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REVIEW ARTICLE

Ebola virus disease in nonendemic countries

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The 2014 West African outbreak of Ebola virus disease was unprecedented in its scale and has resulted in transmissions outside endemic countries. Clinicians in nonendemic countries will most likely face the disease in returning travelers, either among healthcare workers, expatriates, or visiting friends and relatives. Clinical suspicion for the disease must be heightened for travelers or contacts presenting with compatible clinical syndromes, and strict infection control measures must be promptly implemented to minimize the risk of secondary transmission within healthcare settings or in the community. We present a concise review on human filoviral disease with an emphasis on issues that are pertinent to clinicians practicing in nonendemic countries.

Introduction

The largest outbreak of Ebola virus disease (EVD) in history has renewed interest in filoviruses and has provided an unprecedented impetus to the development of new therapeutics and vaccines for this highly lethal infection. Hemorrhagic fevers caused by Ebola and Marburg viruses—also collectively known as filoviral hemorrhagic fever (FHF)—previously caused dramatic, albeit limited, outbreaks in central Africa. Their impact on global health was rather small (except in the realm of biological warfare research) because of the high mortality rate, lack of effective antiviral therapies and vaccines, and potential for person-to-person transmission.[1] The 2014 West African outbreak of EVD proved that these filoviruses should no longer be considered as merely regional problems. A short review of EVD and its clinical relevance to the nonendemic countries is presented. The current epidemic is caused by *Zaire ebolavirus*; however, references will also be made to the related *Marburgvirus*, which shares many virological, clinical, and epidemiological characteristics.

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Virology and pathogenesis

The order *Mononegavirales* consists of enveloped, nonsegmented, negative-sense, single-stranded RNA viruses. The Family *Filoviridae* comprises three genera: *Cuevavirus*, *Ebolavirus*, and *Marburgvirus*.[2] In 2011, the sole species of *Cuevavirus*, *Lloviu cuevavirus*, was described. It was discovered during an investigation of massive die-offs of *Miniopterus schreibersii* bats in France, Spain, and Portugal in 2002; and the virus was detected in bat carcasses collected from northern Spain.[3] The genus *Ebolavirus* (*EBOV*) includes five species, *Bundibugyo ebolavirus* (*BEBOV*), *Reston ebolavirus* (*REBOV*), *Sudan ebolavirus* (*SEBOV*), *Taï Forest ebolavirus* (*TAFV/CIEBOV*; previously called *Ivory Coast ebolavirus* or *Côte d'Ivoire ebolavirus*), and *Zaïre ebolavirus* (*ZEBOV*). The genus *Marburgvirus* consists of one species: *Marburg marburgvirus* (*MARV*).

The name "filovirus" describes a unique morphological characteristic of the viruses. The virions are generally filamentous (in Latin, *filum* means "thread") with a diameter of approximately 80 nm and a highly variable length of 800–14,000 nm. They may also appear as branched filaments, short rods, U-shaped, circular, or hairpin-shaped.[4,5] The genomes of *EBOV* and *MARV* are approximately 19 kb and consist of seven genes (from the 3′ to 5′ end): nucleoprotein (NP), VP35, VP40, glycoprotein (GP), VP30, VP24, and RNA-dependent RNA polymerase (L).[4,6] *Ebolavirus* expresses an additional protein through transcriptional editing of the GP gene. In addition to GP, a smaller secreted glycoprotein (sGP) is produced and excreted extracellularly.[4,7]

At 20°C, *EBOV* and *MARV* are stable and resist desiccation, which probably explains their stability in aerosol droplets.[7,8] They are however inactivated by heat and common disinfectants such as detergents, phenolics, and hypochlorites.[7] The usual heat treatment of clinical samples at 56°C for 30 minutes may fail to render the specimen noninfectious. Thermal inactivation at 60°C for 60 minutes or 75°C for 30 minutes is necessary.[9–11] Gamma irradiation readily inactivates the filoviruses, although this method may not be readily available in routine clinical or laboratory settings.[12]

