fluid (CSF) findings are lymphocytic pleocytosis and elevated protein with normal glucose, in keeping with other viral central nervous system infections. Chest radiographs may show scattered infiltrates.

Magnetic resonance imaging (MRI) of brain imaging may show multiple, small asymmetric focal lesions in subcortical and deep white matter, presumably areas of micro-infarction; but similar findings are noted in other viral encephalitides.[18] EEG shows diffuse slow waves, and in some cases periodic bitemporal sharp waves.

Viral isolation is not done as Nipah virus is classified as a biohazard.

ELISA can establish the diagnosis of Nipah virus infection. Both an IgM capture ELISA and an indirect IgG ELISA and highly specific PCR assays can detect viral sequences in tissue or CSF specimens.

PATHOLOGY

Post-mortem CNS findings in patients who died from Nipah encephalitis show widespread ischemia, thrombosis, and infarction with areas of necrotizing vasculitis and syncytia. Viral inclusions are seen adjacent to vasculitic vessels.[19]

TREATMENT

Ribavirin has shown in vitro activity against HeV and NiV. Clinical trials have been inconclusive. Treatment is supportive. Airway protection should be initiated with the onset of neurologic decline. Antithrombotic agents have been used based on pathologic findings of ischemia and infarction in autopsy specimens, but have not been studied.

PREVENTION

Human disease has been associated with infection in intermediate species such as horses with HeV and swine with NiV. The most crucial way of limiting future human disease is early recognition of illness in intermediate animal hosts.

GENDER

In the first Nipah outbreak among pig farmers, the male to female ratio was 4.5 to 1, reflecting that males are more

likely to have direct exposure to the pigs. Otherwise there has been no significant difference in gender susceptibility.

Early abortion and stillbirths have been reported in sows. No data are available in human women.

Chikungunya Fever

Chikungunya fever, seen in Africa and Asia, is caused by an alphavirus, a large group of viruses that cause fever, rash, and polyarthritis. Alphaviruses, previously known as 'group A arboviruses' (arthropod-borne viruses) comprise a genus within the family Togaviridae. New World alphaviruses are EEE, WEE, and VEE. Old world alphaviruses of major importance, in addition to chikungunya, include O'nyong-nyong virus in Africa, Mayaro virus in South America, and Ross River virus in Australia and Oceania.

Alphaviruses occur in distinct geographic regions based on the range of their respective arthropod vectors. Epidemics of chikungunya prior to 2005 have occurred periodically based on serologic surveys, but disease has usually been sporadic. The virus was first isolated during an epidemic in Tanzania in 1952 and 1953.[20]

Chikungunya is endemic in parts of Africa, South-East Asia and on the Indian sub-continent. In 2005–2006 a major outbreak occurred in the Indian Ocean, starting in Kenya and moving to the Comoro Islands, Mauritius, the Seychelles, Madagascar, Mayotte, and Reunion and finally reaching India, where 1.4 million cases were reported. In some areas the attack rates reached 45%. Asymptomatic chikungunya infection was rare; almost everyone infected became ill. In Reunion, of 265 000 cases of chikungunya there were 237 deaths. Deaths were more common in the elderly and people with other underlying diseases.[21] More than 1000 European and American travelers to India during the epidemic returned home with chikungunya fever. Despite the relatively low fatality rate, widespread epidemics are responsible for considerable morbidity and substantial economic loss.

Chikungunya, from the Makonde language of Mozambique, means 'that which bends up,' which describes the crippling symptoms of the infection. Aedes mosquitoes are known to be the principal vectors of chikungunya: *Aedes aegypti* in Africa, *Aedes albopictus* (the Asian tiger mosquito) in Reunion, and other species in sylvatic cycles. Non-human primates appear to be the reservoir, but dense areas of infected humans may also provide a reservoir. High levels of viremia (10^9 virus particles per milliliter of serum) make transmission from person to person possible.[21] Epidemics usually occur during the tropical rainy season and abate during the dry season.[22]

The vast size of the epidemic that spread to India was attributed to a new variant of chikungunya virus.[23]

Between July and September 2007, 247 cases of chikungunya infection were reported in Italy, the first reports of infection in Europe.

CLINICAL MANIFESTATIONS

After an incubation pesriod of 1 to 12 days, chikungunya presents as a very acute illness with severe polyarticular arthralgias, shaking chills, and fever as high as 40 °C. The illness is biphasic, with the fever abating and then returning, described as a 'saddle-back' fever curve.[24] Other symptoms may include myalgias, headache, photophobia, retro-orbital pain, pharyngitis, nausea, and vomiting.

The arthralgias in chikungunya infection favor the wrists and ankles or previously injured joints, and are worse after a period of rest. Pressure on the wrist produces intense pain, often considered to be a diagnostic sign.[21] There may be joint swelling but usually effusions are absent. Patients remain as immobile as possible.[25] In HLA-B27 positive patients, joint involvement may be permanent.[26]

In the early phase of illness there may be a flush over the face and neck which evolves to a more widespread maculopapular and sometimes pruritic rash, including the palms and soles.

The illness may last from a week to as long as several months. Although rarely fatal, convalescence from chikungunya fever may be prolonged, up to a year.

Patients presenting with chikungunya infection may be indistinguishable from patients with other alphavirus infections known also to cause fever, rash, and polyarthritis, such as Mayaro, O'nyong-nyong, and Ross River viruses. Other illnesses in the differential diagnosis are dengue, parvovirus, hepatitis B prodrome, juvenile rheumatoid arthritis, and rubella.

LABORATORY FINDINGS

Laboratory results include lymphopenia and thrombocytopenia, the latter sometimes severe enough to cause bleeding gums and epistaxis. Hepatic enzymes are commonly elevated, and the erythrocyte sedimentation rate is usually markedly elevated. Chikungunya virus may be rapidly detected via a reverse transcription loop-mediated isothermal amplification assay (RT-LAMP). Diagnosis may also be aided by antibody capture IgM ELISA which can be arranged through public health authorities.[27]

TREATMENT

No specific treatment is available for any alphavirus infections. Supportive care, analgesics, and antipyretics may mitigate symptoms. Aspirin should be avoided. No vaccine is commercially available.[28]

GENDER DIFFERENCES

Infection rates appear to be equal in males and females. Much of the information on pregnancy, fetal, and neonatal exposure to chikungunya comes from the Reunion Island outbreak in 2005. No increase in birth defects were associated with chikungunya during pregnancy. In a study of 160 pregnant mothers infected with chikungunya, 3 of

9 miscarriages before 22 weeks gestation were attributable to the virus. The greatest risk of vertical transmission of chikungunya appears to be at delivery. Of the remaining 151 infected women who carried to term, 33 were viremic at delivery. Almost half the newborns (48.5%) born to those viremic mothers had neonatal chikungunya infection. Cesarean section did not protect against transmission. Infected neonates were asymptomatic at birth and became ill within 3–7 days.[29] Although preliminary reports suggested that 90% of affected newborns recovered quickly without sequelae, a retrospective analysis of 38 neonates showed a high rate of morbidity. Complications included seizures with abnormal brain MRI findings in 14 of 25 infants, hemorrhagic symptoms, hemodynamic disorders with abnormal echocardiographic findings, and one death from necrotizing enterocolitis.[30]

Of the remaining 118 women who had been infected during their pregnancies but were non-viremic at delivery, all gave birth to healthy newborns.[29,31] There is no evidence the virus is transmitted through breastfeeding.

Hantavirus

Hantavirus infection was first identified during the Korean War when several thousand US and UN forces became ill with fever, hypotension, renal failure, and DIC. The name given to the syndrome was hemorrhagic fever with renal syndrome (HFRS). Symptoms and signs of clinical illness were fever, hypotension, thrombocytopenia, DIC, and renal failure. The etiologic agent was named Hantaan, after the Hantaan River in Korea. Hantavirus is an RNA virus belonging to the bunyavirus family.

In 1993 in New Mexico, 3 persons died in the Four Corners region of the southwestern US. Four Corners is the intersection of four states: Utah, New Mexico, Arizona, and Colorado. Another cluster of 5 deaths, also in the Four Corners region, led to a public health investigation by state, local health organizations and the Centers for Disease Control and Prevention (CDC). All of the cases had a similar clinical illness of fever, chills, and myalgias, then cough, shortness of breath, and progression to cardiovascular collapse and respiratory failure. The mortality rate was approximately 80%.

After only a month, the investigators identified the etiologic virus as well as the deer mouse as both reservoir and vector. The new virus was originally called Sin Nombre (Spanish for 'the virus with no name'), later it was identified as a hantavirus. The syndrome in the Four Corners outbreak was named hantavirus cardiopulmonary syndrome (HCPS).

The Four Corners 1993 outbreak was thought to be caused by a preceding wet, mild winter (El Nino) which led to a steep increase in the food supply for mice and a tenfold increase in their numbers in the Four Corners region.[32] Increased rodent density in turn led a higher percentage of infected mice who readily entered homes and farm buildings. Deer mice shed virus in their urine, droppings and saliva. Transmission to humans occurs when they breathe in contaminated air. Although the greatest risk factor appeared to be living in a rodent infested dwelling, in one study, simply entering a long-closed-up building was an important means of exposure.

Hantavirus can cause two distinct clinical entities, HCPS and HFRS. Old World viruses cause HFRS and occur worldwide, especially in Asia. China has approximately 100 000 cases of HFRS each year. These hantaviruses have been called Seoul virus, Dobrava virus, and Puumala virus.

In the New World, 13 different hantaviruses have been identified. Some cause HCPS and some cause HCPS with renal failure. Dozens of HCPS cases have been reported in Alberta, Canada. In South America, at least four strains of hantavirus have been reported to cause HCPS. One of them, the Andes virus, causes person-to-person transmission and high pediatric mortality.

Infection occurs by inhalation of aerosolized virus from feces, urine or saliva of infected rodents. Immune reactivity rather than direct viral injury is likely responsible for plasma leakage in HFRS and HCPS. In HCPS, fulminant pulmonary edema ensues from damage to pulmonary endothelium. Cardiogenic shock in HCPS appears to result from an as yet unidentified myocardial depressant.

Mortality rate in the 1993 outbreak in the southwestern US was 80%. Most of the deaths occurred within 24 hours of admission to hospital. Because of recognition of the disease and earlier diagnosis, more aggressive intervention has dropped the death rate considerably.

By the end of 2000, 227 HCPS cases were reported in 31 states west of the Mississippi. The mean age of cases was 38 years, indicating a predilection in young adults. Most of the cases were rural and occurred in the spring and fall, when residents were exposed to rodents during seasonal planting and harvesting.

CLINICAL PRESENTATION

After exposure, there is an incubation period of 2–3 weeks, when the prodrome of fever chills and myalgias begins, lasting 3–10 days. HCPS is heralded by hypoxemia and tachycardia leading to precipitous clinical deterioration. Patients who recover may have no residual deficits apart from several months of fatigue and decreased exercise tolerance.

