Ebola Virus Disease

Steven J. Hatfill, M.D., M.Sc., M.Med.
Trevor Nordin
Geoffrey L. Shapiro

ABSTRACT

The Ebola virus (EBOV) is the cause of an emerging disease that may be harbored across a much larger geographic range then previously assumed. The present large outbreak of EBOV illustrates how an emerging disease may start and spread, the difficulty of its containment, and the sociopolitical factors that may appear during an emerging disease outbreak.

EBOV targets the body's immune system, causes harmful inflammatory responses such as a cytokine storm, leads to apoptosis of many cell types including vascular endothelium and lymphocytes, and in fatal cases terminates in the multiple organ dysfunction syndrome (MODS) and multiple organ failure.

Uncertainties in the scientific data on the transmission of this virus raise concerns about current published Centers for Disease Control and Prevention (CDC) guidance for health worker protection.

Introduction to Emerging Infectious Disease

An emerging disease is an infectious disease that has newly appeared in a population, or is rapidly increasing in incidence or geographic range. During the past 30 years some 41 new emerging infectious organisms or strains have jumped from their animal hosts into humans, and it is almost certain that other previously unknown infectious diseases will emerge into the human population in the near future.

There are approximately 1,407 organisms (fungi, bacteria, parasites, protozoa and viruses) that can infect humans. Roughly 58% of these are considered to be animal diseases, and most of these have an Old World origin as a result of man's development of agriculture and animal domestication. Over time, some of these infections have become confined to humans and absent from animals.[1,2] In addition, there are now some 177 pathogens considered to cause newly emerging or re-emerging diseases. Examples in the period 2012–2014 include the Middle East respiratory syndrome coronavirus (MERS-CoV), the Bas-Congo rhabdovirus that causes hemorrhagic fever, the Sierra Leone-Liberia Ebola outbreak, the continuing Kasai Oriental Province monkeypox, cases of chikungunya virus and dengue virus in Florida, the outbreak of serious enterovirus respiratory disease in the U.S. Midwest, and a case of plague in China.

The RNA viruses are particularly prone to emergence because of their inherent rapid mutation rate, but they do not exist in a vacuum.[3] Risk of human infection depends on the virus, human/animal interactions, and human population migration. Human population in Africa has doubled in 27 years while native animal habitats have been destroyed or fragmented, and wild animal food sources made less diverse. In addition, intensive domestic animal breeding facilitates viral mixing and increased targets for spillover from wild viruses.[4,5]

The risk to man continues to grow globally. At present, an average of three to four new pathogen species are detected in the human population every year.[6]

Ebola and Other Filoviruses

The Ebola virus (EBOV) has a non-segmented linear negative-sense RNA genome of approximately 19,000 base pairs. It encodes seven structural proteins: nucleoprotein (NP), polymerase cofactor (VP35), (VP40), GP, transcription activator (VP30), VP24, and RNA polymerase (L).

Taxonomically, EBOV belongs to the new *Filoviridae* virus family established in 1982, and amended in 2011.[7,8] Filoviruses form filamentous viral particles (see Figure 1). The *Filoviridae* include the original Ebola virus and Marburg virus that cause similar lethal hemorrhagic fevers in humans and nonhuman primates. Both viruses are World Health Organization (WHO) risk group 4 pathogens, requiring biosafety level 4 (BSL-4) containment.

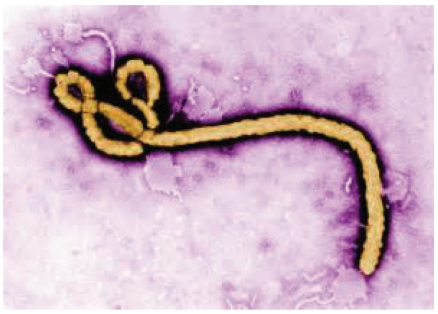

Figure 1. Ebola Zaire Filovirus (electron micrograph, Fred Murphy, U.S. CDC)

One recent addition to the *Filoviridae* family is the genus *Cuevavirus*, which includes only one species at this time, Lloviu cuevavirus, the Lloviu virus (LLOV).[9] Isolated from a dead insectivorous bat in Spain, LLOV is a distant relative of the Ebola and Marburg viruses. This new filovirus is endemic in France, Portugal, and Spain, and its genome differs from that of Marburg virus by 50% or more at the nucleotide level.

Origin of the Filoviruses

The first documented outbreaks of hemorrhagic fever caused by a filovirus occurred more than 45 years ago, but the amount of virus genetic diversity in the strains indicate that the *Filoviridae* are much older.

The rate of genetic change in the *Filoviridae* is 100 times slower than in influenza A in humans, but roughly the same magnitude as in hepatitis B. Extrapolating backwards using these rates indicates that EBOV and Marburg virus diverged from a common viral ancestor approximately 700–850 years ago.[10] Other estimates place this divergence at several thousand years ago.[11]

The most recent common ancestry can be traced back only within the last 50 years for Reston ebolavirus and Zaire ebolavirus species, suggesting the existence of genetic bottlenecks. Examination of the *Filoviridae* genetic sequence data, including the recently described LLOV, show these viruses all share another common ancestor originating approximately 10,000 years ago (see Figure 2).[10]

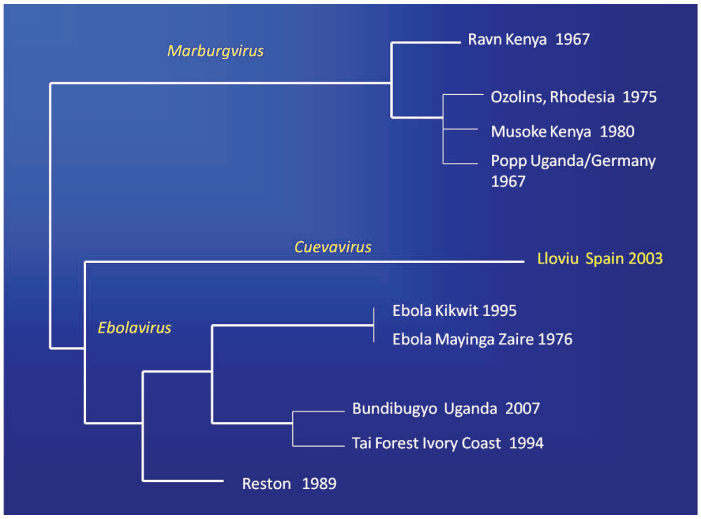

Figure 2. Family Tree Based on Gene Sequences in Filoviruses (adapted from Carroll et al.)[10]

However, the filovirus lineage goes back much farther. Paleoviruses (genomic fossils) of viruses that are closely related to the EBOV have been found in the genome of mammals. Sections of the NP and the L genes from EBOV have been identified as endogenous sequences in the genomes of bats[12] and the Chinese hamster.[13] It is thought that these gene sequences are at least tens of millions of years old.

Another related disease, snake inclusion body disease (IBD), is a fatal infectious viral disease of snakes typified by behavioral abnormalities, wasting, and secondary infections. Viral isolation studies have characterized a divergent arenavirus associated with the disease that has envelope glycoproteins more similar to those of filoviruses than to those of other arenaviruses.[14]

History of Ebola Outbreaks

The first recognized filovirus outbreak was in Europe with the Marburg virus in 1967. This occurred in Germany and Yugoslavia among vaccine workers handling tissue specimens from imported African green monkeys. Thirty-one people were infected, with a 23% fatality rate. The virus was named after the German town of Marburg, where the German outbreak occurred.

The first outbreak of EBOV happened almost 10 years later in 1976, with simultaneous Ebola strain outbreaks in Yambuku in northern Zaire (now the Democratic Republic of Congo, DRC), and Southern Sudan. The Zaire virus was named after the Ebola River. It caused 500 diagnosed cases with a 92% fatality rate. The simultaneous Sudan ebolavirus outbreak had a 50% fatality rate.[15,16]

In 1990, a mysterious outbreak of fatal illness occurred in Philippine-imported Cynomolgus crab-eating macaque monkeys at a primate holding facility in Reston, Va. Researchers eventually determined this was a strain of EBOV of Asian origin, and they designated it as the Reston ebolavirus (REBOV). REBOV is the only species of EBOV that does not cause disease in humans, and it has provided major clues to the pathogenesis of the lethal ebolavirus strains.[17-19]

Repeated outbreaks and new strains of EBOV include the Ivory Coast ebolavirus, named for a single human case that occurred in 1994.[20] Outbreaks have also decimated mountain gorilla populations, and multiple EBOV outbreaks have occurred in Gabon between 1994 and 1996 as well as small outbreaks in other parts of Africa.[21-26]

In 2007 a new strain of EBOV emerged in Western Uganda in the township of Bundibugyo.[27] This marked the discovery of a fifth strain of the virus, the Bundibugyo ebolavirus. This outbreak lasted 2 months, with 149 suspected cases and 37 deaths. On Mar 21, 2014, a large outbreak began in Guinea, Liberia, Nigeria, and Sierra Leone. This is the first large outbreak in West Africa.[28]

During EBOV's known history, a few secondary cases have entered South Africa (with one health worker death), Europe, and most recently the U.S.

Filovirus Ecology

Early Ebola infectivity studies were conducted to look for potential hosts in the representatives of different classes and orders of vertebrates, invertebrates, potential African arthropod vectors, and even plants. Replicating EBOV was recovered from experimentally infected insectivorous bats (*Tadarida* spp.) in the laboratory, but no histopathologic lesions were observed, and there was no evidence of widespread tissue infection. In one experiment, the virus was seen in the endothelial cells of lung tissue of a bat sacrificed on day 8 post inoculation. In addition, virus was recovered from the feces of a fruit bat on day 21 post inoculation.[29] This is significant, as the presence of EBOV implies that respiratory, oral, or guano spread of infection could occur in the confined spaces where bats roost. Isolation of the virus from bat feces suggests the existence of a mechanism for Ebola transmission to other animals. This study spurred further efforts to demonstrate bats as a reservoir host for the *Filoviridae*.