The genetics and molecular biology of the filoviral genome have been previously reviewed.[4,7] In addition to the essential functions for viral replication and assembly, many viral proteins exert their effects on the host immune system and may contribute to the pathogenesis of the infection (Table 1).[4,7,13–15,26,191,192] For example, VP35 and VP24 inhibit the normal antiviral activities of type I interferons at multiple steps of the pathway, whereas sGP may contribute to immune evasion by absorbing anti-GP antibodies (i.e., antigenic subversion).[13–15] Because of the essential roles of many viral proteins in replication and assembly, some viral proteins (e.g., VP30 and VP35) are potential targets for antiviral agent development.[16,17]

In recent years, the pathogenesis of FHF has been better elucidated.[18] Filoviruses are pantropic with the ability to infect different host cell types. The initial cells in which the viruses replicate are likely dendritic cells, macrophages, monocytes, and Kupffer cells at the site of entry. A large number of lectins (e.g., DC-SIGN and L-SIGN) and immunorecognition receptors [i.e., triggering receptors expressed in myeloid cells (TREM)] can serve as receptors for the viruses.[19] After the initial multiplication in the aforementioned cell types, the viruses are transported to the reticuloendothelial system (e.g., lymph nodes, spleen, liver) and other organs where infection of other cell types occur. The resulting massive necrosis and end organ damage are reflected in the histopathology of human and primate infection models with necrosis in the liver, kidneys, lungs, lymphoid tissues, and other organs.[20,21] In addition, various mechanisms contribute to the development of coagulopathy and disseminated intravascular coagulation, which is a hallmark of viral hemorrhagic fevers. Patients with FHF develop significant platelet dysfunction (which is not merely accountable for by thrombocytopenia), and this is contributed to by platelet activation and decreased vascular endothelium production of prostacyclin.[22] Another important target in the pathogenesis of FHF is the endothelium. Human and primate endothelial cells are susceptible to infection by *EBOV*, although direct cyopathic effects are not an important factor in the development of vasculopathy and coagulopathy.[23] The release of vasoactive

Table 1 Filovirus genes and their functions.[4,7,13–15,26,191,192]

Viral genes and proteins	Function in the viral life cycle	Effects on hosts
Nucleoprotein	Viral nucleocapsid assembly; budding	May be a main virulence mechanism
VP35	Viral nucleocapsid assembly	Type I interferon antagonist
VP40 (matrix protein)	Viral nucleocapsid assembly; budding; structural integrity of viral particles	
Glycoprotein	GP: a transmembrane protein; viral attachment to and entry into host cells Likely receptors: cell surface lectins	GP: induces proinflammatory cytokines sGP: possibly contributes to immune evasion by antigenic subversion
VP30	RNA-binding protein, stabilizes nascent RNA; activates RNA transcription; regulates the replication cycle	
VP24 (matrix protein)	Viral assembly; budding	Inhibits interferon signaling and activation May be a main virulence mechanism
RNA-dependent RNA polymerase (L)	Viral transcription	

GP = glycoprotein; NP = nucleoprotein; sGP = secreted glycoprotein.

factors (e.g., nitric oxide) or cytokines and chemokines from monocytes and macrophages (e.g., tumor necrosis factor-alpha, interferons, monocyte chemoattactant protein-1, interleukin-8) could be important in the genesis of vascular damage.[23] The combined effects of increased secretions of proinflammatory cytokines, activation of the coagulation cascade, consumption and/or reduced production of protein C, thrombocytopenia and platelet dysfunction, hepatic damage with impaired production of coagulation factors, and vascular damage contribute to the development of coagulopathy in FHF.[24]

Protective humoral and cellular immune responses can be demonstrated in patients who survive, and antibody levels persist for years.[25] In addition to the various immune evasion mechanisms alluded to previously [e.g., binding of anti-GP antibodies by sGP (i.e., immune subversion), suppression of innate immune responses, and inhibition of interferon pathways], other potential virulence and pathogenic mechanisms of filoviruses such as the NP and VP24 proteins have been identified.[26] The generation of antibodies towards GP is critical for the protective efficacy of vaccines.[27]