Because the differential diagnosis is so broad and early HCPS can mimic (among other diseases) influenza, congestive heart failure, bacterial pneumonia, pneumonic plague and tularemia, obtaining a history of rodent exposure or exposure to rodent excreta is essential.

LABORATORY FINDINGS

Laboratory findings may show elevated AST and LDH during the prodrome. WBC is usually elevated with a left shift. A falling platelet count reliably precedes the cardiopulmonary collapse of HCPS patients. Hemoconcentration may

occur because of capillary leak. Progressive lactic acidosis and severe hypoxemia are ominous signs.

Serologic assays can be done using ELISA for circulating IgM and IgG. Western blot uses a nucleocapsid antigen for the detection of hantavirus antibodies. A very sensitive test is a rapid immunoblot strip assay (RIBA) that detects hantavirus antibodies during the acute clinical phase of the illness.

One third of patients on initial chest x-ray have pulmonary edema.

GENDER DIFFERENCES

Males account for 60% of HCPS cases, probably reflecting a greater occupational exposure to deer mice.

Hantavirus appears to be rare in pregnancy. A pregnant woman with HFRS was reported in 1992. Another report of a pregnant woman who presented after 6 days of high fever and 2 days of no fetal movement gave birth to a stillborn 3200 g infant. She was diagnosed with HFRS based on a high IgM titer to hantavirus. The mother recovered with aggressive care and hemodialysis.[33]

A small review of five pregnant women with HCPS included one death. There were two fetal losses which, at autopsy, showed no evidence of hantavirus infection either microscopically or with immunhistochemical studies. The three surviving children similarly had no evidence of infection, suggesting that transplacental transmission of hantavirus does not occur.[34] Like any life-threatening illness accompanied by hypoxemia, hantavirus infected mothers may give birth to infants who have suffered hypoxemic damage in utero.

Monkeypox

Along with vaccinia (cowpox) and variola (smallpox) virus, monkeypox virus is in the family of DNA viruses Poxviridae, genus Orthopoxvirus. Monkeypox, enzootic among squirrels and monkeys in rainforests of western and central Africa, creates a vesicular illness similar to variola. The disease was first found in 1958 in laboratory monkeys. A smallpox-like illness in humans in Africa in 1970 led to the first report of monkeypox.[35] Transmission from person to person and mortality from monkeypox is much lower than from smallpox.

Fifty-nine cases of monkeypox in humans were reported from western and central African rainforests in the decade from 1970 to 1980. The mortality rate was 17%. All the human monkeypox cases had been in contact with small forest animals. Transmission occurs from bites or contact with blood, body fluid, vesicles or respiratory droplets of the infected animals.

Between 1981 and 1986, WHO surveillance revealed more than 400 additional monkeypox virus infections in humans. Most were children under 10, and the attack rate correlated with time spent outdoors. The secondary cases

numbered eight times higher in people who had not received smallpox vaccine versus than those who had.[36]

Between 1996 and 1998 a very large outbreak of monkeypox occurred in the Democratic Republic of Congo. The large number of cases was attributed to military unrest in the region which drove people deeper into the rainforests, and a population of predominately non-smallpox vaccinated people. From 1998 to 2002, 1625 more cases of monkeypox were reported in the Democratic Republic of Congo.[37]

In 1997, wild animals caught in the DRC were tested for the monkeypox virus. Several animals were found to have neutralizing antibodies against the monkeypox virus: domestic pig, Gambian rat, elephant shrew, and several species of squirrel.

Prior to 2003, monkeypox virus infection had never been reported in the Western hemisphere.[38] Early in June 2003, the Centers for Disease Control and Prevention (CDC) announced the first evidence of community-acquired monkeypox in the United States. By July 30, 2003, a total of 72 cases had been reported to CDC from six states.

The index case was a 3-year-old Wisconsin girl who developed fever and cellulitis after a prairie dog bite, initially, thought to be an isolated event. However, 2 weeks later the girl's mother became ill and a sample from one of the mother's skin lesions demonstrated a poxvirus on electron microscopy. Another report of a sick meat inspector who distributed exotic animals led to an investigation. Most of the 72 cases became ill after contact with sick pet prairie dogs with monkeypox.

The introduction of monkeypox into Wisconsin was traced to a distributor in Illinois, who had received a shipment of exotic animals imported into the United States through Texas from Ghana, West Africa. On arrival to the United States, imported prairie dogs were housed at the same distribution facility as Gambian giant rats, along with other exotic animals. The prairie dogs likely acquired the virus from the Gambian rats.

CLINICAL ILLNESS

From seroepidemiologic data, most monkeypox infections are asymptomatic. Symptomatic monkeypox in humans resembles smallpox, but patients are less ill and more likely to have lymphadenopathy. The incubation period is approximately 12 days. Despite early reports, secondary cases are unusual, as opposed to smallpox, which is extremely contagious.

Symptoms of monkeypox virus infection are fever, headache, myalgias, and fatigue, followed in 1–3 days by a rash which starts on the trunk and then spreads peripherally. Palms and soles are usually involved, as is the face. Patients may also have mucous membrane lesions as large as 1 cm. Initially the rash is maculopapular and then evolves over a 2–4 week period to vesicles. As the vesicles heal, they umbilicate, become pustular, and then form eschars which desquamate. Patients are ill for as long as 4 weeks, but may still have healing vesicular lesions once they feel well.

Mortality in African cases has ranged from 1% to 10% but risk is lower in the United States, where nutrition and access to medical care are better.[39]

Smallpox vaccination with vaccinia virus confers significant protection against infection with monkeypox virus, 85% or higher. Both smallpox and monkey-pox are orthopoxviruses, and the vaccinia immunization induces cross-immunity to monkeypox virus.

Two other infections may mimic monkeypox: varicella and smallpox. Smallpox has been eradicated worldwide, and is therefore unlikely in the absence of a laboratory accident or bioterrorism. Monkeypox vesicles look alike at each stage of the illness, whereas in varicella the lesions are all at different stages of development.

DIAGNOSIS

If the diagnosis of monkeypox is being considered, local health authorities and the CDC should be notified. Diagnostic methods include virus isolation, real-time PCR, ELISA, immunofluorescent antibody assay, and electron microscopy.

GENDER DIFFERENCES

Information about gender differences and pregnancy in monkeypox illness is extremely limited. One case from Zaire (Democratic Republic of Congo) reported a woman at 24 weeks gestation with fever and a rash. Monkeypox virus was isolated. At 30 weeks, she gave birth to a 1500 g infant with a generalized skin rash consistent with monkeypox.[35]

In the 2003 outbreak in the United States, there were pregnant mothers in several of the affected households. The CDC recommended that anyone exposed either to a sick prairie dog or an infected person receive the smallpox (vaccinia) vaccine, whether they were pregnant or not.

TREATMENT

Treatment is largely supportive. No information is available on post-exposure smallpox vaccination. Cidofovir has both in vitro activity against monkeypox virus and in vivo activity in some animal studies.[40] No data are available regarding vaccinia immune globulin.

West Nile Virus

West Nile virus is perhaps the best example of the introduction, establishment, and distribution of a new zoonosis in densely inhabited urban areas. Its emergence in 1999 in the United States and its rapid spread across the country demonstrates that arboviruses can pose a threat in temperate climates.

West Nile virus (WNV), transmitted to humans by mosquitoes, is a single-stranded RNA virus in the genus *Flavivirus*, a group of zoonotic or arthropod-borne viruses.[41] WNV is related antigenically to the Japanese encephalitis virus (JEV) complex, which includes several neurotropic viruses associated with human encephalitis. These include JEV, St Louis encephalitis, Murray Valley encephalitis, and Kunjin, an Australian subtype of WNV.

The virus was first identified in 1937 in the West Nile area of Uganda. Until 1999, the virus was found only in Africa, Asia, the Middle East, and Europe. Human outbreaks were rare and associated with mild illness, usually in soldiers, children, and healthy adults. However, in 1957 in Israel an outbreak in nursing homes associated with severe neurologic disease with fatalities led to recognition of WNV as a cause of severe human meningoencephalitis. Leading up to the identification of WNV infection in the United States, outbreaks in Romania (1996) and Russia (1999) involved hundreds of cases with severe neurologic symptoms, suggesting that WNV disease was increasing not only in frequency but severity.

The first human outbreak of WNV in the United States began in 1999 with 62 reported cases and 7 deaths in New York, New Jersey, and Connecticut.[42] To date, the number of WNV cases appears to have peaked in 2001 at 9862 cases, 2866 with neuroinvasive disease.[43]

Peak incidence of human disease in North America occurs in late August to mid-September. Sporadic cases occur year-round in the south. The seasonality is due to mosquitoes' emergence in the spring in temperate climates.[44] WNV is spread by infected Culex mosquitoes who feed on infected birds who act as amplifying hosts. The mosquitoes carry virus in their salivary glands and in turn infect susceptible bird species, thus maintaining the bird-mosquito-bird transmission cycle. Humans, horses, and other vertebrates are incidental hosts and unlikely to be sources of transmission, since viremia is low grade and brief. In a very small number of cases, WNV has been spread through blood transfusions and organ transplants.

Wild birds develop sustained high levels of viremia but generally are not ill. In the United States and Israel, WNV causes high mortality in avian populations. The presence of dead birds may herald an outbreak of human disease.

Migration of birds and/or the Culex mosquito are the likely explanation for dissemination of WNV to the United States.

CLINICAL ILLNESS

Eighty percent of WNV infections are asymptomatic. The clinical illness with WNV can be divided into two categories: West Nile fever and West Nile neuroinvasive disease. Most of the remaining 20% of infected individuals will develop West Nile fever, with symptoms of headache, myalgias, and nausea in addition to fever. The incubation is 3–14 days. Signs of illness are occasional adenopathy and a rash that lasts a few days to several weeks. This is usually a self-limited illness lasting 3–6 days, indistinguishable from other viral infections.

One out of 150 infected persons, usually elderly, will become seriously ill with neuroinvasive West Nile disease. Patients present with any combination of high fever,

headache, neck stiffness, stupor, coma, convulsions, and blindness. Patients presenting with encephalitis associated with muscle weakness and flaccid paralysis should be strongly suspected to have WNV infection.[45] The illness lasts for several weeks and may leave patients with permanent neurologic sequelae such as muscle weakness, concentration problems, confusion and depression.

In addition to encephalitis, cranial nerve palsies, myelitis, and aseptic meningitis have been described. West Nile poliomyelitis, an acute flaccid paralysis without fever, occurs rarely. Most patients with the polio presentation of WNV recover incompletely and are left with profound residual deficits.

Although central nervous system disease is most serious, other organs may become involved including muscles, liver, pancreas, and heart. Fatal hemorrhagic fever has been reported, but is rare.