Bats in the order *Chiroptera* are the most diverse and

widely distributed nonhuman mammalian species in the world. Several bat species are reservoir hosts of zoonotic viruses. This includes both insectivorous bat species and some megachiropteran fruit bats.[30] Bats have been shown to be natural reservoirs of a variety of emerging and highly virulent viruses such as Hendra, Malayan Nipah, SARS (severe acute respiratory syndrome), MERS, and the Australian bat Lyssa viruses, and there is a high rate of detection of a large number of previously unknown viral sequences in bat specimens. Viral isolation from a common species of fruit bat (*Rousettus aegyptiacus*) in Gabon has shown bats to be a primary reservoir host for the Marburg virus.[31]

Sampling studies conducted between 2001 and 2003 in Gabon and the DRC found evidence of asymptomatic infection by EBOV in three species of fruit bat, indicating that these animals are likely the reservoir for this deadly virus.[32]

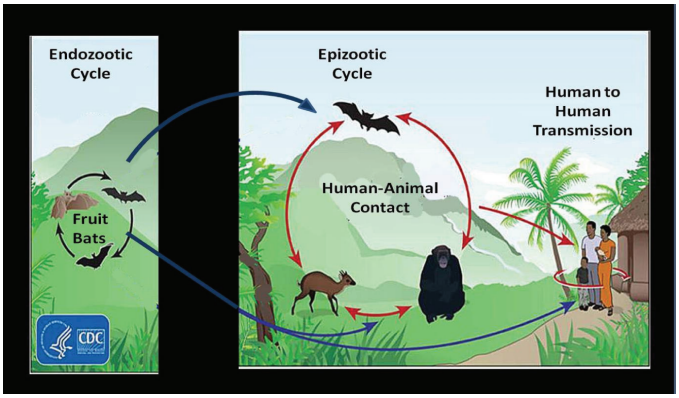

Figure 3. Cycle of Transmission
[Source: U.S. Centers for Disease Control and Prevention]

Indirect evidence also exists. Between May and November 2007, EBOV re-emerged in the Occidental Kasaï province of the DRC, causing 186 deaths. The local African population described a massive annual fruit bat migration by the Lulua River, and these were extensively hunted by villagers as a source of protein. Contact tracing demonstrated that the putative first human victim in this outbreak bought freshly killed bats from hunters to eat.[33]

The association of select bat species with EBOV may have ramifications for future outbreaks. During April 2010–March 2011, scientists tested 276 bats from the Faridpur, Rajbari, Lalmonirhat, and Comilla districts in Bangladesh. Five (3.5%) bats were positive for antibodies against Ebola Zaire and Reston viruses; no virus was detected by polymerase chain reaction (PCR). This is a disturbing indication that the filoviruses might be harbored across a much larger geographic range then previously assumed.[34] Unconfirmed reports of Ebola Zaire in nonhuman primates in North Borneo require confirmation.

Humans and great apes are end hosts for EBOV,[35] and while fruit bats appear to be a major natural reservoir, the involvement of other species in Ebola transmission remains unclear. Dogs and pigs are so far the only domestic animals identified that can be infected with Ebola Zaire.[36]

In 2009, a survey in Gabon found a greater than 30% seroprevalence for EBOV in dogs during the 2001–2002 outbreak. As noted below, pigs in the Philippines have been reported to be infected with the nonhuman pathogen Ebola Reston virus, suggesting that other interim or amplifying hosts may exist.[37] While canine infections appear to be asymptomatic,[36] pigs experimentally infected with the lethal Ebola Zaire can develop clinical disease and can transmit this lethal virus to naïve pigs and macaques; however, any porcine role during the EBOV outbreaks in Africa still requires clarification.[37]

If domestic animals do indeed prove to play a role in transmission of lethal African Ebola strains to man through viral shedding, it may be necessary to develop veterinary vaccines.

The cycle of transmission is illustrated in Figure3.

The Pathogenesis of Ebola Virus Disease (EVD)

Amid the current background of inconclusive data concerning the timing of viral shedding via the skin and body fluids and the experimental demonstration of fomite and aerosol droplet transmission of the *Filoviridae*, it is useful to examine the pathogenesis of Ebola virus disease (EVD).

EBOV Binding and Entry

The primary target of the Ebola virus is the mononuclear phagocytic system. As the virus spreads through the organism, the spectrum of target cells increases to include endothelial cells, fibroblasts, hepatocytes, and many other cells.[38] There is significant evidence that the Ebola outer glycoprotein (GP) plays an important role in this cell tropism, and the spread and pathogenesis of infection.[39]

Initially the Ebola viral spike glycoprotein (GP) mediates viral entry into both macrophages and dendritic antigen presenting cells (APC). Filovirus entry is by this spike glycoprotein binding to receptors on the target cell's surface.[39] Unedited GP mRNA yields the nonstructural glycoprotein sGP, which is extensively secreted in a soluble form from infected cells. As will be discussed later, this sGP appears to exert a profound suppression of the body's defensive antibody response.[39] Different strains of EBOV show variations in the processing of the cleavability of the glycoprotein, and this may account for differences in pathogenicity, as has been observed with influenza viruses and paramyxoviruses.

Cell Surface Receptors for the EBOV Glycoprotein

The Niemann–Pick C1 (NPC1) protein is a cholesterol transporter protein. This appears to be the main receptor for Ebola GP binding and entry of Ebola virions into the host cell for replication.[40]

The second candidate EBOV GP receptor is the TIM-1 (T-cell immunoglobulin and mucin domain-1) protein. TIM-

1 was shown to bind to the receptor binding domain of the EBOV glycoprotein, to increase the receptivity of Vero cells. Silencing its effect with siRNA prevented infection of Vero cells. A monoclonal antibody against the IgV domain of TIM-1 blocked EBOV binding and infection.[41]

Together, these studies suggest that cells and tissues with high NPC1 and TIM-1 expression may be major sites of viral infection and sites of significant shedding of mature infectious viral progeny. This includes not only the antigen-presenting cells and macrophages in the airway and skin, but also from tissues with high TIM-1 expression levels that are known to be seriously affected by EBOV lysis (trachea, cornea, and conjunctiva). This conceivably could be a factor in human-to-human virus transmission events and transmission from infected patients to medical workers.

Initial Viral Infection

Mucous membrane antigen-presenting cells (APCs) provide the initial targets for filovirus infection. Macrophages, monocytes, Kupffer cells, and dendritic cells (DCs) are all targets of filovirus infection in vivo.[38,42,43] The most specialized APCs in the body are the dendritic cells found in tissues that are in contact with the external environment, such as the skin (Langerhans cells) and the inner lining of the nose, lungs, stomach, and intestines. They can also be found in an immature state in the blood. Once activated, they migrate to the lymph nodes, where they interact with T cells and B cells to initiate and shape the adaptive immune response.

Infection of these cell types is accompanied by significant progeny virus production and the release of a variety of pro-inflammatory mediators that increase monocyte production, and lymphocyte and macrophage extravasation and migration into inflamed tissue. This provides more targets for infection. Fatal EBOV infections are associated with the hypersecretion of numerous cytokines (interleukins IL-1β, IL-1RA, IL-6, IL-8, IL-15 and IL-16), chemokines, and growth factors (MIP-1α, MIP-1β, MCP-1, M-CSF, MIF, IP-10, GRO-α and eotaxin).[42] This is not accompanied by an increase in interferon IFNα2 secretion.

Initially the pro-inflammatory response promotes vascular permeability and expression of a transmembrane glycoprotein called tissue factor (TF). However, levels of these mediators rise rapidly after the onset of symptoms in non-survivors, and inflammatory mediators enter the general circulation reaching very high levels in the two days before death, creating a "cytokine storm." Shortly before death, average pro-inflammatory mediator levels range between 5 and 1,000 times higher than those observed in both healthy individuals and survivors.[44-45]

Systemic Inflammatory Response Syndrome (SIRS)

Clinical and laboratory data suggest that EVD patients suffer from the systemic inflammatory response syndrome (SIRS), a generalized inflammatory state affecting the small blood vessels of the body, first described in 1983. This is an immune response to a severe infection, and is characterized as a subset of a "cytokine storm" with dysregulation and an abnormal production of select lymphokines and cytokines that flood the systemic circulation. This precipitates an inflammatory state of the small blood vessels,[46] with the vascular endothelium responding by the production of nitric oxide. Capillary beds throughout the body "leak," causing hypotension that once initiated may be progressively refractory to intravenous fluid therapy and vasopressors.[47]

The abnormal systemic inflammation of the small blood vessels may progress to an active process of fibrin deposition, platelet aggregation, coagulopathies, and liposomal release from stagnant leukocytes inside the vascular system.[48] The inflamed capillary beds may become progressively occluded by small fibrin microthrombi, leading to organ microcirculatory damage and cellular hypoxemia. This is accompanied by the release of progressively increasing amounts of TF into the circulation from dying and necrotic tissue areas.[49]

Unabated, the vascular inflammation of SIRS may progress into renal failure, the respiratory distress syndrome, possible gastrointestinal bleeding, and central nervous system dysfunction as a part of a multiple organ dysfunction syndrome or MODS.

Massive Lymphocyte Apoptosis

"Bystander" lymphocyte apoptosis is a phenomenon associated with several viral infections of man and animals, most notably HIV-1. While filoviral replication occurs in cells of the mononuclear phagocyte system, there is no evidence of viral replication in human lymphocytes. However, inside the lymph nodes, filoviral antigen becomes co-localized with apoptotic lymphocytes. Examination of cell populations in lymph nodes shows a concomitant depletion of CD8+ T cells and plasma cells.[50-52]

These findings suggest that the lymphopenia and lymphoid depletion associated with filoviral infections result from lymphocyte apoptosis induced by a number of factors that may include release of various chemical mediators during the early stages in the disease course, with pronounced intravascular and extravascular lymphocyte apoptosis. This massive "bystander" apoptosis of natural killer and T cells further impairs immunity.