Epidemiology and transmission

Ebolavirus and *MARV* are geographically restricted to and cause outbreaks in sub-Saharan Africa; however, *REBOV* is found in nonhuman primates and pigs in the Philippines (Table 2).[5,68,80,176,193,194] The first filovirus was discovered in 1967 after an outbreak of Marburg hemorrhagic fever infection in Germany. This infection originated from *Cercopithecus aethiops* monkeys that were imported from Uganda.[28] In 1976, the first natural outbreak of *ZEBOV* occurred in northern Zaïre (now called the Democratic Republic of the Congo).[29] In 1976, *SEBOV* was discovered in an outbreak in Nzara in Sudan.[30] In 2007–2008, *BEBOV* caused an outbreak in Uganda, followed by another outbreak in the Democratic Republic of the Congo in 2012.[31,32] In 1994, *TAFV* was discovered in a Swiss biologist who acquired the infection in the Republic of Côte d'Ivoire (i.e., the Ivory Coast).[33] In 1989, *REBOV* was first discovered in monkeys (*Macaca fascicularis*) that were exported from the Philippines to Reston, Virginia, USA The infected monkeys were subsequently exported to Italy (1992–1993) and to Texas (1996). Cynomolgus macaques in the Philippines are naturally infected, as are pigs. The *REBOV* virus appears to be nonpathogenic to humans.[34–36]

Human filovirus infections are primary zoonotic diseases with a high propensity for interpersonal transmission. The primary animal reservoir of *EBOV* and *MARV* are bats (especially fruit bats).[37] Bats support long-term viral replication without developing the disease.[38] Virological and serological studies have confirmed filoviral infection in diverse bat species.[37,39,40] In the Philippines, China, and Bangladesh, there is also evidence of *REBOV* (and possibly other filoviral) infection in bats.[41–43] Cases of FHF have been epidemiologically linked to contact with bat carcasses, spelunking among tourists, and outbreaks among gold miners who work in caves.[44–46] Peak seasons of bat *MARV* infection have been correlated with the incidence of human "spillover"infections.[47] Primates are also naturally infected with filoviruses, although they are likely to be infected by natural reservoir hosts (e.g., bats) and are not considered important reservoirs.[48,49] Primates, nevertheless, remain important vectors for the introduction of the disease into humans in rural Africa, and wild animal mortality sometimes precede human outbreaks of EVD.[50] Other mammals that can be infected by EVD include pigs (especially by *REBOV* and *ZEBOV*) and dogs; however, their role in causing human EVD outbreaks is uncertain.[51–53]

Human infections typically occur with a few patterns of transmission. Inhabitants of endemic areas, especially individuals dwelling in forests with occupational exposure to wild animals (e.g., hunters and people handling bushmeat), may develop symptomatic or subclinical infections, presumably because of the exposure to animal reservoirs of the virus. In some cases of human infection, a history of direct exposure to animal carcasses (such as primates, bats, duiker antelopes, and porcupines) or indirect exposure to bats in caves has occurred.[44–46,54,55] These single or multiple introductions to human populations may result in short chains of human transmission. When FHF patients are admitted to healthcare facilities where infection control measures are inadequate, a large outbreak may occur with transmission to other patients and healthcare workers. Hospitals have been a major amplifying and disseminating factor in many previous outbreaks of FHF in Africa.

Direct person-to-person transmission of *EBOV* and *MARV* occurs via blood or body fluids by percutaneous inoculation or by mucosal exposures.[56–58] During an outbreak of *SEBOV*, the virus was detected by viral culture or reverse transcription polymerase chain reaction (RT-PCR) in patients' saliva, skin swab, stool, tears, breast milk, and semen, but not in environmental samples.[59] In view of the persistence of the viruses in semen and breast milk at 91 days and 15 days, respectively, after illness onset,[58,59] the use of condoms and withholding breastfeeding are recommended during convalescence.