West Nile infection in the elderly may simply reflect waning immunity with advancing age, however, proclivity to neuroinvasion may be based on functional or structural CNS changes.[44] In addition to age, alcohol abuse and diabetes have been associated with West Nile encephalitis;[46] as had solid organ transplantation.[47]

LABORATORY FINDINGS

General laboratory findings are non-specific. Hyponatremia may be present in encephalitis patients. Cerebrospinal fluid shows a lymphocytic pleocytosis, elevated protein and normal glucose. CT and MRI brain imaging are useful only to rule out other processes, as imaging findings in WNV neurologic disease are non-specific. The best diagnostic method is a four-fold or greater change in serum IgM antibody to WNV or IgM antibody-capture ELISA in CSF.

Diagnosis relies on a high index of suspicion. WNV should always be considered in patients with otherwise unexplained febrile illness, encephalitis, and/or meningitis, or flaccid paralysis during mosquito season. Closely related arboviruses cross-react in serologic tests. To pinpoint the etiologic agent it may be necessary to conduct tests using a battery of closely related viruses.

TREATMENT

Treatment is supportive. In milder cases, patients will recover on their own. For severe disease, hospitalization with intravenous fluids and ventilatory support may be necessary.

PREVENTION

Prevention is simply avoiding mosquito bites, which occur most commonly at dusk and dawn when mosquitoes are most active. Use of insect repellents (DEET), pants that tuck into socks, long sleeves with cuffs, and insect screens on doors and windows are all effective deterrents. Clothing should be sprayed with permethrin- or DEET-containing products. Standing water in flower pots, buckets, rain barrels, pet dishes, and bird baths are invitations for mosquito infestation. Tire swings should have holes so rain water drains out and children's wading pools should be empty and stored sideways when not in use. No human vaccine against WNV is currently available.

GENDER DIFFERENCES

In surveillance reports, the number of cases of WNV in men has been slightly greater than women, but not statistically significant. This may be explained by greater numbers of men than women working outdoors. Severity of illness correlates with age rather than gender.

Limited information about WNV in pregnancy is available, with few case reports. Flavivirus infections during pregnancy have been rarely associated with spontaneous abortion and neonatal illness, but no known birth defects.[48]

It is not clear whether pregnant women are more susceptible to infection with WNV or whether they become more ill than non-pregnant women. In 2002 a woman developed WNV encephalitis during her 27th week of pregnancy. At 38 weeks she delivered an infant with chorioretinitis, cystic destruction of cerebral tissue, and laboratory evidence of congenitally acquired WNV infection. This appears to be the only case of documented vertical transmission of WNV.[49]

In another case of WNV meningoencephalitis during pregnancy, the mother was induced at 32 weeks for pre-eclampsia and fetal growth restriction. Her infant did not have serologic evaluation for WNV, making it unclear whether maternal hypertension or WNV or both led to the growth restriction.[50] In four other cases of reported WNV infections in pregnancy, all delivered full-term infants with grossly normal appearance and negative laboratory findings of WNV.

In 2003 the CDC developed a registry to track pregnant women with WNV infection. During 2003 and 2004, 77 pregnant women with WNV had 72 live infants, 4 miscarriages and 2 elective abortions. None of the 72 infants followed to date have had conclusive laboratory evidence of WNV infection. However, the sensitivity of IgM testing for WNV in newborns is unknown. Three infants born to mothers ill with WNV within 3 weeks of delivery had symptomatic WN disease at birth.[51]

If WNV is diagnosed during pregnancy, a detailed ultrasound examination should be done in the first few weeks after the mother becomes ill. If an infant is born to a mother who had documented WNV, a thorough evaluation of the infant is recommended with careful physical exam, serologic testing for WNV, hearing evaluation, and pathological examination of the placenta. If the infant appears to be ill, the infant should undergo brain CT scan, neurologic and ophthalmologic specialist consultations, complete blood work including WNV serology, and close follow-up through the first six months with repeat evaluations as indicated.[52]

One probable case of WNV transmitted from mother to infant via breast milk has been reported.[53]

EMERGING BACTERIAL INFECTIONS

Bartonellosis

In 1909 A.L. Barton described organisms that adhered to red blood cells. The organism was named *Bartonella bacilliformis* and was the only species identified in this genus until 1993, when Dolan and colleagues isolated *Bartonella henselae* (previously named *Rochalimae henselae*), a curved, pleomorphic Gram-negative intracellular bacillus, from lymph nodes of patients with cat scratch disease.[54] The clinical syndrome of cat scratch disease had been recognized for nearly a century but prior to Dolan's work had been a disease without a known etiology.

Bartonella organisms were originally thought to be rickettsiae but differ in that the former can grow on artificial media. At least a dozen species have now been identified within the genus *Bartonella*.

In a tragic story in infectious disease lore, in 1885 a Peruvian medical student, Daniel Alcides Carrion, injected himself with the pus of a lesion from a patient who had verruga peruana, a strange purplish eruption. Three weeks later Carrion developed Oroya fever, showing that verruga peruana (the eruptive phase) and Oroya fever (the hematic phase) were two stages of the same disease. Carrion died several weeks after becoming ill. The causative organism of this two-stage disease, now also called Carrion's disease, is *Bartonella bacilliformis*. This is a rare disease found only in the Andes in Peru, Ecuador, and Columbia and is transmitted by sandflies.[55]

Bartonella species first came to clinical attention in the United States in 1990 when they were identified as the cause of unusual opportunistic infections in patients with acquired immunodeficiency syndrome (AIDS).[56]

CLINICAL ILLNESS

Bartonellosis encompasses a spectrum of infectious diseases ranging from mild lymphadenopathy seen in cat scratch disease to life-threatening disease in the immunocompromised host. One pathogenic process unique to this genus is endothelial cell proliferation and neovascularization, a syndrome called peliosis hepatis.

The commonest manifestation of disease caused by Bartonella species, usually *Bartonella henselae*, is cat scratch disease (CSD). The host for this organism is the cat, and cat fleas are the vector which spread the organism to other cats. There is no evidence of transmission of Bartonella from cat fleas to humans.

Patients with cat scratch disease (CSD) present 1 week to 2 months after a bite or scratch from a domestic or feral cat, usually a kitten, with regional tender adenopathy proximal to the injury. A primary cutaneous inoculation site may be seen at the site of the bite or scratch, developing into a papule or pustule a week after exposure. Constitutional symptoms are mild and non-specific and include low-grade fever and malaise. The disease is seasonal, the majority of cases occurring in the fall and early winter, presumably due to a midsummer rise in kitten births and increased flea infestation.

CSD is usually self-limited and is one of the common causes of prolonged fever in children and fever of unknown etiology in adults. In 1993 the Centers for Disease Control and Prevention reported approximately 22 000 cases of CSD annually, although many more cases may be unrecognized.[57]

Atypical presentations of CSD occur in 10% of cases and may include encephalopathy with seizures, neuroretinitis with sudden blindness, joint pain, and atypical pneumonia. Abdominal pain may signal CSD granulomatous hepatitis and splenitis, a self-limited condition in healthy hosts. Parinaud oculoglandular syndrome, an uncommon presentation (5% of cases), consists of a granulomatous conjunctivitis (caused usually by the patient's own hand spreading the organism to the eye) associated with ipsilateral preauricular lymphadenopathy.

Bartonella quintana is found worldwide and was first described during World War I as responsible for causing trench fever in soldiers in Europe, Mesopotamia, and Egypt. Trench fever was the most prevalent disease among Allied troops. After World War I, trench fever seemed to disappear, but reemerged in the German army in Russia during World War II, with attack rates up to 30%. The organism is spread by the body louse, *Pediculus humanus corporis*. After a two-week incubation period, the illness begins with a sudden high fever, headache, and myalgias. The fever typically lasts five days and remits, but may relapse several times for five days, hence the name *quintana*. An unusual symptom of hyperesthesia of the shins may aid in diagnosis.

Over the past decade, a contemporary *B. quintana* infection emerged in various US cities and abroad and was dubbed urban trench fever. This disease primarily affects inner-city dwellers, chronic alcohol abusers, and political refugees.

Trench fever is almost always self-limited and affected patients recover without treatment.[58]

Bartonella is an increasingly important cause of culture-negative endocarditis. Six different species have been identified, but the majority of endocarditis cases are caused by *B. quintana* and *B. henselae*.[59] Body louse infestation, contact with cats, and underlying valvular heart disease are the major risk factors for Bartonella endocarditis. A predilection exists for the aortic valve. A high rate (60%) of valve replacement appears to be necessary. Most cases occur on native valves, but aggressive prosthetic valve endocarditis with rapid valve destruction has been reported.[60]

Bacillary angiomatosis, first described in 1983 in HIV-infected patients and organ transplant recipients, is a vascular proliferative disease usually involving the skin, but also reported in liver, spleen, bone, brain, and other organs. Cutaneous lesions are papular, purple to red-black in color, and highly vascular. Both *B. henselae* and *B. quintana* have

been identified as causative agents.[61] Bacillary angiomatosis occurs in advanced AIDS patients with a median CD4 lymphocyte count of <50 cells/microliter.[62]

Disseminated, severe, progressive disease may also occur in patients with other forms of immunosuppressive disease or alcoholism. Close observation and treatment with antibiotics are indicated in these populations.

Also seen in HIV-infected individuals and transplant recipients, peliosis hepatis caused by *Bartonella henselae* is a vascular proliferation of hepatic capillaries that create blood-filled spaces in the liver.[63]

LABORATORY FINDINGS

As the spectrum of disease attributed to Bartonella is further defined, reliable laboratory methods to identify these unique organisms will become increasingly important.

Diagnosis of CSD can be confirmed with a four-fold rise in antibody levels, first IgM, followed by IgG.

Bartonella species are rod-shaped and slightly curved fastidious bacteria which are difficult to isolate from tissue and therefore require a high clinical suspicion and communication with the microbiology laboratory. Growth requires at least 3 weeks in 5% carbon dioxide. Histopathology, when available, may demonstrate the organisms using a Warthin–Starry silver stain. Gram and acid-fast stains are almost always negative.

Indirect immunofluorescence assay (IFA) and ELISA are the two most common serologic tests for Bartonella. Western immunoblot appears to be sensitive and specific for Bartonella endocarditis. PCR methods appear promising as well.

In bacillary angiomatosis, diagnosis is confirmed by biopsy and histopathology, which shows vascular proliferation along with numerous bacilli that take up the modified silver stain.

TREATMENT

Incision and drainage of cat scratch lymph nodes should be avoided. Thin-needle aspiration is much less likely to lead to fistulas.

Bartonella infections respond to doxycycline, erythromycin, and the newer macrolides, azithromycin, and clarithromycin. In a healthy host, CSD is self-limited and may not require treatment. However, once a patient presents to a physician with CSD, therapy is typically given because the patient is uncomfortable and early treatment may reduce the risk of more complicated or disseminated disease. For severe infections, rifampin or gentamicin can be added to doxycycline. Duration of therapy may be 2 months or longer for patients with peliosis hepatis or disseminated disease.

Pregnant women with bartonellosis should be treated with erythromycin.

For endocarditis with *Bartonella* sp. the recommended regimen is ceftriaxone 2 g daily for 6 weeks plus gentamicin

1 mg/kg every 8 hours for 2 weeks with dosage adjustment to achieve peak serum concentration of 3–4 μg/ml and trough of <1 μg/ml. Doxycycline 100 mg twice daily either intravenously or orally may be added.