As a consequence, EBOV disseminates to other cell types throughout the body, and severe illness results from a complex pathophysiology that enable the virus to suppress innate and adaptive immune responses, infect and kill a broad variety of cell types, and elicit a strong harmful inflammatory response.[53]

Role of the Secreted Ebola Glycoprotein (sGP)

As previously mentioned, unedited GP mRNA from replicating EBOV inside its host cell yields the nonstructural glycoprotein sGP, which is extensively secreted in a soluble

form from infected cells. This secreted sGP can function to absorb anti-GP antibodies produced by the infected host. More importantly, instead of simply passively absorbing host antibodies, sGP actively subverts the host immune response to induce cross-reactivity with epitopes it shares with processed membrane-bound GP[54]

This immune subversion by sGP is by a mechanism that is different from viruses that use secreted antigens as antibody decoys, and it might be a factor in future EBOV vaccine designs.

Progressive Endothelial Cell Dysfunction and Apoptosis

With respect to the vascular instability and the hemorrhagic disease caused by EBOV infection, data indicate that the vascular endothelium is a major target cell early in infection. Animal and tissue culture studies have provided insight into the pathogenetic role of the endothelium in contributing to the coagulation disorders that characterize Ebola hemorrhagic fever in primates.

Early studies were conducted using human tonsil tissue explants maintained in a National Aeronautics and Space Administration (NASA) three-dimensional tissue culture system[55] and in raft histoculture.[56,57] Human tonsil tissue obtained post-tonsillectomy was dissected into 3 mm cube blocks and separately infected with live Ebola Zaire and Ebola Reston, with incubation for 4 days at 37°C in RPMI-1640/10% FCS and a pCO_2 equivalent to human arterial blood. Massive endothelial cell apoptosis in the human tissue blocks was apparent within 36 hours, but only in the Ebola Zaire-infected human tissue. Tonsillar explants infected with Ebola Reston showed no evidence of endothelial apoptosis, as noted by S. Hatfill and P. Jahrling (unpublished results, 1998).

Later definitive studies further implicated the importance of the EBOV glycoprotein for induction of cytotoxicity and injury in vascular cells. Gene transfer of GP into explanted human or porcine blood vessels caused massive endothelial cell loss within 48 hours that led to a substantial increase in vascular permeability. GP derived from the Reston strain of virus, which causes disease in nonhuman primates but not in man, did not disrupt the vasculature of human blood vessels. In contrast, the Zaire GP induced massive endothelial cell disruption and cytotoxicity in nonhuman primate and human blood vessels. Subsequent studies suggested that human cardiac microvascular endothelial cells underwent death by apoptosis, or programmed cell death.[58]

The induction of endothelial apoptosis by EBOV was confirmed in 2000. Gene transfer of Ebola Zaire GP into explanted human and porcine blood vessels caused massive endothelial cell apoptosis within 48 hours. Ebola Reston GP did not disrupt the vasculature of human and porcine blood vessels. Apoptosis-inducing activity linked to a serine-threonine-rich mucin-like domain of the Ebola transmembrane GP. Endothelial cell cytotoxicity during synthesis of the virion GP of Ebola Zaire is a major viral determinant of Ebola pathogenicity.[59]

The Onset of Disseminated Intravascular Coagulation (DIC)

Disseminated intravascular coagulation (DIC) involves widespread activation of the clotting cascade leading to the formation of blood clots in the small blood vessels throughout the body. This compromises tissue blood flow, predisposing to organ damage that triggers further blood vessel microthrombi formation. An unending cycle may be initiated: as the coagulation process consumes clotting factors and platelets, normal clotting is disrupted, and severe bleeding can occur from various body sites.[60,61]

One critical mediator of DIC is the release of TF into the general circulation. TF is present on the surface of many cell types (including endothelial cells, macrophages, and monocytes) and is not normally in contact with the general circulation, but is exposed to the circulation after vascular damage. TF is released in response to exposure to cytokines, particularly IL-1, the tumor necrosis factor, and it plays a major role in development of DIC in viral infections.[62]

An increased level of TF is a consistent finding in EVD and likely plays a major pathogenic role in precipitating a consumptive coagulopathy that exceeds clearance by the body's fibrinolytic system.[44] It is of note that D-dimer formation and DIC appears to play a larger role in EVD than in Marburg virus disease.[63-65]

End-Stage EVD and Multiple Organ Dysfunction Syndrome (MODS)

Multiple organ dysfunction syndrome (MODS) is the consequence of continuing severe systemic vascular inflammation with generalized increased capillary permeability, capillary leak, and edema.[66] In MODS, organ dysfunction is precipitated by capillary changes in permeability, blood flow, and the development of microvascular stasis and microthrombi.

By definition, MODS is characterized by progressive dysfunction of six organ systems. Inflammatory cytokine-induced damage to the capillaries causes a change in their permeability, resulting in water and serum proteins leaking into the interstitial tissue spaces. With widespread leakage of blood volume, blood pressure becomes increasingly difficult to maintain with intravenous fluids and vasopressor drugs.

Hepatic dysfunction characterized by hyperbilirubinemia and depressed albumin production by the liver occurs early. As hepatocytes die, acute hepatic failure ensues. Ammonia and amide levels in the body rise, and an end-stage encephalopathy may be induced as a result of elevated plasma NH_3, with a reduction in the Glasgow Coma Score.

Renal risk increases from a combination of endothelial dysfunction, SIRS-induced endothelial damage, and progressive hemodynamic shock. This is characterized by oliguria as measured by hourly urine output less than 40-60 ml/hr, and increased plasma BUN, creatinine, and potassium, with decreased urine urea, creatinine, and potassium.

In addition, patients may exhibit multiple metabolic and endocrine abnormalities, including hyperglycemia and

increased insulin requirements. Eventually, lung interstitial spaces and alveoli may be involved. Some degree of myocardial depression may occur, affecting the right side of the heart in particular. Blood supply to the bowel may become compromised with a resulting bloody diarrhea, and the patient may develop ischemic colitis. The resulting transudation of Gram-negative bacteria from the gut lumen into the general circulation may precipitate terminal septic shock and death.

Because of the damage to the capillary microcirculation, it is difficult to reverse the established organ failure. Therapy therefore is limited to maintaining adequate tissue perfusion and adequate tissue oxygenation. The chance of survival diminishes as the number of different organs involved increases, and the mortality rate of MODS has changed little since its recognition the 1980s.[67]

Clinical Features of Ebola Virus Disease (EVD)

Beginning with the 1967 Marburg filovirus outbreak, some 30 epidemics, isolated cases, and accidental laboratory infections with the *Filoviridae* have been described in the medical literature. With the exception of the 2000 outbreak of EVD in the Sudan and Uganda, reports of epidemics in Central Africa have provided little controlled or objective clinical data other than the case fatality rate. In an attempt to identify significant gaps in the clinical data, Kortepeter et al. made an extensive case review to determine the basic clinical and laboratory features of the filoviral hemorrhagic fevers. The most detailed information was found in descriptions of patients treated in industrialized countries.[68]

EVD is the human disease caused by caused by four of the five current viruses classified in the genus *Ebolavirus*, family *Filoviridae*, order *Mononegavirales*. These four viruses are Bundibugyo virus (BDBV), (Zaire) Ebola virus (EBOV), Sudan virus (SUDV), and the Taï Forest virus (TAFV). The fifth ebolavirus, Reston virus (RESTV), is not pathogenic to humans but is pathogenic to nonhuman primates and pigs.

In EVD, symptoms usually begin suddenly with a flu-like stage characterized by fever, headaches, and joint, muscle, and abdominal pain. Less common symptoms include: sore throat, chest pain, hiccups, shortness of breath, and difficulty swallowing.[69]

The "classical" described average time between contracting the infection and the start of symptoms is eight to 10 days, varying between two and 21 days, although in light of current data, this should be reassessed.

Data collected in 1995 during the EVD outbreak (subtype Zaire) in Kikwit, DRC, suggested occasional longer incubation times. Using maximum likelihood inference and assuming a log-normally distributed incubation period, the mean incubation period was estimated to be 12.7 days (standard deviation 4.31 days). This suggests that 4.1% of patients may have incubation periods longer than 21 days. To reduce the risk of new cases, 25 days should be used when investigating the index case of an outbreak, determining the duration of contact surveillance, and deciding on the end of an outbreak.[70]

Along with the elevated body temperature, early complaints include nausea, vomiting, diarrhea, and loss of appetite.[71] The clinical course is summarized in Figure 4.

There is a 60%-to-70% drop in CD4+ and CD8+ peripheral lymphocyte populations during the first four to six days of acute illness, caused by massive "bystander" apoptosis of these non-infected cells via the CD95 (Fas) pathway. This is accompanied by the appearance of inflammatory mediators in the general circulation.