More than 80% of patients in the 1995 Kitwit outbreak in the Democratic Republic of the Congo had secondary cases in the household; the risk was highest for people with direct physical contact with the body fluids of symptomatic patients and exposure to patients in the late stages of the disease; this finding can be explained by the high viral load at this phase of disease.[58] Reusing needles or other medical instruments without adequate sterilization, needle stick injuries, lack of isolation facilities, and inadequate and inappropriate use of personal protective equipment all contribute to explosive hospital outbreaks. African burial customs of washing dead bodies and transporting bodies without barrier precautions further transmit the disease in the community.[29,58,60,61] Infected pigs can transmit *REBOV* to nonhuman primates via the airborne route and nonhuman primates can be infected experimentally by the inhalation of droplets (droplet size of 0.8–1.2 μm), although parenterally infected primates do not transmit the infection via the airborne route.[62–64] True airborne transmission of *EBOV* between humans has likewise not been documented. Up to 40% of patients in some *EBOV* outbreaks may have no known contact or exposure history, although it remains unclear whether this is because of fomites, true airborne transmission, or inadequate investigation.[57,58,61,65]

Table 2 Outbreaks of human filovirus infection.[5,68,80,176,193,194]

Virus strain	Year	Location	No. of cases	Case-fatality rate (%)
Endemic situation				
Zaïre ebolavirus	1976	Zaïre (now called the Democratic Republic of the Congo)	318	88
	1977	Zaïre	1	100
	1994	Gabon	52	60
	1995	Zaïre	315	81
	1996	Gabon	37	57
	1996—1997	Gabon	60	74
	2001—2002	Gabon	65	82
	2001—2002	Republic of the Congo	57	75
	2002—2003	Republic of the Congo	143	89
	2003	Republic of the Congo	35	83
	2007	Democratic Republic of the Congo	264	71
	2008—2009	Democratic Republic of the Congo	32	47
	2014 (as of January 14, 2015)	Guinea, Liberia, Sierra Leone, Nigeria, Senegal, Mali	20,741	40
	2014	Democratic Republic of the Congo	66	74
Sudan ebolavirus	1976	Sudan	284	53
	1979	Sudan	34	65
	2000—2001	Uganda	425	53
	2004	Sudan	17	41
	2011	Uganda	1	100
	2012	Uganda	11	36
	2012—2013	Uganda	6	50
Bundibugyo ebolavirus	2007—2008	Uganda	149	25
	2012	Democratic Republic of the Congo	36	36
Marburgvirus	1980	Kenya	2	50
	1987	Kenya	1	100
	1998—2000	Democratic Republic of the Congo	154	83
	2004—2005	Angola	252	90
	2007	Uganda	4	25
	2012	Uganda	15	27
	2014	Uganda	1	100
Imported, nosocomial, or laboratory-acquired in nonendemic countries				
Zaïre ebolavirus	1996	South Africa ex-Gabon	2	50
	1996	Russia	1 (laboratory accident)	100
	2004	Russia	1 (laboratory accident)	100
	2014 (as of 29 October 2014)	USA ex Liberia and Guinea	2	50
	2014 (as of 29 October 2014)	USA (healthcare workers)	2	0
	2014 (as of 29 October 2014)	Spain ex-Sierra Leone	1	100
	2014 (as of 29 October 2014)	Spain (healthcare worker)	1	0
	2014 (as of January 14, 2015)	UK ex-Sierra Leone	1	0
Sudan ebolavirus	1976	England	1 (laboratory accident)	0
Taï Forest ebolavirus	1994	Switzerland ex-Côte d'Ivoire	1	0

(*continued on next page*)

Table 2 (*continued*)