Penicillins and first and second generation cephalosporins are not active against these organisms and should not be used.[66] Fluoroquinolone activity against *Bartonella* spp. is inconsistent and therefore not recommended for treatment.

GENDER DIFFERENCES

The male-to-female ratio is 3:2. Eighty percent of patients with CSD are under 21 years old. Bartonellosis during pregnancy has been associated with a more severe course and high rates of maternal and perinatal mortality in immunocompetent women. In one report a pregnant patient developed life-threatening anasarca and cardiac tamponade.[64]

Overall, more than 70% of Bartonella endocarditis cases have occurred in men. This male predominance may be related to infestation with body lice associated in homeless and alcoholic men leading to infection with *B. quintana*.[65]

Enterohemorrhagic *E. coli* 0157

Escherichia coli are lactose fermenting Gram-negative rods, and exist as part of the normal flora of the human colon. *E. coli* strains are the most frequent bacterial causes of diarrhea, causing several distinct clinical diarrheal syndromes. In the clinical microbiology laboratory, different strains of *E. coli* are not distinguishable from one another except for enterohemorrhagic *E. coli* (EHEC 0157).

Enterotoxigenic *E. coli* (ETEC) are the most common cause of diarrhea in children under 2 in the developing world. They are also responsible for most cases of travelers' diarrhea.[67] In June 1998 a large foodborne outbreak of diarrheal disease caused by ETEC occurred in Cook County, Illinois. A delicatessen was identified as the common source. As many as 3300 persons developed gastroenteritis.[68]

The illness caused by ETEC requires a large inoculum. Incubation is short, and onset of nausea and watery diarrhea is rapid. Usually the illness lasts 24 hours but may last a few days. It is almost always self-limited. Therapy consists mainly of oral rehydration. Antibiotics are not indicated.

Enteropathic *E. coli* (EPEC) have caused sporadic outbreaks of diarrhea, usually in neonates. The illness can be severe and persistent, particularly in developing countries.

Enteroinvasive *E. coli* (EIEC) are closely related to Shigella, and are uncommon causes of disease.

Enteroaggregative *E. coli* (EAEC) were identified in the late 1980s and cause persistent diarrhea in children in developing and industrialized regions, HIV-infected adults, and international travelers.

This section will focus on enterohemorrhagic *E. coli* (EHEC).

In 1982, two outbreaks of bloody diarrhea occurred in Oregon and Michigan related to ingestion of hamburgers

at a fast-food chain. A previously unidentified serotype of *E. coli*, 0157:H7, was isolated from the patients with diarrhea and the hamburger meat, but not from stool cultures of healthy controls.[69] This new class of *E. coli* was termed enterohemorrhagic *E. coli* (EHEC). Subsequently, EHEC have been responsible for large outbreaks and sporadic cases of diarrhea in the United States and around the world.

EHEC differ from other groups of diarrhea-associated *E. coli* because they produce Shiga toxin. Therefore, they are sometimes called STEC (Shiga-toxin producing *E. coli*). In the United States, most of the EHEC strains have continued to be 0157:H7, as in the 1982 outbreak. The majority of Shiga-toxin producing EHEC in other countries are non 0157:H7, and non 0157:H7 serotypes are increasing in prevalence in the United States as well.[70] An outbreak in Montana in 1994 was an *E. coli* 0104 serotype.

The inoculum required for EHEC with serotype 0157:H7 is very small, only 10–100 organisms, compared to clinical infection with Salmonella which requires more than 10^5 organisms. Therefore only a few EHEC 0157:H7 need to survive for transmission from food to humans.

More than half the cases of EHEC due to *E. coli* 0157:H7 have been traced to ground beef. Patients have also been infected from produce such as apples and radish sprouts.[71] Human-to-human transmission occurs in 14% of cases, 9% are waterborne, and in 21% of cases, the source can not be identified.

Cattle are the most important reservoir for *E. coli* 0157:H7. Ten percent of healthy cattle excrete the organism in their stool. Beef becomes contaminated during slaughter or processing when it comes in contact with intestinal contents from an infected animal. Despite efforts to reduce *E. coli* 0157:H7 by screening beef at meatpacking plants, spread of infected beef continues to occur.

In June 2002 a recall was issued by one meat-packing plant of 350 000 pounds of culture-positive ground beef. Enough of this contaminated beef had been distributed, however, to cause 28 cases of EHEC in seven states. In mid-July 2002, one of the largest recalls in US history, 18.6 million pounds of fresh and frozen beef was recalled.

In 2003 *E. coli* 0157:H7 accounted for 3% of all acute foodborne illness in the United States.[72] In one study, if the stool sample was visibly bloody, 39% of isolates were *E. coli* 0157:H7.[73]

In Washington state in 2005 an outbreak occurred in persons consuming raw milk from a particular farm. Fresh spinach was the cause of an outbreak in 100 people in late summer 2006. Half were hospitalized and three died. Cases of EHEC have been traced to petting zoos as well as a contaminated building.

In 2008, several grocery chains were forced to recall beef after illness due to *E. coli* 0157 occurred in several states. The contamination was linked to a meat-processing plant in Nebraska which had been shut down three times in 2002 and 2003 by the US Department of Agriculture, and

cited again in 2004, 2005, and 2006. Five million pounds of beef were recalled. As of July 15, 2008, 44 confirmed cases of *E. coli* were reported, with 21 hospitalizations. Patients ranged in age from 2 to 78 with a median age of 20.[74]

Between June 2 and August 6, 2008 seven people in Massachusetts were sickened with *E. coli*. Their beef had been purchased from an upscale retailer known for its high prices and presumed high quality. The beef from the Massachusetts outbreak was traced to the same supplier in Nebraska. An additional 1.2 million pounds of beef were recalled.

CLINICAL MANIFESTATIONS

The incubation period for infection with enterohemorrhagic *E. coli* can range from 1 to 9 days but is usually 3–4 days. Hemorrhagic colitis is the most common and typical syndrome. Fever is usually absent but patients complain of abdominal pain. Abdominal tenderness is present on examination. Hospitalization is required in 23–47% of patients with acute diarrhea. Mortality rates are 1–2% in uncomplicated cases, but may be higher in elderly patients.[75]

LABORATORY FINDINGS

The peripheral WBC is usually elevated. There is blood in the stool.

Up to 9% of all EHEC infections have been seriously complicated by hemolytic uremic syndrome (HUS), a triad of microangiopathic hemolytic anemia, acute renal failure, and thrombocytopenia. HUS begins within 5–13 days after the diarrhea. In children under age 10 HUS complicates approximately 15% of cases of EHEC.[76]

Thrombotic thrombocytopenic purpura (TTP) is a disorder related to HUS in which patients have, in addition to the HUS triad, fever and neurologic symptoms. Fifty percent of HUS patients require dialysis. Mortality is 3–5%. Up to 10% will have residual renal or neurologic disease.

At least 70% of postdiarrheal HUS in the United States has been linked to EHEC infection, and 80% of these are caused by *E. coli* 0157:H7. In Australia, patients with postdiarrheal HUS usually have non-0157 *E. coli*.[77]

TREATMENT

Current treatment is supportive with monitoring for complications, especially in patients with HUS. Antiperistaltic agents are contraindicated as they increase risk of systemic complications. Antibiotic therapy is of no established benefit. One study in 71 children under 10 years old showed a steep rise in risk of HUS following antibiotic therapy.[78]

No vaccine is currently available.

PRECAUTIONS

Safeguards to minimize *E. coli* infection include refrigerating meat as soon as possible after purchase, cooking

ground beef to an internal temperature of 160 degrees, and re-refrigerating meat within two hours of cooking. Ground meat that is pink should not be eaten. If an undercooked hamburger is served in a restaurant, the diner should request that the meat be cooked through and served with a new bun on a clean plate.

Assiduous universal and contact precautions should be in place to prevent transmission to hospital staff and other patients. Household spread to siblings may be mitigated by admitting infected children to hospital. The importance of handwashing by children and staff in daycare and school settings cannot be overemphasized.

Higher standards in meat processing plants and close government monitoring are essential to reduce future cases of EHEC.

GENDER DIFFERENCES

Although male:female attack rates differ in various outbreaks, no consistent pattern is noted, nor is there a difference in incidence of HUS based on gender. No specific information on EHEC in pregnancy or in the peripartum period is available.

Exclusive breastfeeding of young infants appears to confer protection against severe ETEC diarrhea[79] and Shigella.[80]

Methicillin-resistant *Staphylococcus aureus*

Methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria are defined as organisms having an minimum inhibitory concentration (MIC) to oxacillin of 4 mg/l or greater or an MIC to methicillin of 16 mg/l or greater.

MRSA was first isolated in England in 1961[81] shortly after the antibiotic methicillin was introduced. The isolates recovered during that decade were likely a single clone, but by 2002 five MRSA clones worldwide had been reported. Emergence of MRSA was probably due to antibiotic selection pressure.

Epidemiologically, MRSA infections have been divided into HA-MRSA (healthcare-associated MRSA) and CA-MRSA (community-acquired MRSA).

HA-MRSA has been a growing problem worldwide in hospitalized patients since the 1960s and often causes severe, invasive disease. Between 1995 and 2001 in the United States the proportion of MRSA isolates increased from 22% to 57% in over 24 000 cases of nosocomial *S. aureus* bacteremia. Nosocomial MRSA infections are responsible for longer hospital stays, higher mortality, and higher costs than patients with methicillin-sensitive *S. aureus* (MSSA). Risk factors for HA-MRSA infections include antibiotic use, surgery, intravenous devices, prosthetic devices such as artificial joints and heart valves, intensive care unit stays, hemodialysis, and exposure to other patients with MRSA. The most common mode of transmission of HA-MRSA is contaminated hands of healthcare workers. However, fomites such as stethoscope

ear tips and surfaces proximate to infected patients can also serve as reservoirs. In one study, environmental surfaces had an MRSA contamination rate of 59% in the hospital rooms of patients with heavy gastrointestinal MRSA colonization and diarrhea.[82,83]

CA-MRSA infection is defined as MRSA infection in an individual without recent hospitalization, surgery, stay in a long-term care facility, dialysis, or indwelling medical devices. The first reports of CA-MRSA were reported in intravenous drug users in the early 1980s.[84] Based on molecular evidence, CA-MRSA strains evolved spontaneously rather than migrating from hospitals to communities. CA-MRSA differs from HA-MRSA in genetic makeup, increased pathogenicity, and antibiotic susceptibility. Most CA-MRSA strains in the United States encoded with the novel *mec*A gene sequence, which until recently, had not been present in HA-MRSA strains. *Mec*A produces PBP2a, a penicillin-binding peptide that decreases beta-lactam affinity for MRSA. *Mec*A is a subset of a larger mobile genetic element called staphylococcal chromosome cassette (SCC*mec*) which governs other differences between HA-MRSA and CA-MRSA.[85]

While CA-MRSA usually retain their susceptibility to many non-beta-lactam agents, HA-MRSA strains do not. CA strains produce virulence factors and destructive toxins not commonly found in HA strains, particularly Panton–Valentine leukocidin. USA 300 and USA 400 are the predominant clones of CA-MRSA infections, with USA 300 most common.[86]

In a population review in three communities, the annual incidence of CA-MRSA during 2001–2 was 18–25 per 100 000. Twenty-three percent of patients required hospitalization. CA-MRSA infections most often present with skin and soft tissue infections in young, healthy people who neither work nor have been in hospitals or other healthcare settings.[87] These organisms are usually sensitive to non-beta-lactam antibiotics.