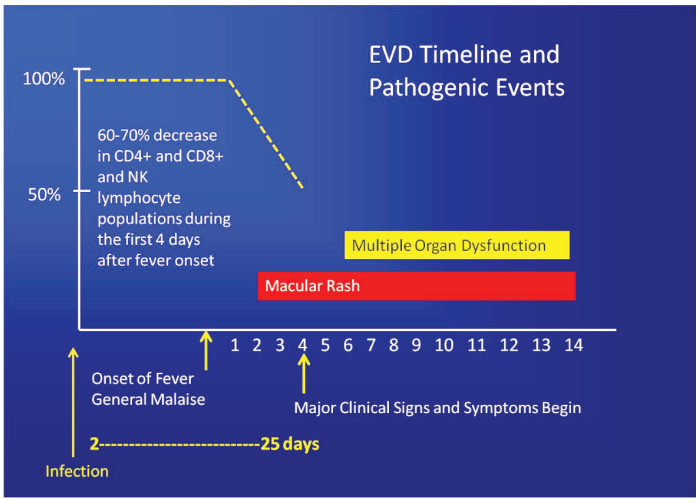

Figure 4. Clinical Course of EVD

At this time there is no evidence for or against mitochondrial DNA trapping in the pathogenesis of early capillary microthrombi generation. Mitochondrial DNA trapping refers to the release of mitochondrial DNA by viable neutrophils to generate neutrophil extracellular traps (NETs). These extracellular "traps" can bind a variety of microorganisms when the neutrophil undergoes short-term toll-like receptor 4 (TLR4) or complement factor 5a (C5a) receptor stimulation. These extracellular DNA structures are released in a reactive oxygen species (ROS)-dependent manner, but do not require the death of the neutrophils involved.[72]

After the initial non-specific prodromal period, there may be an early decrease in liver or kidney function. A macular rash may appear on the face and chest on the second or third day in about half the cases.[68] It is likely that the macular rash coincides with an initial burst of viremia and the onset of SIRS.

Criteria for SIRS include a body temperature less than 36 °C (96.8 °F) or greater than 38 °C (100.4 °F), a heart rate greater than 90 beats per minute, tachypnea greater than 20 breaths per minute or an arterial partial pressure of carbon dioxide less than 4.3 kPa (32 mmHg). White cell counts are less than 4 x 109 cells/L or greater than 12 x 109 cells/L, or show more than 10% immature neutrophils (band forms).

SIRS can be diagnosed when two or more of these criteria are present.[73]

In EVD, TF is markedly increased by the third day of acute illness, and the bleeding phase typically begins within five to seven days after first onset of symptoms. Internal and subcutaneous bleeding may occur in the conjunctiva, and there may be signs of hematemesis, hemoptysis, or melena. Bleeding into the skin may be evident as petechiae, purpura, ecchymoses, and hematomas (especially around needle injection sites). Heavy bleeding is rare and if it occurs it is usually confined to the gastrointestinal tract. Infected cases almost invariably show some indication of impaired blood clotting with altered prothrombin or activated partial thromboplastin time. In general, the development of bleeding symptoms indicates a worse prognosis. There is a dramatic increase in D-dimers at roughly five to seven days after the onset of acute illness. The increase is four times higher in fatal cases, up to 180,000 ng/ml.[74]

If the infected person does not recover, death due to MODS usually occurs within seven to 16 days (usually between days eight and nine) after the first symptoms. This is normally accompanied by a combination of progressive thrombocytopenia, mucous membrane bleeding, and the onset of bloody diarrhea.

Serum alanine aminotransferase (ALT) increases by about the same amount in fatal and non-fatal cases, but aspartate aminotransferase (AST) may markedly increase in fatal cases, demonstrating that other tissues besides the liver are undergoing necrosis. Serum amylase, BUN, and creatinine increase as a result of pancreatic and renal damage, and patients can develop encephalitis and/or pulmonary hemorrhage.

Patient Survival

Approximately 10% to 30% of patients survive the illness by mobilizing an adaptive immune response, and there is some limited evidence, based on background seropositivity in African populations, that mild or symptomless infections may occur in nature. All the EBOV strains (except RESTV) produce a similar illness, but with different case-fatality rates.

There may be prolonged post-recovery sequelae. Three (15%) of the 20 survivors of the 1995 EBOV outbreak in the DRC developed uveitis after being asymptomatic for 1 month. Signs and symptoms included ocular pain, photophobia, hyperlacrimation, and loss of visual acuity. This uveitis improved with topical treatment with 1% atropine and steroids.[75]

Anecdotal reports and one definitive study indicate that survivors may experience other vague problems for months or even years afterwards. One year after an EBOV epidemic in Uganda, 60 of 257 survivors were still suffering from complications of the disease. These included abdominal pains, loss of vision or hearing, impotence, bleeding, psychological problems, pain in the chest and various joints, and frequent severe headache. A common symptom was profound skeletal muscle weakness.[76]

Differential Diagnosis

The initial recognition of EVD is problematic without a high index of suspicion, as the initial symptoms are similar to those of Marburg virus disease, and both infections can be confused with other common tropical diseases, typified by sudden acute onset high fever, muscle pain, and general malaise. These include falciparum malaria, typhoid fever, shigellosis, rickettsial diseases such as typhus, or a Gram-negative septicemia. Occasional diagnostic confusion may occur with borreliosis or enterohemorrhagic *Escherichia coli*.

Definitive Diagnosis

EBOV is found in saliva, stool, semen, breast milk, tears, nasal blood, skin, and mucous membrane swabs during the acute phase of illness. The classic diagnostic technique is by electron microscopy for virus identification in inoculated Vero or MA-104 monkey kidney cell lines.

Electron microscopy is time-consuming and requires significant technical proficiency. However, less complicated diagnostic protocols and reagents exist. These include ELISA (IgM and IgG), an indirect immunofluorescence assay, and an immune (Western) blot assay. Skin swabs or tissue biopsies can be subjected to immunohistochemistry. A typical result is illustrated in the tissue section in Figure 5, which shows a thin paraffin-sectioned mouse spleen three days after infection with mouse-adapted EBOV strain. Strepavidin alkaline phosphatase Immunohistochemistry was performed with Strepavidin-linked antibody specific for the outer EBOV GP glycoprotein (red) and counter-stained.

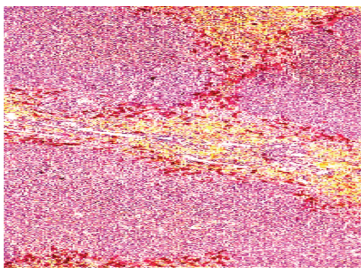

Figure 5. Mouse Spleen Infected with EBOV

Both U.S. Department of Defense classified and unclassified primer sets can be used for reverse transcriptase polymerase chain reaction (RT-PCR), and this molecular method yields a sensitive, rapid, and highly specific testing result.[71] For rapid field testing, Corgenix Medical Corp. has developed a point-of-care rapid diagnostic card test for Ebola and Sudan virus detection.

Clinical Management

Supportive therapy for EVD is directed toward maintaining effective blood volume and pressure, electrolyte balance, and tissue oxygenation. Isolation and strict barrier nursing is necessary to manage EVD patients. Minimum worker protection involves the use of high-efficiency particulate air (HEPA) filters, surgical masks, gloves, gown, booties, and eye protection, along with strict aerosol and droplet precautions. Higher-level worker protection involves the use of positive-pressure air-purifying respirators with liquid-impenetrable Tyvek-type suits sealed to provide a positive internal pressure. Intermediate sophistication is a full-face air-purifying respirator, splash-protective ensemble, booties, gloves, and individual decontamination with household bleach.

Administration of human interferon appears to have little value in treatment, although some early studies indicate that convalescent paired sera may have some effect if given early.

A number of possible treatments are under research and development, and several have been shown to have an effect in animal models of EBOV infection.

CpG immunomodulators are synthetic oligodeoxy-nucleotides that contain immunostimulatory CpG motifs capable of triggering an immunomodulatory cascade on parenteral administration. This responsive immune stimulation involves both B and T cells, as well as natural killer cells and professional antigen-presenting cells. In response to a CpG challenge, the host's immune system is activated in favor of a T-helper 1 (T_h1)-cell response with a regulated pro-inflammatory cytokine production.

Synthetic constructed mouse CpG motifs showed a 100% successful prevention of mouse-adapted EBOV lethality when injected intraperitoneally either 24 hours before or 24 hours after a lethal challenge with mouse-adapted Ebola Zaire virus. Identical results were achieved in guinea pigs when injected with synthetic constructed guinea pig CpG motifs and challenged with a 100% lethal Ebola Zaire virus titer,[77] as first noted by S.J. Hatfill and D.M. Klinman (unpublished results, 1998).

EBOV infection in human and animal models induces over-expression of the procoagulant TF in primate monocytes and macrophages. This suggests that inhibition of the TF pathway could ameliorate this effect of EBOV infection. In a rhesus macaque model of EBOV infection, administered recombinant nematode anticoagulant protein c2 (rNAPc2), a potent inhibitor of TF-initiated blood coagulation, provided a prolonged survival time, with a 33% survival rate over control animals. The administration of rNAPc2 attenuated the coagulation response as evidenced by modulation of various important coagulation factors, including plasma D-dimers, which were reduced in nearly all treated animals with less prominent fibrin deposits and intravascular microthrombi.[78]

In other experiments, a novel synthetic adenosine analogue, BCX4430, was shown to inhibit filovirus infection of human cells. Biochemical, reporter-based, and primer-extension assays indicate that BCX4430 inhibits viral RNA polymerase function, acting as a non-obligate RNA chain terminator. Post-exposure intramuscular administration of BCX4430 protects against EVD and Marburg virus disease in rodent infection models. In addition, BCX4430 completely protected cynomolgus macaques from Marburg virus infection when administered as late as 48 hours after infection.[79]

In addition to chemotherapy, current monoclonal antibody-based (mAbs) therapies seem to be able to reverse the progression of a lethal EBOV infection in nonhuman primates, and most recently in a human case. Novel combinations of mAbs can even fully cure lethally infected animals after clinical symptoms and circulating virus have been detected several days into the infection.[80]

A study demonstrated 100% and 50% survival of EBOV-infected cynomologus macaques with a combination of 3 EBOV-GP-specific monoclonal antibodies (ZMAb) administered at 24 or 48 hours post exposure, respectively. The survivors demonstrated EBOV-GP-specific humoral and cell-mediated immune responses, sufficient to protect survivors against a subsequent EBOV exposure.[81] These new developments have reopened the door for using antibody-based therapies for filovirus infections.