Virus strain	Year	Location	No. of cases	Case-fatality rate (%)
Reston ebolavirus (based on seroprevalence studies)	1989–1990, 1996	USA	0–3% seropositive (188 cases with exposure to monkeys tested)	0
	1992	Italy	0% seropositive (16 cases with exposure to monkeys tested)	0
	1898–1990, 1993, 1996	Philippines	0–17% seropositive (458 cases with exposure to monkeys tested)	0
	2008–2009	Philippines	6% seropositive (332 cases with exposure to pigs tested)	0
Marburgvirus	1967	Germany, Yugoslavia	31 (exposure to monkeys from Uganda)	23
	1975	South Africa ex-Zimbabwe	3	33
	1990	Russia	1 (laboratory accident)	100
	2008	USA ex-Uganda	1	0
	2008	Netherlands ex-Uganda	1	100

UK = United Kingdom; USA = United States of America.

The ongoing EVD outbreak at the time of this writing is unique in two aspects: (1) its unprecedented scale and (2) its origination in West Africa (Guinea) rather than in central Africa. The first case occurred in Guinea in February 2014, and subsequently spread to the African countries of Liberia, Sierra Leone, Nigeria, Senegal, and Mali. In October 2014, the World Health Organization declared the epidemic over in Senegal and Nigeria.[66,67] Further epidemiological investigations suggested the index case was probably a 2-year-old child who died on December 6, 2013 in southern Guinea.[60] The infection was subsequently transmitted from the child to family members, a village midwife, and a healthcare worker; some of these patients later caused outbreaks in different areas of Guinea. Ebola virus disease has caused infection in 838 local healthcare workers of whom 495 (59%) workers died (as of January 7, 2015); four of these infected healthcare workers were citizens of the United States of America, Spain, and the United Kingdom who returned to their respective countries for further management.[68] As of September 14, 2014, the basic reproduction number in this epidemic ranged from 1.71 to 2.02, which is similar to previous estimates of 1.34 to 2.70.[69–71] The virus strain causing the current outbreak is *ZEBOV*, which shows 97% identity to the *EBOV* strains that caused outbreaks in Gabon and the Democratic Republic of the Congo in 2002.[60,72] A concurrent but unrelated outbreak of EVD occurred in the Democratic Republic of the Congo from August 2014 to November 2014; it involved 66 cases and 49 deaths. The index case had a history of exposure to bushmeat.[73,74]

Clinical manifestations

Filoviral hemorrhagic fever is characteristically a highly fatal disease. The average case–fatality ratio of FHF in previously reported outbreaks caused by *ZEBOV*, *SEBOV*, *BEBOV*, and *MARV* in endemic countries (Table 2) are 0.72 (range, 0.40–0.89), 0.50 (range, 0.36–0.65), 0.31 (range, 0.25–0.36), and 0.55 (range, 0.25–0.90), respectively (excluding situations that involved only one case). *Reston ebolavirus* causes asymptomatic infection among contacts of infected primates or pigs.[34] On the other hand, asymptomatic infection was described in 1996, as evidenced by seroconversion in 46% of close contacts of patients, in two *ZEBOV* outbreaks in Gabon.[75] In 64% (7/11) of the seroconverted asymptomatic contacts, RT-PCR of the peripheral blood mononuclear cells was positive for the virus, and viremia persisted up to 2 weeks in some contacts. Another line of evidence for asymptomatic infections comes from seroprevalence studies in Africa, in which 2.2–15.3% of the surveyed population in central Africa was seropositive for *EBOV* with the seroprevalence consistently higher among forest-dwelling populations and hunters.[76–78]