CA-MRSA has now become the most frequent cause of skin and soft tissue infections in emergency rooms in the United States.[88,89]

In men who have sex with men, multidrug-resistant isolates containing a plasmid pUSA03 have been described. These MRSA isolates may be resistant, in addition to beta-lactams, to fluoroquinolones, tetracycline, macrolides, clindamycin, and mupirocin.[90]

Clusters of CA-MRSA skin and soft tissue infections have been reported in aboriginal communities; athletic teams including football, wrestling, fencing, and canoeing; daycare centers; military personnel; men having sex with men; prison inmates; and prison guards. Suboptimal hygiene, lacerations, abrasions, shaving, shared gym equipment, tattoos, incarceration, close physical contact with other MRSA carriers, and HIV infection[91] have all been identified as risk factors, but are poorly predictive. Farm animals (notably pigs) and even family pets have all been indentified as sources. Many patients presenting with CA-MRSA lack any obvious risk factors or exposure.[92]

Four children died of fulminant CA-MRSA in Minnesota and North Dakota from 1997 to 1999.[93] Countless other outbreaks of CA-MRSA have been reported. Some of them will be described here.

In Ohio, Kentucky, and Vermont in 2004 and 2005 44 tattoo recipients from 13 unlicensed tattoo parlors developed CA-MRSA infections. Thirty-four cases were primary. Ten cases were secondary through direct contact with a primary case. Only one patient had hepatitis C; all the others had no underlying disease. Symptoms occurred 4–22 days after receiving the tattoo. Adherence to hygiene among the tattooists was poor. Three of the tattooists in Ohio had recently been in correctional facilities. Some tattooists used homemade equipment including guitar-string tattoo needles and computer ink-jet cartridges for dye.[94]

The Los Angeles County Jail is the largest jail in the United States, with 165 000 incarcerated individuals each year. In 2002, 928 inmates were diagnosed with MRSA wound infections. Sixty-six required hospitalization. At least 10 of them developed invasive disease, including bacteremia, endocarditis or osteomyelitis.[95]

Several publicized reports of CA-MRSA have been in college or professional football teams. Eleven cases of CA-MRSA (type USA300) skin and soft tissue infections, boils being the most common, occurred in 2003 in a Los Angeles team of 107 players. Linemen, who have frequent and aggressive close physical contact during play, were identified as a high-risk subgroup.[96]

The distinctions between CA-MRSA and HA-MRSA are no longer well-defined, because individuals may be colonized in one setting and develop infection in the other. For example, in one study of over 200 patients discharged home from hospital, 49% of them developed new MRSA infections outside the hospital within 18 months after discharge.[97]

Conversely, patients who acquire CA-MRSA in the community may be hospitalized and transmit their strains to other in-patients.

Colonized individuals serve as a reservoir for MRSA. They also have a higher risk for MRSA infection. The commonest site of colonization is the anterior nares. Colonized individuals may also have MRSA on their hands, axillae, anovaginal areas, and (in infants) umbilici.

Clinical Presentation

The clinical presentation of CA-MRSA is usually a boil or abscess, often mistakenly diagnosed both by patients and physicians as a spider-bite.[98] The area is red, swollen, and tender. Drainage may be yellow pus or pus mixed with blood. There may be a surrounding area of cellulitis. Patients with skin and soft tissue infections do not usually appear ill.

If fever, chills or malaise are present or the patient has localizing signs apart from the skin or soft tissue, appropriate investigations should be performed. Blood cultures, radiographs, echocardiography, and vigorous debridement should all be employed when clinically appropriate.[99]

More severe invasive disease with CA-MRSA does occur. Pneumonia, endocarditis, osteomyelitis, necrotizing fasciitis, and death due to overwhelming sepsis have all been reported.

Treatment

First-line treatment in uncomplicated cases may simply be incision and drainage. All abscess or debrided material should be sent for culture and susceptibility testing, without exception. Numerous cases have become unnecessarily complicated because no culture is sent, the presence of MRSA is not suspected, and patients are treated with ineffective antibiotics. If cellulitis is present, or the affected area is phlegmonous and not ready to be drained, antibiotics should be given.

Beta-lactam agents are no longer appropriate empiric therapy for skin and soft tissue infections because of the increasing prevalence of MRSA. Because local antibiotic patterns differ, and certain populations may have resistance to multiple non-beta-lactam antibiotics, the clinician must tailor treatment accordingly.

Several antibiotic options are available for suspected or known CA-MRSA. Double strength trimethoprim-sulfamethoxazole twice daily with or without rifampin 600 mg daily in non-sulfa-allergic patients is first-line therapy. Patients must be warned about sulfonamide toxicity, including fever and rash, which, if it occurs can be severe. Doxycycline 100 mg twice daily may be used, with tetracycline or minocycline as alternatives. Rifampin may be used in combination with sulfonamides or tetracycline, but never alone because of rapid development of resistance.

Ninety-six percent of CA-MRSA strains are sensitive to clindamycin. Clindamycin has the advantage of inhibiting bacterial toxin production including Panton–Valentine leukocidin and other virulence factors. However, if in vitro testing shows an MRSA isolate to be susceptible to clindamycin but resistant to erythromycin, the isolate when exposed to clindamycin may acquire resistance via an inducible macrolide-lincosamide-streptogramin B (iMLSb) phenotype.[100] Also, some theoretical concern exists regarding clindamycin as a bacteriostatic rather than bacteriocidal agent.

At least a third of CA-MRSA strains are resistant to fluoroquinones. Even if in vitro testing indicates susceptibility, resistance to ciprofloxacin can readily develop during treatment.[101] Beta-lactams should not be used, and oral vancomycin is not absorbed and is therefore not appropriate therapy.

Linezolid, a relatively new synthetic antibiotic in the oxazolidinone class, is available for oral as well as intravenous use.[102] It is highly effective but its use is limited by cost, drug interactions, toxicity and development of resistance, and should be limited to patients who either are allergic to or fail older agents.[103] Nasal mupirocin ointment may help to eradicate nasal colonization, but resistance to this topical agent is increasingly reported.

For hospitalized patients, intravenous vancomycin is usually effective but reports of CA-MRSA with intermediate or high MICs to vancomycin are increasing.[104,105] Daptomycin, a cyclic lipopeptide bactericidal antibiotic, can be used for complicated skin and soft tissue infections, bacteremia, and endocarditis due to MRSA. Daptomycin cannot be used for pulmonary infection because it is inactivated by pulmonary surfactant.[106] Tigecycline, a broad-spectrum glycylcycline antibiotic derived from minocycline, is approved for skin and skin-structure infections due to MRSA.[107] Quinupristin–dalfopristin, a streptogramin antibiotic approved for vancomycin-resistant enterococcal infections, has activity against MRSA and vancomycin-intermediate *Staphylococcus aureus* (VISA) isolates.[108]

Group A streptococci are capable of causing skin and soft tissue infections similar to *S. aureus*. If, in addition to MRSA infection, group A streptococcal infection is suspected, a beta-lactam agent should be added to trimethoprim-sulfa, tetracyclines, and fluoroquinolones until culture data are available. Clindamycin, linezolid, daptomycin, quinupristin–dalfopristin, and intravenous vancomycin are all appropriate anti-streptococcal agents.

Prevention of spread of CA-MRSA is based on proper hygiene, hand washing, covering open wounds, not sharing personal razors or towels, and routine cleaning of equipment.

A number of new agents are under development for the treatment of MRSA infections. Dalbavancin is one of several glycopeptide agents being studied. It has a long half-life, permitting weekly dosing.[109] Two new cephalosporins, ceftaroline and ceftobiprole, appear to be effective against MRSA, as does a new carbapenem.[110] A new topical cationic peptide, omiganan pentahydrochloride, is also being studied for MRSA catheter-associated infections.[111] Tefibazumab, a monoclonal antibody, targets a surface protein of *S. aureus*, preventing the organism from binding to human fibrinogen.[112]

GENDER DIFFERENCES

Because of the risk groups of men having sex with men and a male predominance in many other risk groups such as prisoners, military recruits, and football players, more CA-MRSA cases are reported in men than women. While there is no evidence that susceptibility is greater, opportunity for exposure is greater.

MRSA infection outbreaks have been documented in pregnant and postpartum women and in infants in neonatal intensive care units. In one 6 month study of 2963 rectal and vaginal specimens from pregnant women between 35 and 37 weeks gestation, 17% were positive for *S. aureus*. Only 2.8% of the *S. aureus* isolated were MRSA cases (14), an overall MRSA prevalence of 0.05%. Thirteen of the 14 were found to be CA-MRSA based on their susceptibility to several common non-beta-lactam antibiotics.[113] Another study of 288 expectant mothers also found MRSA to be uncommon (2.1%) and no transmission to vaginally delivered newborns occurred.[114]

One study over a 3-year period of more than 5700 mothers showed an overall MRSA colonization rate of 3.5%. No invasive neonatal MRSA infection occurred among study infants. Colonization by MSSA and MRSA were significantly more common among women colonized with group B streptococcus (GBS) than among GBS-negative women.[115]

CA-MRSA has been reported as the etiologic agent in mastitis in postpartum women.[116] One case report demonstrated passage of MRSA to 2 of 3 pre-term triplets from contaminated breast milk delivered by nasogastric tube. One of the infants developed sepsis on day 14 of life, the other was less ill with conjunctivitis. The mother had no clinical evidence of infection.[117]

A retrospective cohort study of 57 pregnant women with CA-MRSA infection at Parkland Memorial Hospital in Dallas, Texas, showed no evidence of increased risk for chorioamnionitis or neonatal sepsis. One-fifth of patients had a history of drug abuse. Co-morbid conditions were HIV infection, asthma, and diabetes. The CA-MRSA infected women were significantly more likely to be multiparous and have had a previous cesarean delivery when compared to the general obstetric population.[118]

EMERGING PRION INFECTION

Variant Creutzfeldt–Jakob Disease

In 1982 Prusiner coined the term *prion* for agents causing transmissible neurodegenerative diseases. A prion is defined as a small misfolded proteinaceous infectious pathogen resistant to normal decontaminating procedures.[119] Diseases caused by prions are unique in that they are sporadic, genetic, and transmissible. Prions do not elicit any specific immunologic response in the host. They are not eradicable by conventional inactivation or sterilization procedures. They have long incubation periods and cause inexorable progression to dementia and usually death.