Ebola Vaccines

There is no vaccine or treatment currently licensed to counteract EBOV infections. A variety of DNA, protein sub-unit, and several viral vector approaches, both replicating and non-replicating, have been tested as potential vaccines. Their efficacy has been tested in nonhuman primate EBOV infection models, and several appear to confer protection against lethal EVD.[82]

Combinational protein vaccines using EBOV-like particles (eVLP) composed of virus protein (VP40), glycoprotein GP, and nucleoprotein, have been shown to protect rodents and nonhuman primates from lethal EBOV infection. This is a new direction for the development of a candidate vaccine for EBOV infection. Data indicates that eVLP administration triggers a host response through a Toll-like receptor (TLR) signaling pathway and a Type I IFN signaling pathway to initiate an early innate protective immune response.[83]

Human-to-Human Transmission Routes

Perhaps the greatest uncertainty in the pathogenesis of EBOV infection concerns the time, duration, and amount of viral shedding from infected patients, most particularly with respect to aerosol transmission. While classical epidemiologic evidence indicates that aerosol exposure is not an important means of virus transmission in human-to-human epidemics of EVD, infective virions are present in the oral fluid of infected patients,[84] and experimental studies

have verified that EBOV can be effectively transmitted by oral or conjunctival droplet exposure in nonhuman primate models.[85]

Field observations during epidemic human outbreaks of EVD indicate that secondary transmission is linked to improper needle hygiene, direct contact with infected tissue or fluid samples, and close unprotected contact with infected patients. However, while it is presumed that the virus infects through either the skin or contact with mucous membranes, the only two routes of exposure that have been extensively experimentally verified in animal models are parenteral inoculation, droplet exposure, and aerosol inhalation.

While epidemiologic evidence suggests that aerosol exposure is not an important method of virus transmission in natural outbreaks of human EVD, the Ebola Zaire (Mayinga) virus has been shown to be effectively transmitted by oral or conjunctival droplet exposure in nonhuman primates,[86] and aerosol models of filovirus transmission have been developed in knock-out guinea pigs and nonhuman primates. In addition, mouse-adapted EBOV models of airborne transmission have been developed that show infection in all aerosol-challenged mice, as well as lung lesions in two of the three strains tested.[87]

Concern over the possibility of EBOV aerosol transmission first emerged following the 1989 outbreak of RESTV at a primate quarantine facility in Reston, Va.[17,19,88] At the time, some scientists thought that the infection was aerosol transmitted between monkeys, and several animal care workers showed serological conversion. Although RESTV proved to be non-pathogenic to humans. CDC nevertheless assigned the strain a BSL-4 mandate.

This concern was reinforced in 2008 when RESTV was detected in pigs in the Philippines and specific RESTV antibodies were found in pig farmers, confirming their exposure.[36] Later experimental challenge studies in 5-week-old pigs, with exposure of animals to 106 TCID50 of the 2008 swine isolate via the oronasal route, showed virus replication in internal organs and viral shedding from the nasopharynx in the absence of clinical signs of disease.

These observations confirm not only that asymptomatic infection of pigs with RESTV occurs, but that affected animals pose a transmission/seroconversion risk to farm, veterinary, and abattoir workers.[89]

Extending these studies, researchers documented the possibility that pigs are susceptible to infection with the Ebola Zaire virus. EBOV was shown to be able to replicate and induce disease in domesticated Landrace pigs, and the infection can be transmitted to naïve animals after mucosal exposure.

Replication of EBOV to high titers was observed mainly in the respiratory tract, and the infected animals developed severe lung pathology. Shedding from the oronasal mucosa was detected for up to 14 days after infection, and transmission was confirmed in all naïve pigs cohabiting with inoculated animals.

These results indicate that pig farms in outbreak areas must now be considered potential sites of virus amplification, and the attendant risk must be managed.

Although Zaire Ebola is transmitted by unprotected physical contact with infected persons, few data exist on which specific bodily fluids are infected or on the actual risk of fomite transmission. To address this problem in 2007, researchers analyzed clinical specimens taken from 26 laboratory-confirmed human cases of EVD, as well as environmental specimens collected from an Ebola isolation ward. Virus was detected by culture and/or reverse-transcription polymerase chain reaction in 16 of 54 clinical specimens, including saliva, stool, semen, breast milk, tears, nasal blood, and a skin swab. EBOV was also detected in two of 33 environmental specimens from the isolation ward.

The researchers concluded that human EBOV infection is accompanied by viral shedding in a wide variety of bodily fluids during the acute period of illness, but that the risk of transmission from fomites in an isolation ward, and from convalescent patients, is low when currently recommended infection-control guidelines for the viral hemorrhagic fevers are followed.[90,91]

The presence of live EBOV in the skin of infected cases and the clinical possibility of fomite transmission via skin shedding were reinforced in 1995 during an EBOV outbreak in the DRC. During the outbreak, researchers examined the possibility of using immunohistochemistry (IHC) testing of formalin-fixed postmortem skin specimens as a positive alternative diagnostic method for EVD. Testing by IHC showed abundant viral antigens and EBOV particles in the skin of EVD patients. This reaffirms an epidemiologic role for contact transmission in EVD.[92]

New data demonstrate EBOV stability in respiratory droplets and liquids, and on environmental surfaces.[93]

Unanswered Questions

As part of their biological warfare program, the former U.S.S.R. successfully weaponized large quantities of the Marburg filovirus. However, Soviet scientists found it difficult to stabilize the aerosol decay rate of concentrated EBOV as a small dry-particle preparation suitable for offensive aerosol dissemination (K. Alibek, personal communication, 1998).

Concerning natural EBOV transmission, more data are required on virion stability in environmental samples, to include bat guano at cave temperatures and Chiroptera saliva-contaminated fruit, together with a better understanding of viral shedding and stability in demonstrated EBOV-associated fruit bat species.

Also lacking is a detailed study of the timing and amount of viral shedding in human EBOV patients as they progress through the disease. This should include viral quantization in skin, saliva, and respiratory droplets, and the stability of EBOV particles when disseminated in these media. This should include a further study of live virus shedding in

recovered survivors.

While it must be emphasized that airborne droplet and fomite transmission between humans has not been evident in case description and cohort studies of EBOV outbreaks in Africa,[94] aerosol droplet transmission has been demonstrated in animal models. It is therefore irresponsible for government health officials to emphatically state that aerosol transmission does not occur, when uncertainty remains in humans, and animal data suggest that this form of EBOV transmission to humans may be possible under certain conditions.

Current U.S. Doctrine for Civilian Infection Control

Civilian emergency responders in all disciplines, including emergency medical services (EMS), law enforcement, and fire and rescue departments and agencies, have robust infection-control procedures and protocols in place, often in the form of standard operating guidelines, to minimize the chance of infectious disease transmission in the field. During outbreaks, containment depends on a high index of suspicion and following standard precautions and disinfection procedures including isolation of patients.[95]

EVD is only one of a number of highly contagious viral hemorrhagic fevers that are well known to spread from person to person, and to be able to cause nosocomial outbreaks with a high case fatality rate. Outside of the the *Filoviridae*, there are the *Arenaviridae* (Lassa fever and more exceptionally the Junin and Machupo virus), and the *Bunyaviridae* (Crimean-Congo hemorrhagic fever). So far there have been only a limited number of imported cases of viral hemorrhagic fever in industrialized countries.

The *Filoviridae* continue to be a major concern with the evident increasing number of outbreaks in Africa. Therefore clinicians should consider the possibility of a viral hemorrhagic fever in an acutely ill patient just returning from Africa or South America with pyrexia for which there is no obvious cause. Such patients should be questioned for risk factors for viral hemorrhagic fever.

Current CDC doctrine emphasizes that by using universal/standard precautions when managing patients and handling blood and body fluids, together with barrier nursing techniques, there is little risk secondary cases.[96] The tenets of "universal" or "standard" precautions dictate that those coming in direct or potential contact with airborne, blood-borne, and vector-borne pathogens should employ the proper personal protective equipment (PPE) based on the perceived or actual risks of patient contact. The principle of standard precautions means that emergency medical personnel will assume that patients have the potential to transfer or spread a pathogen that they are carrying, or to which they have been exposed.[94]

Because of the recently reported outbreak of EVD in Africa and the ease and frequency of international travel and immigration, all medical workers should be made aware of the signs and symptoms of EVD and other viral hemorrhagic fevers. This should be suspected in any recent traveler who presents with the possible early signs and symptoms, including acute onset of high fever, muscle pain, and general malaise.

Additionally, EVD should also be suspected in laboratory workers who show symptoms after exposure to animals from endemic areas. Providers should carefully question patients presenting with the previously noted signs and symptoms. Specific questions should include recent travel and possible exposure to ill patients or other vectors. Quickly identifying high-risk patients will allow appropriate measures to be taken.[98]

CDC guidelines recommend disposable non-sterile examination gloves as a minimum level of PPE for situations with low suspicion of coming in contact with contaminants, droplets, or body fluids during assessment, examination, and/or treatment. When there is likelihood of body fluid splashing or coughing, EMS providers will also don surgical masks, eye protection (glasses, visors, or splash guards), and even fluid-impervious gowns along with the examination gloves.[99]

CDC's first EBOV guidelines were insufficient and were quickly modified. At the time of this writing, no specific fever threshold is specified, but lack of fever appears to be used as a criterion for excluding a high-risk situation. Some data suggest, however, that fever is not a presenting complaint in 12% of human EVD cases.[100]

High-risk situations, such as being in contact with and providing care to known infectious patients, may call for additional PPE such as properly fitted and fit-tested HEPA masks that offer enhanced droplet protection in conjunction with impervious gowns, eye protection, and gloves.

Additional PPE might be required in certain situations (e.g., copious amounts of blood, other body fluids, vomit, or feces present in the environment), including but not limited to double gloving, disposable shoe covers, and leg coverings.

Certain agencies may opt to increase the protection level to include air-purifying respirators (APR) or positive air purifying respirators (PAPR).

Care must also be given to ensure that disposable equipment is properly disposed of, and that all remaining exposed equipment and surfaces are properly cleaned and decontaminated. EMS providers should also pay close attention to prevent cross-contamination, and notify the receiving facility as soon as possible so appropriate arrangements can be made to turn over patient care without exposing others.