The incubation period of EVD varies from 2 days to 21 days (commonly, 6–10 days), but a recent analysis of the cases in the 1995 *ZEBOV* outbreak in Kitwit suggested that the mean incubation period was 12.7 days, and the maximum incubation period was up to 25 days.[79] A biphasic illness has been described with an apparent remission of 1–2 days in between.[5,6,80–82] The disease usually begins abruptly with nonspecific symptoms such as fever (93–95% of patients, *ut infra*), malaise (85–95%), headache (52–74%), sore throat, odynophagia or dysphagia (56–58%), hiccoughs (5–17%), and nonproductive cough (7–26%). Abdominal pain (62–68%) or nausea and vomiting (68–73%) often precede the onset of diarrhea (84–86%), usually 5 days after the onset of illness). The abdomen can be tender on palpation. In the absence of supportive therapy, diarrhea and vomiting may lead to fluid depletion

and electrolyte disturbances such as hypokalemia. Other symptoms include arthralgia or myalgia (50–79%), chest pain (5–10%), and conjunctival injection (42–47%). A diffuse erythematous rash (14–16%) appears towards the end of the 1st week which will later desquamate. The nonpruritic rash appears first on the trunk, and then spreads to the entire body with sparing of the face. Three early symptoms of bilateral conjunctival injection, rash, and sore throat are suggestive of EVD in the differential diagnosis.[82] After the appearance of the rash, patients may either gradually recover or, in severe cases, progress over 7–14 days to the full-blown hemorrhagic fever syndrome with petechiae (8%), gum bleeding (0–15%), melaena (8–16%), hemoptysis (0–11%), hematemesis (0–13%), epistaxis (0–2%), hematuria (7–16%), menorrhagia, bleeding at venipuncture sites (5–8%), and show features of disseminated intravascular coagulation.[82,83] The typical hemorrhagic fever picture, however, occurs only in approximately 40% of the patients (range, 17–71%).[82,84] Other manifestations in the late stage include evidence of multiorgan failure such as circulatory shock, obtundation, tachypnea, renal shutdown, convulsion, delirium, and coma. Fever is often absent at this stage. Death often occurs between Days 6 and 16, whereas patients who survive will show improvement around Days 6 to 11.[6] Intrauterine death is common in pregnant patients. Mortality among pregnant women is substantial but may not be significantly higher than for nonpregnant patients; and pregnant women do not have an increased susceptibility to the infection.[85,86] Survivors tend to improve from the 2nd week of illness. They make a slow recovery during which arthralgia (which could be asymmetric and migratory and often involves the large joints), uveitis, conjunctivitis, orchitis, parotitis, hearing loss, and tinnitus may occur.[80,82] Chronic infection by filoviruses has not been documented, but male patients may shed the virus in the semen for 40–91 days after the onset of illness, and the transmission of *MARV* has occurred via sexual intercourse.[58,59,87,88]

Common laboratory findings include lymphopenia, thrombocytopenia, elevated aminotransferases, hyperproteinemia, proteinuria, and prolonged prothrombin and activated partial thromboplastin time.[6] With the progression of disease, evidence of disseminated intravascular coagulation and renal failure will appear. Disease progression is also associated with worsening lymphopenia and rising antigenemia.[89]

Compared to survivors, patients with fatal EVD are more likely to have tachypnea.[82] Patients with disease also have a significantly higher level of viremia and a much weaker humoral immune response to the infection.[90] Significant differences in a number of cytokine and chemokine levels have also been detected between patients with nonfatal and fatal EVD; in particular, the levels of many proinflammatory cytokines and nitric oxide are higher in nonsurvivors (with the possible exception of *BEBOV*), whereas the level of T cells and $CD8^+$ T cells were lower.[25,89,91–94] A high viral load is of prognostic significance. In *SEBOV* FHF, patients with fatal cases had an average of 10^8–10^9 (up to 10^{10}) copies of RNA/mL of serum, compared to the approximately 10^7 copies of RNA/mlL of serum in survivors.[90] Patients with *MARV* FHF likewise have high levels of viremia in blood with a median level of 4.1×10^4 (range, 2.62×10^2–9.33×10^8)/mL of serum serum.[95]

Laboratory diagnosis

There are no pathognomonic signs or symptoms in the early stages of EVD. The most useful history is epidemiological linkage to possible cases by travel history or by contact with known or suspected cases. Laboratory investigations are necessary to confirm the diagnosis. Other differential diagnoses (*vide infra*) should be excluded, as appropriate.