Classic Creutzfeldt–Jakob disease (CJD) is the most common human prion disease, but remains a rare disease with approximately 1 case per million people worldwide. Ninety percent of cases of CJD are sporadic. A small number of familial cases have been described, allowing greater understanding of the abnormal host protein (PrP) gene in pathogenesis.

The age of onset for CJD is 57–62 years. Equal numbers of males and females are afflicted. CJD may progress in just a few weeks from dementia and myoclonus to akinetic mutism and death in 4 months. Although prion diseases are not contagious through usual human contact, person-to-person spread can occur with direct inoculation or transplantation of infectious material such as dural, liver, and corneal transplants; use of dura mater in embolization procedures; use

of prion-contaminated growth hormone or pituitary gonado-tropin from human cadavers; and contaminated neurosurgical equipment. Nearly 100 cases of iatrogenic CJD have occurred in patients who received cadaveric human growth hormone.[120]

Other known prion disease that affect humans are Gerstmamm–Straussler–Scheinker Syndrome, kuru, and fatal familial insomnia. These, along with CJD and variant CJD, which will be discussed below, share similar neuropathologic features that include neuronal loss, glial cell proliferation, little or no inflammatory response, accumulation of an abnormal host protein (PrP) and presence of small vacuoles in the neuropil. These vacuoles produce a spongiform appearance, leading to the descriptive name of bovine spongiform encephalopathy (BSE), a prion disease in cattle.

Numerous other prion diseases, also known as transmissible spongiform encephalopathies, have been described in animals: scrapie in sheep, feline spongiform encephalopathy, transmissible mink encephalopathy, and chronic wasting disease of deer and elk.

BSE, commonly known as mad-cow disease, is a uniformly fatal neurodegenerative disease in cattle. The affected cows are sometimes referred to as 'downer cows' because they are unable to walk. The largest known outbreak occurred beginning in 1980 in the United Kingdom, where almost 200 000 cattle were infected and almost 5 million cattle were slaughtered in an attempt to eradicate the disease. An exhaustive epidemiologic investigation concluded that normally herbivorous cattle were being fed infected remains of other cattle in the form of meat and bone meal which had been contaminated with bovine brain and spinal cord. Other contributing theories were that a change in British law allowed lower sterilization temperatures for protein meal and that the use of organic solvents in feed preparation had been abandoned. Cattle farming entails using protein supplements as well as antibiotics and hormones. Soya bean meal is used worldwide as a protein supplement, but because soya beans grow poorly in Europe, cattle farmers turned to less expensive forms of protein.

During the mid l990s, cases of what was initially presumed to be CJD began to appear in teenagers and young adults in the UK. However, the early clinical presentation differed from that of classic CJD, beginning with persistent and prominent behavioral and psychiatric disturbances which ranged from anxiety and depression to frank psychosis with visual and auditory hallucinations. These patients were minimally responsive to psychiatric medication. Painful neurologic symptoms such as dysesthesia also occurred early in disease. Onset of hard neurologic signs such as gait disturbance, slurring, and tremor followed several months into the illness. Chorea, dystonia, and myoclonus were seen late in the course. Survival time was longer (14 months on average) in these cases than in classic CJD and median age was much younger (28 years).

By late 1998, a total of 39 cases had been diagnosed. A comparison of biochemical properties of PrP from brains of BSE-infected cattle and patients with this new form of CJD led to the realization that there was a new variant of CJD (vCJD).[121–123]

Although the exact incubation period from BSE prion exposure to the onset of symptoms of vCJD is not known, it can be measured in years.

MRI brain scanning in more than 75% of vCJD patients shows a prominent, symmetrical pulvinar high signal on T2-weighted and/or proton-density-weighted images. This pulvinar sign is not seen in patients with classic CJD.[124] The electroencephalogram is diffusely abnormal and non-specific.

Neuropathologic findings in vCJD differ markedly from those of classic CJD and most resemble the findings in bovine spongiform encephalopathy (BSE). Post-mortem brain examinations from vCJD patients show multiple microscopic, abnormal aggregates surrounded by holes, resulting in a daisy-like appearance described as 'florid plaques.' Immunohistochemical analysis of brain tissue shows marked accumulation of protease-resistant prion protein. Lymphoreticular involvement, such as in the tonsils, Peyer patches and appendix, occurs in vCJD (but not CJD). Therefore, a tonsil biopsy showing a characteristic prion protein by Western blot and immunhistochemistry can help establish the diagnosis of vCJD. Tonsil biopsy has shown 100% specificity and sensitivity in the diagnosis of vCJD.[125]

In 2002, a 22-year-old Florida resident developed symptoms consistent with vCJD. Symptoms began with depression and memory loss that interfered with the patient's job. Within a month, the patient developed involuntary muscle movement, gait disturbance and incontinence. The mother of the patient, a UK resident, took the patient to England where the patient continued to deteriorate, developing confusion, hallucinations, speech abnormalities, bradykinesia, and spasticity. He was referred to the National Prion Clinic in the UK. A Western blot analysis of a tonsil biopsy indicated a protease-resistant prion protein (PrP-res) with the characteristic pattern of vCJD, and other analyses were consistent with 105 other vCJD cases in the UK.[126]

A total of 208 patients from 11 countries have been diagnosed with vCJD between the first case report in 1996 and June 2008. The majority (167) have been from the UK, but a total of 3 cases occurred in US residents. Two of the US residents were likely exposed while living in the UK, the third was likely exposed while living in Saudi Arabia. The Centers for Disease Control and Prevention reports that every case of human vCJD had a history of exposure within a country where there were BSE-infected cattle. The majority of persons with vCJD became infected through consumption of cattle products, but three UK cases have been linked to receiving blood from an asymptomatic, infected donor.[127]

Strong laboratory and epidemiologic evidence suggest that vCJD and BSE are causally linked.[128] Reports of vCJD in humans in the UK following a large epidemic of BSE in cattle with a lag period consistent with the incubation period of prions strongly suggests that vCJD results from bovine

to human transmission of BSE.[129] Most vCJD cases (128 of 138) have occurred in the UK where the highest number of BSE cattle infections have occurred. By 1993, the peak of the BSE epidemic, several hundred thousand BSE-infected cattle might have entered the human food chain. The BSE epidemic in the UK peaked in 1993, and since then the number of BSE cases in cattle has been steadily declining. Fewer cases of cattle BSE may be attributable to bans, beginning in 1988, on use of animal protein in feed.

TREATMENT

To date, all treatment is experimental. Supportive care with antipsychotic medication and sedatives has not been particularly effective for patients with vCJD. Quinacrine, which may prevent conversion of normal prion protein to abnormal prion protein, is currently being evaluated. Pentosan polysulphate may also affect prion production and replication. Flupirtine may have some benefit on cognitive function.[130] Other strategies such as compounds that interact with the abnormal prion structure or immunologic approaches to reduce brain amyloid accumulation are being studied.

GENDER DIFFERENCES

No preponderance in either sex has been reported for vCJD. In other prion diseases, only kuru, a progressive cerebellar ataxia ending in dementia and death related to ritual cannibalism, is known to be more prevalent in women among the Fore tribe in Papua New Guinea.[131]

Approximately 15% of human prion diseases are familial and autosomal dominant. Capability already exists for genetic testing of inherited mutations and polymorphisms in disease-causing genes before clinical disease is present. Ethical issues regarding pre-natal testing will arise for pregnant women with family histories of dementia-associated prion disease. Pre-implantation genetic diagnosis, performed on a single cell from a 3-day-old embryo, is not yet widely available, but is likely to become so. These are difficult issues, yet to be resolved. Geneticists, physicians, medical ethicists, and their patients will have much to consider regarding pre-natal testing for inherited disease.

References

1. Morse SS. Factors in the emergence of infectious diseases. *Emerg Infect Dis.* 1995;1:7–15.
2. Reintjes R, Dedushaj I, Gjini A, *et al*. Tularemia outbreak investigation in Kosovo: case control and environmental studies. *Emerg Infect Dis.* 2002;8:69–73.
3. Gayer M, Legros D, Formenty P, *et al*. Conflict and emerging infectious diseases. *Emerg Infect Dis*. 2007;13:1625–1631.
4. Shirley SM, Kark S. Amassing efforts against alien invasive species in Europe. *PLos Biol.* 2006;4:e279.
5. Jamieson DJ, Jernigan DB, Ellis JE, *et al*. Emerging infections and pregnancy: West Nile virus, monkeypox, severe acute respiratory syndrome, and bioterrorism. *Clin Perinatol.* 2005;32:765–776.
6. Magnus JH, Greene ME, Kishor S. Infectious disease and gender [conference summary]. Emerg. Infect. Dis. 2004 Nov. Available from www.cdc.gov/ncidod/EID/vol10no11/04-0622_06.htm
7. Pinto da Cunha 1997. In: Pan American Health Organization. *Ethnicity and Health.* Washington, DC: PAHO;2002.
8. Cono J, Cragan JD, Jamieson DJ, *et al*. Prophylaxis and treatment of pregnant women for emerging infections and bioterrorism emergencies. *Emerg Infect Dis.* 2006;12(11):1631–1637.
9. Sanz E, Gómez-López T, Martínez-Quintas MJ. Perception of teratogenic risk of common medicines. *Eur J Obstet Gynecol Reprod Biol.* 2001;95:127–131.
10. Jamieson DJ, Ellis JE, Jernigan DB, *et al*. Emerging infectious disease outbreaks: old lessons and new challenges for obstetrician-gynecologists. *Am J Obstet Gynecol.* 2006;194:1546–1555.
11. Centers for Disease Control and Prevention. Updated recommendations for antimicrobial prophylaxis among asymptomatic pregnant women after exposure to *Bacillus anthracis*. *MMWR Morb Mortal Wkly Rep.* 2001;50(43):960.
12. Sawatsky B, Ranadheera C, Weingartl H, *et al*. Hendra and Nipah virus. In: CT Mettenleiter, F Sobrino, eds. *Animal Viruses: Molecular Biology*. Norfolk, UK: Caister Academic Press; 2008.
13. Paton NI, Leo YS, Zaki SR, *et al*. Outbreak of Nipah-virus infection among abattoir workers in Singapore. *Lancet.* 1999; 354:1253–1256.
14. Chua KB, Koh CL, Hooi PS, *et al*. Isolation of Nipah virus from Malaysian Island flying-foxes. *Microbes Infect.* 2002;4:145–151.
15. Anon. Nipah encephalitis outbreak over wide area of western Bangladesh. *Health Sci Bull.* 2004;2(1):7–11.
16. Anon. Nipah virus outbreak from date palm juice. *Health Sci Bull.* 2005;3(4):1–5.
17. Sejvar JJ, Hossain J, Saha SK, *et al*. Long-term neurological and functional outcome in Nipah virus infection. *Ann Neurol.* 2007;62:235.
18. Sarji SA, Abdullah BJ, Goh KJ, *et al*. MR imaging features of Nipah encephalitis. *AJR* 2000;175:437.
19. Wong KT, Shieh WJ, Kumar S, *et al*. Nipah virus infection: pathology and pathogenesis of an emerging paramyxoviral zoonosis. *Am J Pathol.* 2002;161:2153.
20. Charrel RN, de Lamballerie X, Raoult D. Chikungunya outbreaks – the globalization of vectorborne diseases. *N Engl J Med.* 2007;356(8):769–771.
21. Parola P, de Lamballerie X, Jourdan J, *et al*. Novel Chikungunya virus variant in travelers returning form Indian Ocean islands. *Emerg Infect Dis.* 2006;12:1493–1499.
22. Laras K, Sukri NC, Larasati RP, *et al*. Tracking the re-emergence of epidemic chikungunya virus in Indonesia. *Trans R Soc Trop Med Hyg.* 2005;99:128–141.
23. Schuffenecker I, Iteman I, Michaut A, *et al*. Genome microevolution of chikungunya viruses causing the Indian Ocean outbreak. *PLoS Med.* 2006;3:e263.
24. Deller JJ, Russell PK. Chikungunya disease. *Am J Trop Med Hyg.* 1968;17:1007–1011.
25. Kennedy AC, Fleming J, Solomon L. Chikungunya viral arthropathy: a clinical description. *J Rheumatol.* 1980;7:231–236.
26. Fourie ED, Morrison JGL. Rheumatoid arthritis syndrome after chikungunya fever. *S Afr Med J.* 1979;56:130–132.
27. Parida MM, Santhosh SR, Dash PK, *et al*. Rapid and real-time detection of chikungunya virus by reverse transcription loop-mediated isothermal amplification assay. *J Clin Microbiol.* 2007;45:351–357.