EMS personnel should also promptly report any potential exposures to their designated infection-control officer. The CDC recommends that, "for asymptomatic healthcare providers who had an unprotected exposure (i.e. not wearing recommended PPE at the time of patient contact or through direct contact to blood or body fluids) to a patient with EVD should receive medical evaluation and follow-up care

including fever monitoring twice daily for 21 days after the last known exposure, and hospitals should consider policies ensuring twice daily contact with exposed personnel to discuss potential symptoms and document fever checks. The provider may continue to work while receiving twice daily fever checks, based upon hospital policy and discussion with local, state, and federal public health authorities."[101]

Even though the U.S. has had strong infection control procedures and guidelines in use since the emergence of other infectious diseases such as human immunodeficiency virus (HIV) and hepatitis, additional research and data collection are needed in order to gain true consensus on the necessary levels of precautions and treatment best practices for patients infected with hemorrhagic fevers.[102,103]

Air Evacuation of EVD Patients

Air transport of acutely ill EVD patients with high viral loads and potentially high viral shedding presents special problems. The confined internal space of transport aircraft, the difficulties in ensuring complete interior decontamination, airframe vibration, use of recirculated cabin air for pressurization, and the risk of sudden aircraft cabin depressurization, all combine to make this a special medical/microbiological environment.

In the past, aeromedical evacuation of highly contagious patients was managed by a special military unit trained and practiced in transport of infected patients under high biological containment. In 1978, the U.S. Department of Defense created an Aeromedical Isolation and Special Medical Augmentation Response Team (AIT-SMART). This was a rapid-response unit with worldwide airlift capability designed to safely evacuate and manage contagious patients under BSL-4 conditions.

The team used a UK-developed aircraft transit isolator for patient transport, with the interior of the isolator maintained at a pressure negative to the external environment by a HEPA-filtered blower.[104] While moving or attending to the isolator, team members wore protective Tyvek suits and positive-pressure, HEPA-filtered Racal hoods.[105]

Throughout its existence the AIT-SMART was associated with a BSL-4 medical containment suite (MCS) at the U.S. Army Medical Research Institute for Infectious Diseases (USAMRIID) for intensive care unit-level patient care under full BSL-4 biocontainment conditions.[106] The MCS was built in 1969, became operational in 1972, and was the final destination for highly contagious patients transported by AIT-SMART. The unit's aircraft transit isolator could be attached directly to a transfer port to the MCS situated on the external wall of the main USAMRIID building. This allowed movement of the patient into the MCS without exposing the environment to the patient.

The AIT-SMART combined the MCS with several suites of BSL-4 laboratories staffed by highly experienced viral hemorrhagic fever researchers, with full clinical and pathology laboratories, a full experimental animal colony with strain mice, guinea pig, and nonhuman primate models, as well as scientists and physicians highly experienced in disease assessment, pathogenesis, and experimental vaccine development.

In 2010 the AIT-SMART was decommissioned, and this unified capability was lost. The mission was then handed over to one of the U.S. Air Force's Critical Care Air Transport Teams (CCATTs). However, the Air Force's current capabilities do not meet the same standard of BSL-4, but rather represent only enhanced patient isolation.

When asked about continuity of the original AIT-SMART capability, the public affairs office at the 59th Medical Wing, Wilford Hall, Ambulatory Surgical Center, Lackland Air Force Base, did not have any knowledge of AIT-SMART as a specific capability indigenous to CCATT teams (T. Nordin, personal communication, 2014). In addition, there is nothing to replace the full BSL-4 MCS intensive care unit to date. Although this could be reinstituted with airflow reversion changes, there is no longer a frequently practiced BSL-4 team of attending medical specialists to service it.

As an alternative, Emory University Hospital has set up a special Serious Communicable Disease Unit in collaboration with CDC, to isolate individuals exposed to diseases at BSL-3. It is one of four such facilities in the country.

Biocontained Evacuation Concerns for Overseas Military Personnel

U.S. Air Force aeromedical units will soon be using the Gentex® Patient Isolation Unit (PIU) GO19-1000 (see Figure 6), a temporary, single-use, portable structure designed to contain and transport a patient and prevent particulate cross-contamination with the environment and caregivers. Made of a breathable, HEPA-filtration material, the PIU can be used to temporarily isolate a contaminated patient outside or within patient care facilities, or to transport patients within vehicles, such as aircraft, via a litter or gurney.

Figure 6. Gentex® Patient Isolation Unit (PIU) GO19-1000

While long-distance aeromedical evacuation of any military patient is usually assigned to units such as the USAF Critical Care Aeromedical Transport Teams (CCATTs), this is usually the last step in the tactical evacuation process.

In reality, the evacuation process may actually involve a mixture of partisan liaisons, vehicle transport, rotor-power airlift, or even waterborne littoral transport platforms. Forward-operating Special Operations units, civil affairs, military liaisons, and Medical Civil Action Program (MEDCAP) participants are frequently just as involved in the process of evacuation of sick or injured military personnel, as are the personnel involved in the terminal leg of the evacuation to a higher-level medical facility.

Infectious diseases such EVD or other hemorrhagic fevers pose unique, serious challenges for military forward-area evacuation. The potential for secondary infections is extremely high if body fluid isolation techniques are not employed throughout the patient transport chain.

Because of limited medical resources and supplies at forward military locations, early recognition of potential disease among forward-operating forces is critical. In the event of geographical proximity to a known outbreak of EVD, all patients with unexplained acute onset of pyrexia should be considered as a potential source for secondary EVD infection at intake until a filovirus or other hemorrhagic fever can be ruled out.[107]

Because of the uncertainty of the timing of viral shedding once symptoms develop, all febrile patients from Ebola areas should be considered to be highly infectious. Early recognition and quarantine procedures are critical for these patients, together with active surveillance of all accidental exposures in military practitioners or contact teams.[108,109]

Conclusions

Current epidemiological data indicate that once a primary human EBOV infection develops, classical secondary transmission occurs through direct contact with infected symptomatic individuals or their bodily fluids. Transmission in a medical setting can be especially prevalent without proper precautions.

It is assumed, and frequently stated by national health leaders, that humans infected with EBOV are not shedding infective virus during the incubation period before the onset of high fever, muscle pain, and general malaise. There is some uncertainty about this, and further investigation is needed. The potential for live virus shedding from skin also needs closer examination.

Although humans and great apes are EBOV end hosts, dogs and pigs were infected in African village outbreaks, suggesting that other interim or amplifying hosts may exist. Fruit bats are one natural reservoir for EBOV as well as a variety of other highly virulent viruses, and the true viral load and range of these selected bat species needs further research.

The promise of rapid diagnostic immunoassays should be fully explored, and the current lack of a dedicated U.S. military aeromedical isolation team should be reassessed.

The concept of super-spreader cases should always be kept in mind when dealing with any infectious disease outbreak. The current Ebola situation has demonstrated that the U.S. was woefully unprepared to handle one patient with EVD, despite spending billions on biological defense and pandemic preparedness. This should serve as a wake-up call.

Steven J. Hatfill, M.D., M.Sc., M.Med., is an adjunct assistant professor in the Department of Microbiology, Immunology, and Tropical Medicine, and an adjunct assistant professor in Clinical Research and Leadership at The George Washington University, Washington, D.C.

Trevor Nordin is a former U.S. Air Force para-rescue specialist and current adjunct Instructor for the Emergency Health Services Program, The George Washington University, School of Medicine and Health Sciences.

Geoffrey L. Shapiro is the Director of EMS & Operational Medicine Training for The George Washington University Emergency Health Services Program, School of Medicine and Health Sciences in Washington, D.C.

Corresponding author: Geoffrey L. Shapiro, Director, EMS & Operational Medicine Training, Emergency Health Services Program, School of Medicine and Health Sciences, The George Washington University, 2100 Pennsylvania Avenue, NW Suite 369, Washington, DC 20037.

REFERENCES

1. Wolfe ND, Dunavan CP, Diamond J. Origins of major human infectious diseases. *Nature* 2007;447:279-283. doi: 10.1038/nature05775.
2. Lopez AD, Mathers CD, Ezzati N, Jamison DT, Murray CJL. (eds.) *Global Burden of Disease and Risk Factors*. New York, N.Y.: Oxford University Press; 2006.
3. Moya A, Holmes EC, Gonzalez-Candelas F. The population genetics and evolutionary epidemiology of RNA viruses. *Nature Rev Microbiol* 2004;2:279-288.
4. McNeill WH. *Plagues and Peoples*. Garden City, N.Y.: Anchor; 1976.
5. Taylor LH, Latham SM, Woolhouse ME. Risk factors for human disease emergence. *Phil Trans R Soc Lond B Biol Sci* 2001;356:983-989.
6. Woolhouse M, Scott F, Hudson Z, Howey R, Chase-Topping M. Human viruses: discovery and emergence. *Philos Trans R Soc Lond B Biol Sci* 2012;367:2864-2871.
7. Kuhn JH, Becker S, Ebihara H, et al. Proposal for a revised taxonomy of the family *Filoviridae*: classification, names of taxa and viruses, and virus abbreviations. *Arch Virol* 2010;155:2083-2103.
8. Bukreyev AA, Chandran K, Dolnik O, et al. Discussions and decisions of the 2012–2014 International Committee on Taxonomy of Viruses (ICTV) Filoviridae Study Group, January 2012–June 2013. *Arch Virol* 2014;159:821-830.
9. Negredo A, Palacios G, Vázquez-Morón S, et al. Discovery of an ebolavirus-like filovirus in Europe. *PLoS Pathog* 2011;7(10):e1002304. doi: 10.1371/journal.ppat.1002304.
10. Carroll SA, Towner JS, Sealy TK, et al. Molecular evolution of viruses of the family *Filoviridae* based on 97 whole-genome sequences. *J Virol* 2013:2608-2616. doi: 10.1128/JVI.03118-12.
11. Suzuki Y, Gojobori T. The origin and evolution of Ebola and Marburg viruses. *Mol Biol Evol* 1997;14:800–806.