All clinical specimens must be handled with great care from their collection to transport and testing in the laboratories. Laboratory-acquired infection of *EBOV* has occurred via percutaneous exposures.[96] *Marburgvirus* retains its infectivity in dried blood for at least 5 days.[11] Filoviruses can be inactivated by heat [60°C for 60 minutes or 75°C for 30 minutes; or 60°C for 15 minutes in the presence of 0.2% (final concentration) sodium dodecyl sulfate or 0.1% (final concentration) Tween 20] or inactivated by 1% sodium deoxycholate solution, acetone, diethyl ether, 1% formalin, methanol, sodium hypochlorite, glutaraldehyde, 2% peracetic acid, phenolic disinfectants, and osmium tetroxide (used in fixation for electron microscopy).[11] Ultraviolet light is an effective means for surface disinfection. Depending on the type of specimen and testing methodology, treatment of the sample with either Triton X-100, Tween 20, sodium dodecyl sulfate, beta-propiolactone, chloramine B, or 3% acetic acid (pH 2.5) should be done before routine hematological, biochemical, and serological testing.[11] Heat inactivation is recommended for blood sodium, potassium, magnesium, urate, urea, creatinine, bilirubin, glucose, and C-reactive protein; however, other methods of inactivation would be necessary for calcium, phosphate, albumin, transaminases, gamma-glutamyltransferase, and creatine kinase determination.[11]

Standard virological techniques also apply to the diagnosis of FHF. These include viral culture, electron microscopy, serological tests using antigen or antibody detection, and nucleic acid amplification.[5,97] Filoviruses can be cultivated from clinical specimens (especially blood and liver samples); however, because of the associated biohazards, a viral culture is not—and should not be—routinely performed, except in facilities that can handle biosafety level 4 agents. Electron microscopy, which has excellent specificity because of the unique morphology of filoviruses, is also not routinely performed because of biosafety considerations, limited availability of electron microscope facilities in routine diagnostic settings, and the relatively high viral load necessary for visualization.

The detection antibodies [e.g., immunoglobulin M (IgM) or immunoglobulin G (IgG)] is commonly achieved using immunofluorescent assays and enzyme-linked immunoassay (ELISA) against recombinant NP, GP, VP40, VP35, or VP30 antigens.[97–99] The appearance of IgM and IgG antibodies occurs at approximately 2 days and 6–18 days, respectively, after the onset of illness.[6] Various antigen detection assays have been developed for the diagnosis of FHF and some have been used in field situations. The techniques include antigen-capture ELISA, immunofluorescent assay, dot-immunobinding assay, immunofiltration assay with different genus-specific or

species-specific reactivity towards common targets such NP, GP, and VP40 proteins.[97,100,101] A practical limitation of these serological assays is the limited availability of these tests in laboratories in nonendemic countries.

Nucleic acid amplification is the diagnostic test of choice because of its high sensitivity (especially in the early phase of illness); its ability to differentiate between different agents of viral hemorrhagic fever; and its relatively lower biohazard, if the viruses are appropriately inactivated; and because antigen and antibody assays are often unavailable in laboratories in nonendemic countries. When the viral load is determined by quantitative assays, prognostic information can also be obtained. The most commonly used test is RT-PCR. A reverse transcription—loop-mediated isothermal amplification assay has also been developed for *EBOV* and *MARV*.[102,103] All diagnostic nucleic acid amplification tests must be adequately validated before being used clinically. If appropriately validated, the use of multiplex PCR/RT-PCR allows simultaneous detection of multiple pathogens that cause hemorrhagic fever.[104,105] The provision of nucleic acid amplification tests should preferably be centralized in national or regional reference laboratories to ensure adequate biosafety containment and quality of results. The RNA of *EBOV* can be detected in the sera by RT-PCR 24—48 hours earlier than by antigen capture; in some studies, it is detectable on Day 1 of the illness.[6,90] However, the viral load gradually reaches its peak at approximately 3 —7 days after the onset of the disease.[90] Retesting is therefore necessary if RT-PCR is initially negative but clinical suspicion is high, especially when the first sample was obtained within 3 days of the onset of disease. Blood is the most commonly used sample for RT-PCR. Oral fluid specimens can be a viable alternative to blood samples for RT-PCR in situations in which blood taking may be difficult or infeasible.[106] Common targets for nucleic acid amplification include the L, GP, and NP genes.[89,90,95,107,108]