28. Carey DE, Myers RM, DeRanitz CM, et al. The 1964 chikungunya epidemic at Vellore, South India, including observations about concurrent dengue. *Trans R Soc Trop Med Hyg.* 1969;63:434–435.

29. Gerardin P, Barau G, Michault A, et al. Multidisciplinary prospective study of mother-to-child chikungunya virus infections on the island of La Reunion. *PLoS Med.* 2008;5(3):e60.

30. Ramful D, Carbonnier M, Pasquet M, et al. Mother-to-child transmission of chikungunya virus infection. *Pediatr Infect Dis J.* 2007;26(9):811–815.

31. Lenglet Y, Barau G, Robillard PY, et al. *J Gynecol Obstet Biol Reprod (Paris).* 2006;35(6):578–583.

32. Parmenter CA, Yates TL, Parmenter RR, et al. Statistical sensitivity for detection of spatial and temporal patterns in rodent population densities. *Emerg Infect Dis.* 1999;5(1):118–125.

33. Ma RM, Xiao H, Jing XT, et al. Hemorrhagic fever with renal syndrome presenting with intrauterine fetal death. A case report. *J Reprod Med.* 2003;48(8):661–664.

34. Howard MJ, Doyle TJ, Koster FT, et al. Hantavirus pulmonary syndrome in pregnancy. *Clin Infect Dis.* 1999;29(6):1538–1544.

35. Jezek Z, Fenner F. *Human Monkeypox.* New York, NY: Karger; 1988.

36. Heymann DL, Szczeniowski M, Esteves K. Re-emergence of monkeypox in Africa: a review of the past six years. *Br Med Bull.* 1998;54:693–702.

37. Hutin YJ, Williams RJ, Malfait P, et al. Outbreak of human monkeypox, Democratic Republic of Congo, 1996 to 1997. *Emerg Infect Dis.* 2001;7:434–438.

38. Reed KD, Melski JW, Graham MB, et al. The detection of monkeypox in humans in the Western Hemisphere. *N Engl J Med.* 2004;350:342–350.

39. Ligon BL. Monkeypox: a review of the history and emergence in the Western hemisphere. *Semin Pediatr Infect Dis.* 2004;15(4):280–287.

40. DeClercq E. Cidofovir in the treatment of poxvirus infections. *Antiviral Res.* 2002;55:1–28.

41. Peterson LR, Marfin AA, Gubler DJ. West Nile virus. *JAMA.* 2003;290(4):524–528.

42. Nash D, Mostashari F, Fine A, et al. The outbreak of West Nile virus infection in the New York City area in 1999. *N Engl J Med.* 2001;344:1807–1814.

43. Petersen LR, Marfin AA. West Nile virus: a primer for the clinician. *Ann Intern Med.* 2002;137:173–179.

44. Tsai TF, Popovici F, Cernescu C, et al. West Nile encephalitis epidemic in southeastern Romania. *Lancet.* 1998;352:767–771.

45. Bode AV, Sejvar JJ, Pape WJ, et al. West Nile virus disease: a descriptive study of 228 patients hospitalized in a 4-county region of Colorado in 2003. *Clin Infect Dis.* 2006;42:1234–1240.

46. Centers for Disease Control and Prevention. West Nile virus infections in organ transplant recipients – New York and Pennsylvania August–September 2005. *MMWR Morb Mortal Wkly Rep.* 2005;54:1021–1023.

47. Centers for Disease Control and Prevention. West Nile virus activity – United States, November 20–25, 2003. *MMWR Morb Mortal Wkly Rep.* 2003;52:1160.

48. Kerdpanich A, Watanaveeradej V, Samakoses R, et al. Perinatal dengue infection. *Southeast Asian J Trop Med Public Health.* 2001;32:488–493.

49. Centers for Disease Control and Prevention. Intrauterine West Nile virus infection – New York 2002. *MMWR Morb Mortal Wkly Rep.* 2002;51(50):1135–1136.

50. Chapa JB, Ahn JT, DiGiovanni LM, et al. West Nile virus encephalitis during pregnancy. *Obstet Gynecol.* 2003;102(2):229–231.

51. O'Leary DR, Kuhn S, Kniss KL, et al. Birth outcomes following West Nile virus infection of pregnant women in the United States: 2003–2004. *Pediatrics.* 2006;117:e537–e545.

52. Centers for Disease Control and Prevention. Interim guidelines for the evaluation of infants born to mothers infected with West Nile virus during pregnancy. *MMWR Morb Mortal Wkly Rep.* 2004;53(7):154–157.

53. Centers for Disease Control and Prevention. Possible West Nile virus transmission to an infant through breast-feeding – Michigan. *MMWR Morb Mortal Wkly Rep.* 2002;51(39):877–888.

54. Dolan M, Wong M, Regnery R, et al. Syndrome of *Rochalimae henselae* adenitis suggesting cat scratch disease. *Ann Intern Med.* 1993;118:331–336.

55. Maguina C, Garcia P, Gotuzzo E, Cordero L, Spach D. Bartonellosis (Carrion's Disease) in the modern era. *Clin Infect Dis.* 2001;33:772–779.

56. Anderson BE, Neuman MA. *Bartonella* spp. as emerging human pathogens. *Clin Microbiol Rev.* 1997;10(2):203–219.

57. Jackson LA, Perkins BA, Wenger JD. Cat scratch disease in the United States: an analysis of three national databases. *Am J Public Health.* 1993;83(12):1707–1711.

58. Maurin M, Raoult D. Bartonella (Rochalimae) quintana infections. *Clin Microbiol Rev.* 1996;9:275–292.

59. Raoult D, Fournier PE, Drancourt M, et al. Diagnosis of 22 new cases of Bartonella endocarditis. *Ann Intern Med.* 1996;125:646–652.

60. Kreisel D, Pasque MK, Damiano RJ, et al. Bartonella species-induced prosthetic valve endocarditis associated with rapid progression of valvular stenosis. *J Thorac Cardiovasc Surg.* 2005;130:567–568.

61. Kemper C, Lombard C, Deresinski S, et al. Visceral bacillary epithelioid angiomatosis: possible manifestations of disseminated cat scratch disease in the immunocompromised host: a report of two cases. *Am J Med.* 1990;89:216–222.

62. Spach DH, Koehler JE. Bartonella-associated infections. *Infect Dis Clin North Am.* 1998;12:137–155.

63. Perkocha L, Geaghan B, Yen T, et al. Clinical and pathological features of bacillary peliosis hepatis in association with human immunodeficiency virus infection. *N Engl J Med.* 1990;323:1581–1586.

64. Huarcaya E, Maguina C, Best I, et al. Immunological response in cases of complicated and uncomplicated bartonellosis during pregnancy. *Rev Inst Med Trop Sao Paulo.* 2007;49(5):335–337.

65. Raoult D, Fournier PE, Vandnesch F, et al. Outcome and treatment of Bartonella endocarditis. *Arch Intern Med.* 2003;163:226–230.

66. Rolain J, Brouqui P, Koehler J, et al. Recommendations for treatment of human infections caused by *Bartonella* species. *Antimicrob Agents Chemother.* 2004;48:1921–1933.

67. Black RE, Brown KH, Becker S, et al. Contamination of weaning foods and transmission of enterotoxigenic *Escherichia coli* diarrhoea in children in rural Bangladesh. *Trans R Soc Trop Med Hyg.* 1982;76:259–264.

68. Devasia RA, Jones TF, Ward J, et al. Endemically acquired foodborne outbreak of enterotoxin-producing *Escherichia coli* serotype 0169:H41. *Am J Med.* 2006;119:168.

69. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food – selected

sites, United States, 2003. MMWR Morb. Mortal. Wkly. Rep. 53 (2004) 338.

70. Johnson KE, Thorpe CM, Sears CL. The emerging importance of non-0157 Shiga toxin producing *Escherichia coli*. *Clin Infect Dis.* 2006;43:1587–1595.

71. Cody SH, Glynn MK, Farrar JA, *et al*. An outbreak of *Escherichia coli* 0157:H7 infection from unpasteurized commercial apple juice. *Ann Intern Med*. 1999;130:202–209.

72. Frenzen PD, Drake A, Angulo FJ. Economic cost of illness due to *Escherichia coli* 0157 infections in the United States. *Food Prot*. 2005;68(12):2623–2630.

73. Slutsker L, Ries AA, Greene KD, *et al*. Escherichia coli 0157:H7 diarrhea in the United States: clinical and epidemiologic features. *Ann Intern Med*. 1997;126:505–513.

74. Centers for Disease Control and Prevention. Investigation of outbreak of human infections caused by *E. coli* 0157:H7. Atlanta, GA: CDC; July 15, 2008.

75. Waters JR, Sharp JC, Dev VJ. Infection caused by *E. coli* 0157 in Alberta, Canada, and in Scotland: a five-year review, 1987–1991. *Clin Infect Dis*. 1994;19:834–843.

76. Boyce TG, Swerdlow DL, Griffin PM. Current concepts: *Escherichia coli* 0157:H7 and the hemolytic-uremic syndrome. *N Engl J Med*. 1995;333:364–368.

77. Elliott EJ, Robins-Browne RM, O'Loughlin EV, *et al*. Nationwide study of haemolytic uraemic syndrome: clinical, microbiological, and epidemiological features. *Arch Dis Child*. 2001;85:125–131.