12. Taylor DJ, Dittmar K, Ballinger MJ, Bruenn JA. Evolutionary maintenance of filovirus-like genes in bat genomes. *BMC Evol Biol* 2011;11:336. doi: 10.1186/1471-2148-11-336.
13. Taylor DJ, Leach RW, Bruenn J. Filoviruses are ancient and integrated into mammalian genome. *BMC Evol Biol* 2010;10:193. doi: 10.1186/1471-2148-10-193.
14. Stenglein MD, Sanders C, Kistler AL, et al. Identification, characterization, and in vitro culture of highly divergent arenaviruses from boa constrictors and annulated tree boas: candidate etiological agents for snake inclusion body disease. *mBio* 2012;3(4):e00180-12. doi: 10.1128/mBio.00180-12.
15. Johnson KM. Ebola haemorrhagic fever in Zaire, 1976. *Bull World Health Organ* 1978; 56:271-293.
16. Smith DIH. Ebola haemorrhagic fever in Sudan, 1976. *Bull World Health Organ* 1978;56:247-270.
17. Jahrling PB, Geisbert TW, Dalgard DW, et al. Preliminary report: isolation of Ebola virus from monkeys imported to USA. *Lancet* 1990;335:502-505.
18. Le Guenno B, Formenty P, Wyers M, et al. Isolation and partial characterization of a new strain of Ebola. *Lancet* 1995;345:1271-1274.
19. Rollin PE, Williams RJ, Bressler DS, et al. Ebola (subtype Reston) virus among quarantined nonhuman primates recently imported from the Philippines to the United States. *J Infect Dis* 1999;179:S108-S114.
20. Formenty P, Hatz C, Le Guenno B, et al. Human infection due to Ebola virus, subtype Côte d'Ivoire: clinical and biologic presentation. *J Infect Dis* 1999;179:S48-S53.
21. Khan AS, Tshioko FK, Heymann DL, et al. The reemergence of Ebola hemorrhagic fever, Democratic Republic of the Congo, 1995. *J Infect Dis* 1999;179:S76-S86.
22. Georges AJ, Leroy EM, Renaut AA, et al. Ebola hemorrhagic fever outbreaks in Gabon, 1994–1997: epidemiologic and health control issues. *J Infect Dis* 1999;179:S65-S75.
23. Leroy EM, Rouquet P, Formenty P, et al. Multiple Ebola virus transmission events and rapid decline of Central African wildlife. *Science* 2002;303:387-390.
24. Wittmann TJ, Biek R, Hassanin A, et al. Isolates of Zaire ebolavirus from wild apes reveal genetic lineage and recombinants. *Proc Natl Acad Sci USA* 2007;104:17123-17127.
25. Baron RC, McCormick JB, Zubeir OA. Ebola virus disease in southern Sudan: hospital dissemination and intrafamilial spread. *Bull World Health Organ* 1983;1:997-1003.
26. Towner JS, Sealy TK, Khristova ML, et al. Newly discovered Ebola virus associated with hemorrhagic fever outbreak in Uganda. *PLoS Pathog* 2008;4. doi: 10.1371/journal.ppat.1000212.
27. Dixon MG, Schafer IJ. Ebola viral disease outbreak—West Africa, 2014. *MMWR* 2014;63:548-551.
28. Swanepoel R, Leman PA, Burt FJ, et al. Experimental inoculation of plants and animals with Ebola virus. *Emerg Infect Dis* 1996;2(4).
29. Van der Poel WH, Lina PH, Kramps JA. Public health awareness of emerging zoonotic viruses of bats: a European perspective. *Curr Opin Virol* 2011;1:649-657.
30. Towner JS, Pourrut X, Albariño CG, et al. Marburg virus infection detected in a common African bat. *PLoS* 2007;2(8). doi: 10.1371/journal.pone.0000764..
31. Leroy EM, Kumulungui B, Pourrut X, et al. Fruit bats as reservoirs of Ebola virus. *Nature* 2005;438:575-576.
32. Leroy EM, Epelboin A, Mondonge V, et al. Human Ebola outbreak resulting from direct exposure to fruit bats in Luebo, Democratic Republic of Congo, 2007. *Vector Borne Zoonotic Dis* 2009;9:723-738.
33. Olival KJ, Islam A, Yu, M, et al. Ebola virus antibodies in fruit bats, Bangladesh. *Emerg Infect Dis* 2013;19(2).
34. Wang LF, Walker PJ, Poon LL. Mass extinctions, biodiversity and mitochondrial function: are bats 'special' as reservoirs for emerging viruses? *Vector Borne Zoonotic Dis* 2006;6(4):315-324.
35. Weingartl HM, Nfon C, Kobinger G. Review of Ebola virus infections in domestic animals. *Dev Biol* (Basel) 2013;135:211-218.
36. Barrette RW, Metwally SA, Rowland JM, et al. Discovery of swine as a host for the Reston ebolavirus. *Science* 2009;325:204-206.
37. Kobinger GP, Leung A, Neufeld J, et al. Replication, pathogenicity, shedding, and transmission of Zaire ebolavirus in pigs. *J Infect Dis* 2011;204:200-208.
38. Martinez O, Leung LW, Basler CF. The role of antigen-presenting cells in filoviral hemorrhagic fever: gaps in current knowledge. *Antiviral Res* 2012;93:416-428.
39. Feldmann H, Volchkov VE, Volchkova VA, Klenk HD.The glycoproteins of Marburg and Ebola virus and their potential roles in pathogenesis. *Arch Virol* 1999;15(Suppl):159-169.
40. Carette JE, Raaben M, Wong AC, et al. Ebola virus entry requires the cholesterol transporter Niemann-Pick C1. *Nature* 2011;477:340-343.
41. Kondratowicz AS, Lennemann NJ, Sinn PL, et al. T-cell immunoglobulin and mucin domain 1 (TIM-1) is a receptor for Zaire Ebolavirus and Lake Victoria Marburgvirus. *Proc Nat Acad Sci USA* 2011;108:8426-8431.
42. Wauquier N, Becquart P, Padilla C, Baize S, Leroy EM. Human fatal Zaire Ebola virus infection is associated with an aberrant innate immunity and with massive lymphocyte apoptosis. *PLoS Negl Trop Dis* 2010;4(10):pii: e837. doi: 10.1371/journal.pntd.0000837.
43. Zhang AP, Abelson DM, Bornholdt ZA, et al. The ebolavirus VP24 interferon antagonist: know your enemy. *Virulence* 2012;3:440-445.
44. McElroy AK, Erickson BR, Flietstra TD, et al. Ebola hemorrhagic fever: novel biomarker correlates of clinical outcome. *J Infect Dis*. 2014;210:558-566.
45. Wong G, Kobinger GP, Qiu X. Characterization of host immune responses in Ebola virus infections. *Expert Rev Clin Immunol* 2014;10:781-790.
46. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med* 1992;20: 864-874.
47. Feldmann H, Bugany H, Mahner F, et al. Filovirus-induced endothelial leakage triggered by infected monocytes/macrophages. *J Virol* 1996;70:2208-2214.
48. Tsiotou AG, Sakorafas GH, Anagnostopoulos G, Bramis J. Septic shock: current pathogenetic concepts from a clinical perspective. *Med Sci Monit* 2005;11(3).
49. Engelmann B, Luther T, Müller I. Intravascular tissue factor pathway—a model for rapid initiation of coagulation within the blood vessel. *Thromb Haemost* 2004;89:3-8.
50. Baize S, Leroy EM, Georges-Courbot M-C, et al. Defective humoral responses and extensive intravascular apoptosis are associated with fatal outcome in Ebola virus-infected patients. *Nature Med* 1999;5:423-426.
51. Baize S, Leroy EM, Mavoungou E, Fisher-Hoch SP. Apoptosis in fatal Ebola infection. Does the virus toll the bell for the immune system? *Apoptosis* 2000;5:5-7.
52. Gupta M, Spiropoulou C, Rollin PE. Ebola virus infection of human PBMCs causes massive death of macrophages, CD4 and CD8 T cell subpopulations in vitro. *Virology* 2007;364:45-54.
53. Bray M, Geisbert TW. Ebola virus: the role of macrophages and dendritic cells in the pathogenesis of Ebola hemorrhagic fever. *Int J Biochem Cell Biol* 2005;37:1560-1566.
54. Mohan GS, Li W, Ye L, Compans RW, Yang C. Antigenic subversion: a novel mechanism of host immune evasion by Ebola virus. *PLoS Pathog* 2012;8(12):e1003065. doi: 10.1371/journal.ppat.1003065.
55. Barzegari A, Saei AA. An update to space biomedical research: tissue engineering in microgravity bioreactors. *Bioimpacts* 2012;2(1):23-32.
56. García-Gareta E. Collagen gels and the 'Bornstein legacy': from a substrate for tissue culture to cell culture systems and biomaterials for tissue regeneration. *Exp Dermatol* 2014;23:473-474.
57. Ozbun MA, Patterson NA. Using organotypic (raft) epithelial tissue cultures for the biosynthesis and isolation of infectious human papillomaviruses. *Curr Protoc Microbiol* 2014;34:14.
58. Ray RB, Basu A, Steele R, et al. Ebola virus glycoprotein-mediated anoikis of primary human cardiac microvascular endothelial cells. *Virology* 2004;321:181-188.
59. Yang ZY, Duckers HJ, Sullivan NJ, et al. Identification of the Ebola virus glycoprotein as the main viral determinant of vascular cell cytotoxicity and injury. *Nat Med* 2000;6:886-889.
60. Levi M. Disseminated intravascular coagulation. *Crit Care Med* 2007;35:2191-2195.
61. Levi M, ten Cate H. Disseminated intravascular coagulation. *N Engl J Med* 1999;341:586-592.
62. Antoniak S, Mackman N. Multiple roles of the coagulation protease cascade during virus infection. *Blood* 2014;123:2605-2613.