78. Wong CS, Jelacic S, Habeeb RL, *et al*. The risk of the hemolytic-uremic syndrome after antibiotic treatment of *E. coli* 0157 infections. *N Engl J Med*. 2000;342:1930–1936.

79. Clemens JD, Rao MR, Chakraborty J, *et al*. Life-threatening enterotoxigenic *E. coli* diarrhea in Bangladeshi infants and children. *Pediatrics*. 1997;100(6):e2.

80. Clemens JD, Stanton BF, Stoll B. Breast-feeding as a determinant of severity in shigellosis: evidence for protection throughout the first three years of life in Bangladeshi children. *Am J Epidemiol*. 1986;123:710–720.

81. Enright MC. The evolution of a resistant pathogen – the age of MRSA. *Curr Opin Pharmacol*. 2003;3:474–479.

82. Smith MA, Mathewson JJ, Ulert IA, *et al*. Contaminated stethoscopes revisited. *Arch Intern Med*. 1996;156:82–84.

83. Boyce JM, Havill NL, Otter JA, *et al*. Widespread environmental contamination associated with patients with diarrhea and methicillin-resistant *S. aureus* colonization of the gastrointestinal tract. *Infect Control Hosp Epidemiol*. 2007;28:1142–1147.

84. Saravolatz LD, Markowitz N, Arking L, *et al*. Methicillin-resistant *S. aureus*. Epidemiologic observations during a community-acquired outbreak. *Ann Intern Med*. 1982;96:11–16.

85. Baba T, Takeuchi F, Kuroda M, *et al*. Genome and virulence determinants of high virulence CA-MRSA. *Lancet*. 2002;359(9320):1819–1827.

86. King MD, Humphrey BJ, Wang YF, *et al*. Emergence of community acquired MRSA USA300 clone as the predominant cause of skin and soft-tissue infections. *Ann Intern Med*. 2006;114:309–317.

87. Fridkin SK, Hageman JC, Morrison M, *et al*. Methicillin-resistant *Staphylococcus aureus* disease in three communities. *N Engl J Med*. 2005;352:1436–1444.

88. Frazee BW, Lynn J, Charlebois ED, Lambert L, *et al*. High prevalence of methicillin-resistant Staphylococcus aureus in emergency department skin and soft tissue infections. *Ann Emerg Med*. 2005;45(3):311–320.

89. Moran GJ, Krishnadasan A, Gorwitz RJ, *et al*. Methicillin-resistant *S. aureus* infections among patients in the emergency department. *N Engl J Med*. 2006;355(7):666–674.

90. Diep BA, Chambers HF, Graber CJ, *et al*. Emergence of multidrug-resistant, community-associated methicillin-resistant *S. aureus* clone USA300 in men who have sex with men. *Ann Intern Med*. 2008;148:249–257.

91. Mathews WC, Caperna JC, Barber RE, *et al*. Incidence of and risk factors for clinically significant MRSA infection in a cohort of HIV-infected adults. *J Acquir Immune Defic Syndr*. 2005;40:155–160.

92. Miller LG, Perdreau-Remington F, Bayer AS, *et al*. Clinical and epidemiologic characteristics cannot distinguish community-associated methicillin-resistant *S. aureus* infection from methicillin-susceptible *S. aureus* infection: a prospective investigation. *Clin Infect Dis*. 2007;44:471–482.

93. Centers for Disease Control and Prevention. Four pediatric deaths from community-acquired methicillin resistant *S. aureus* – Minnesota and North Dakota, 1997–1999. *MMWR Morb Mortal Wkly Rep*. 1999;48:707.

94. Centers for Disease Control and Prevention. Methicillin-resistant *Staphylococcus aureus* skin infections among tattoo recipients – Ohio, Kentucky, and Vermont 2004–2005. *MMWR Morb Mortal Wkly Rep*. 2006;55(24):677.

95. Centers for Disease Control and Prevention. Public health dispatch: Outbreaks of community-associated methicillin-resistant *Staphylococcus aureus* skin infections – Los Angeles County, California, 2002–2003. *MMWR Morb Mortal Wkly Rep*. 2003;52(05):88.

96. Nguyen DM, Mascola L, Brancoft E. Recurring methicillin-resistant *Staphylococcus aureus* infections in a football team. *Emerg Infect Dis*. 2005;11(4):526–532.

97. Huang SS, Platt R. Risk of methicillin-resistant *S. aureus* infection after previous infection or colonization. *Clin Infect Dis*. 2003;36:281–285.

98. Dominguez TJ. It's not a spider bite, it's community-acquired methicillin-resistant *S. aureus*. *J Am Board Fam Pract*. 2004;17:220–226.

99. Miller LG, Perdrea-Remington F, Rieg G, *et al*. Necrotizing fasciitis caused by community-associated methicillin-resistant *S. aureus* in Los Angeles. *N Engl J Med*. 2005;352:1445–1453.

100. Siberry GK, Tekle T, Carroll K, *et al*. Failure of clindamycin treatment of methicillin-resistant *Staphylococcus aureus* expressing inducible clindamycin resistance in vitro. *Clin Infect Dis*. 2003;37:1257–1260.

101. Trucksis M, Hooper DC, Wolfson JS. Emerging resistance to fluoroquinolones in staphylococci: an alert. *Ann Intern Med*. 1991;114:424–426.

102. Moellering RC. Linezolid: the first oxazolidinone antimicrobial. *Ann Intern Med*. 2003;138:135–142.

103. Stevens DL, Herr D, Lampiris H, *et al*. Linezolid versus vancomycin for the treatment of methicillin-resistant *Staphylococcus aureus* infections. *Clin Infect Dis*. 2002;34:1481–1490.

104. Soriano A, Marco F, Martinez J, *et al*. Influence of vancomycin minimum inhibitory concentration on the treatment of MRSA bacteremia. *Clin Infect Dis.* 2008;46(2):193–200.

105. Schairer J, Sankri-Tarbichi AG, Fairfax MR, *et al*. Methicillin-resistant *Staphylococcus aureus* infection with intermediate sensitivity to vancomycin: a case report and literature review. *J Intensive Care Med.* 2008 Aug 12, [Epub ahead of print].

106. Weis F, Beiras-Fernandez A, Schelling G. Daptomycin, a lipopeptide antibiotic in clinical practice. *Curr Opin Investig Drugs.* 2008;9(8):879–884.

107. Tigecycline, Med. Lett. Drugs Ther. 47 (2005) 73–74.

108. Batts DH, Lavin BS, Eliopoulos GM. Quinupristin/dalfopristin and linezolid: spectrum of activity and potential roles in therapy – a status report. *Curr Clin Top Infect Dis.* 2001;21:227–251.

109. Raad I, Darouiche R, Vazquez J, *et al*. Efficacy and safety of weekly dalbavancin therapy for catheter-related bloodstream infection caused by gram-positive pathogens. *Clin Infect Dis.* 2005;40:374–380.

110. Sader HS, Fritsche TR, Jones RN. Antimicrobial activity of ceftaroline and ME1036 tested against clinical strains of C-MRSA. *Antimicrob Agents Chemother.* 2008;52(3):1153–1155.

111. Fritsche TR, Rhomberg PR, Sader HS, *et al*. In vitro activity of omiganan pentahydrochloride tested against vancomycin-tolerant, -intermediate and -resistant *S. aureus*. *Diagn Microbiol Infect Dis.* 2008;60(4):399–403.

112. Weems JJ, Steinberg JP, Filler S, *et al*. Phase II randomized, double-blind, multicenter study comparing the safety and pharmacokinetics of tefibazumab to placebo for treatment of *Staphylococcus aureus* bacteremia. *Antimicrob Agents Chemother.* 2006;50:2751–2755.

113. Chen KT, Huard RC, Della-Latta P, *et al*. Prevalence of methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* in pregnant women. *Obstet Gynecol.* 2006;108:482–487.

114. M. Reusch, P. Ghosh, C. Ham, *et al*. Prevalence of MRSA colonization in peripartum mothers and their newborn infants. Scand. J. Infect. Dis. 40 (8) 667–671.

115. Andrews WW, Schelonka R, Waites K, *et al*. Genital tract methicillin-resistant *Staphylococcus aureus*: risk of vertical transmission in pregnant women. *Obstet Gynecol.* 2008;111(1):113–118.

116. Saiman L, O'Keefe M, Graham PL, *et al*. Hospital transmission of CA-MRSA among postpartum women. *Clin Infect Dis.* 2003;37:1313–1319.

117. Behari P, Englund J, Alcasid G, *et al*. Transmission of methicillin-resistant *Staphylococcus aureus* to preterm infants through breast milk. *Infect Control Hosp Epidemiol.* 2004;25(9):778–780.

118. Laibi VR, Sheffield JS, Roberts S, *et al*. Clinical presentation of CA-MRSA in pregnancy. *Obstet Gynecol.* 2005;106:461–465.

119. Pruziner SB. Novel proteinacious infectious particles cause scrapie. *Science.* 1982;216:136–144.

120. Brown P, Preece MA, Will RG. Friendly fire in medicine: hormones, homografts and Creutfeldt–Jakob disease. *Lancet.* 1992;340:24–27.

121. Collinge J. Variant Creutzfeldt–Jacob disease. *Lancet.* 1999;354(9175):317–323.

122. Will RG, Ironside JW, Zeidler M, *et al*. A new variant of Creutzfeldt–Jakob disease in the UK. *Lancet.* 1996;347:921–925.

123. Spencer MD, Knight RS, Will RG. First hundred cases of variant Creutzfeldt–Jakob disease: retrospective case note review of early psychiatric and neurological features. *BMJ.* 2002;324:1479–1482.

124. Zeidler M, Sellar RJ, Collie DA, *et al*. The pulivinar sign on magnetic resonance imaging in variant Creutfeldt–Jakob disease. *Lancet.* 2000;355:1412–1418.

125. Hill AF, Butterworth RJ, Joiner S, *et al*. Investigation of variant Creutzfeldt–Jakob disease and other human prion diseases with tonsil biopsy samples. *Lancet.* 1999;353:183–189.

126. Department of Health. *Monthly Creutzfeldt–Jakob disease statistics*. London: DoH; October 2002.

127. Centers for Disease Control and Prevention. *Fact Sheet: Variant CJD*. Atlanta, GA: CDC; 2007.

128. Belay ED, Potter ME, Schonberger LB. Relationship between transmissible spongiform encephalopathies in animals and humans. *Task Force Report for the Council for Agricultural Science and Technology*. Washington, DC: Council for Agricultural Science and Technology. 2000:136.

129. Hill AF, Desbruslais M, Joiner S, *et al*. The same prion strain causes vCJD and BSE. *Nature.* 1997;389:448–450.

130. Otto M, Cepek L, Ratzka P, *et al*. Efficacy of flupirtine on cognitive function in patients with CJD: a double-blind study. *Neurology.* 2004;62(5):714–718.

131. Gajdusek DC. Unconventional viruses and origin and disappearance of kuru. *Science.* 1977;197:943–960.