63. Hensley LE, Alves DA, Geisbert JB, et al. Pathogenesis of Marburg hemorrhagic fever in cynomolgus macaques. *J Infect Dis* 2011;204(Suppl 3):S1021-1031. doi: 10.1093/infdis/jir339.
64. Bray M, Hatfill S, Hensley L, Huggins JW. Hematological, biochemical and coagulation changes in mice, guinea pigs and monkeys infected with a mouse-adapted variant of Ebola Zaire virus. *J Comp Path* 2001;125:243-253.
65. Geisbert TW, Young HA, Jahrling PB, et al. Mechanisms underlying coagulation abnormalities in Ebola hemorrhagic fever: overexpression of tissue factor in primate monocytes/macrophages is a key event. *J Infect Dis* 2003;188:1618-1629.
66. Johnson D, Mayers I. Multiple organ dysfunction syndrome; a narrative review. *Can J Anaesth* 2001;48:502-509.
67. Irwin RS, Lilly CM, Rippe JM. *Manual of Intensive Care Medicine*. Philadelphia, Pa.: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2013.
68. Kortepeter MG, Bausch DG, Bray M. Basic clinical and laboratory features of filoviral hemorrhagic fever. *J Infect Dis* 2011;204(Suppl 3): S810-816. doi: 10.1093/infdis/jir299.
69. Jeffs B. A clinical guide to viral haemorrhagic fevers: Ebola, Marburg and Lassa. *Trop Doct* 2006;36(1):1-4.
70. Eichner M, Dowell SF, Firese N. Incubation period of Ebola hemorrhagic virus subtype Zaire. *Osong Public Health Res Perspect* 2011;2(1):3-7.
71. World Health Organization. *Ebola and Marburg Virus Disease Epidemics: Preparedness, Alert, Control, and Evaluation*. Interim Version 1.1. WHO/HSE/PED/CED/2014.05; June 2014. Available at: http://www.who.int/csr/disease/ebola/PACE_outbreaks_ebola_marburg_en.pdf. Accessed Oct 7, 2014.
72. Yousefi C, Mihalache E, Kozlowski E, Schmid I, Simon HU. Viable neutrophils release mitochondrial DNA to form neutrophil extracellular traps. *Cell Death Differ* 2009;16:1438-1444. doi: 10.1038/cdd.2009.96.
73. Parsons M. Cytokine storm in the pediatric oncology patient. *J Pediatr Onc Nurs* 2010;27(5):253–258.
74. Rollin PE, Bausch DG, Sanchez A. Blood chemistry measurements and D-dimer levels associated with fatal and nonfatal outcomes in humans infected with Sudan Ebola virus. *J Infect Dis* 2007;196(Suppl 2):S364-S371.
75. Kibadi K, Mupapa K, Kuvula K, et al. Late ophthalmologic manifestations in survivors of the 1995 Ebola virus epidemic in Kikwit, Democratic Republic of Congo. *J Infect Dis* 1999;179(Suppl 1):S13-S14.
76. Wendo C. Caring for the survivors of Uganda's Ebola epidemic one year on. *Lancet* 2001;358:1350.
77. Klinman DM. Immunotherapeutic uses of CpG oligodeoxynucleotides. *Nature Rev Immuno*l 2004;4:249-259. doi:10.1038/nri1329.
78. Geisbert TW, Hensley LE, Jahrling PB, et al. Treatment of Ebola virus infection with a recombinant inhibitor of factor VIIa/tissue factor: a study in rhesus monkeys. *Lancet* 2003;362:1953-1958.
79. Warren TK, Wells J, Panchal RG, et al. Protection against filovirus diseases by a novel broad-spectrum nucleoside analogue BCX4430. *Nature* 2014;508:402-405.
80. Qiu X, Kobinger GP. Antibody therapy for Ebola: is the tide turning around? *Hum Vaccin Immunother* 2014;10:964-967.
81. Qiu X, Audet J, Wong G, Fernando L, et al. Sustained protection against Ebola virus infection following treatment of infected nonhuman primates with ZMAb. *Sci Rep* 2013;3:3365.
82. Marzi A, Feldmann H. Ebola virus vaccines: an overview of current approaches. *Expert Rev Vaccines* 2014;13:521-531.
83. Ayithan N, Bradfute SB, Anthony SM, et al. Ebola virus-like particles stimulate type I interferon and proinflammatory cytokine expression through the toll-like receptor and interferon signaling pathways. *J Interferon Cytokine Res* 2014;34:79-89.
84. Formenty P, Leroy EM, Epelboin A, et al. Detection of Ebola virus in oral fluid specimens during outbreaks of Ebola virus hemorrhagic fever in the Republic of Congo. *Clin Infect Dis* 2006;42:1521-1526.
85. Jaax NK, Davis KJ, Geisbert TJ, et al. Lethal experimental infection of rhesus monkeys with Ebola-Zaire (Mayinga) virus by the oral and conjunctival route of exposure. *Arch Pathol Lab Med* 1996;120:140-155.
86. Twenhafel NA, Shaia CI, Bunton TE, et al. Experimental aerosolized guinea pig-adapted Zaire ebolavirus (variant: Mayinga) causes lethal pneumonia in guinea pigs. *Vet Pathol* 2014 May 14. pii: 0300985814535612. [Epub ahead of print].
87. Zumbrun EE, Abdeltawab NF, Bloomfield HA, et al. Development of a murine model for aerosolized ebolavirus infection using a panel of recombinant inbred mice. *Viruses* 2012;4:258-275.
88. Geisbert, TW, Jahrling PB. Use of immunoelectron microscopy to show Ebola virus during the 1989 United States epizootic. *J Clin Pathol* 1990;43:813-816.
89. Marsh GA, Haining J, Robinson R, et al. Ebola Reston virus infection of pigs: clinical significance and transmission potential. *J Infect Dis* 2011;204(Suppl 3):S804-S809. doi: 10.1093/infdis/jir300.
90. Bausch DG, Towner JS, Dowell SF, et al. Assessment of the risk of Ebola virus transmission from bodily fluids and fomites. *J Infect Dis* 2007;196(Suppl 2):S142-S147.
91. Centers for Disease Control and Prevention. Ebola (Ebola Virus Disease). Available at: www.cdc.gov/vhf/ebola/index.html. Accessed Oct 7, 2014.
92. Zaki SR, Shieh WJ, Greer PW, et al. A novel immunohistochemical assay for the detection of Ebola virus in skin: implications for diagnosis, spread, and surveillance of Ebola hemorrhagic fever. Commission de Lutte contre les Epidémies à Kikwit. *J Infect Dis* 1999;179(Suppl 1):S36-S47.
93. Piercy TJ, Smither SJ, Steward JA, Eastaugh L, Lever MS. The survival of filoviruses in liquids, on solid substrates, and in a dynamic aerosol. *J Applied Microbiol* 2010;109:1531-1539. doi: 10.1111/j.1365-2672.2010.04778.x.
94. Borio L, Inglesby T, Peters CJ, et al. Hemorrhagic fever viruses as biological weapons: medical and public health management. *JAMA* 2002;287:2391-2405.
95. Georges AJ, Baize S, Leroy EM, Georges-Courbot MC. Ebola virus: what the practitioner needs to know. *Med Trop* (Mars) 1998;58:177-186.
96. Colebunders R, Van Esbroeck M, Moreau M, Borchert M. Imported viral haemorrhagic fever with a potential for person-to-person transmission: review and recommendations for initial management of a suspected case in Belgium. *Acta Clin Belg* 2002;57:233-240.
97. Unknown. *Silent War: Infection Control for Emergency Responders*. 2nd ed. FPP IFSTA; 2002.
98. Visser LG, Schippers EF, Swaan CM, van den Broek PJ. How to treat a patient with indications for an infectious viral hemorrhagic fever. *Ned Tijdschr Geneeskd* 2002;16;146:2183-2138.
99. Centers for Disease Control and Prevention. Guidelines for Environmental Infection Control in Health Care Facilities, Recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC); 2003.
100. WHO Ebola Response Team. Ebola virus disease in West Africa—the first 9 months of the epidemic and forward projections. *N Engl J Med* 2014;371:1481-1495. doi: 10.1056/NEJMoa1411100.
101. CDC. Identify, Isolate, Inform: Ambulatory Care Evaluation of Patients with Possible Ebola Virus Disease (Ebola). Available at: http://www.cdc.gov/vhf/ebola/pdf/ambulatory-care-evaluation-of-patients-with-possible-ebola.pdf. Accessed Nov 20, 2014.
102. Sodhi A. Ebola virus disease. *Postgrad Med* 1996;99(5):75-76, 78.
103. Bühler S, Roddy P, Nolte E, Borchert M. Clinical documentation and data transfer from Ebola and Marburg virus disease wards in outbreak settings: health care workers' experiences and preferences. Viruses 2014;6:927-937.
104. Clayton AJ. Containment aircraft transit isolator. *Aviat Space Environ Med* 1979;50:1067-1072.
105. Christopher GW, Eitzen EM Jr. Air evacuation under high-level biosafety containment: the aeromedical isolation team. *Emerg Infect Dis* 1999;5:241-246.
106. Marklund LA. Patient care in a biological safety level-4 (BSL-4) environment. *Crit Care Nurs Clin N Am* 2003;15:245-255.
107. U.S. Air Force. Airman's Manual: Air Force Manual 10-100; 2004.
108. U.S. Army. Soldier's Manual of Common Tasks: Warrior Skills Level 1. STP 21-1-SMCT; 2012.
109. Department of the Navy. Office of the Chief of Naval Operations. Environmental Readiness Program Manual. OPNAV M-5090.1; 2014.