Clinical management and vaccine development

Treatment of FHF is primarily supportive owing to the unavailability of approved, specific antiviral agents. Concurrent infections such as malaria or bacterial sepsis should be treated, as appropriate. Fluid and electrolyte replacement, blood product transfusion, renal replacement therapy, and ventilatory support such as extracorporeal membrane oxygenation should be administered, as necessary.[6,109]

Various experimental therapeutic approaches have been attempted in experimental animals or clinically; however, no randomized controlled trials prove their efficacy. Examples include recombinant inhibitor of factor VII (rNAPc2), recombinant human activated protein C, and interferon-beta.[110—112] As in cases of other severe viral infections, convalescent plasma from recovered patients has been used to treat FHF. This was deployed with apparent benefits in the 1995 EVD outbreak in Kitwit.[113] The World Health Organization (WHO) published a guideline on the collection and preparation of convalescent plasma for use in the outbreak situation; however, the WHO recognizes the uncertainties in the efficacy of this treatment.[114] Cocktails of monoclonal antibodies have similarly been used recently with some success in reducing the mortality of *EBOV* infection in nonhuman primates. These antibodies have been produced in plants and in mice.[115—117] Based on these studies, an optimized cocktail of plant-derived monoclonal antibodies, called the ZMapp, was produced; it protected 100% of rhesus macaques infected up to 5 days with *EBOV*.[118] These antibodies have been used experimentally for treating human *EBOV* patients in the 2014 West African outbreak, although the actual benefit to human EVD remains to be confirmed.

A second approach to the treatment of FHF lies in the development of specific antiviral agents. A current nucleotide analogue is favipiravir (T-705), which was developed and approved in Japan for the treatment of influenza. Favipiravir inhibits viral RNA-dependent RNA polymerase of the influenza virus. It was subsequently found to exhibit *in vitro* antiviral activities against certain other RNA viruses such as bunyaviruses, arenaviruses, flaviviruses, alphaviruses, norovirus, and *EBOV*.[119—123] Animal studies also demonstrate the efficacy of favipiravir in the treatment of Junín virus, arenavirus, and *EBOV* hemorrhagic fevers, and the drug was used to treat human EVD in the 2014 West African epidemic.[124—128] A dosing regimen of favipiravir for use in a clinical trial for the treatment of EVD has been published.[129] Another nucleotide analogue, brincidofovir (a lipid conjugate of cidofovir), was previously developed to treat infections due to DNA viruses such as adenoviruses, herpesviruses, and orthopoxviruses; the drug was granted Emergency Investigational New Drug Applications in October 2014 by the United States (US) Food and Drug Administration for evaluation in EVD treatment, and a clinical trial was started in January 2015 in Monrovia, Liberia.[130—132] The nucleoside analogue BCX4430 was recently shown to inhibit RNA polymerase of negative- and positive-sense RNA viruses via chain termination effects.[133] Its *in vivo* activity against *MARV* was demonstrated in guinea pigs and cynomolgus macaques. The development of BCX4430 for human clinical trials will require a long time.

Another approach involves the screening of currently available nonantimicrobials for their activities on filoviruses. Compounds such as selective estrogen receptor modulators (e.g., clomiphene, toremifene), amiodarone, dronedarone, and verapamil have antifilovirus activity in cell cultures and/or murine models.[134,135] In addition, RNA interference using small interfering RNAs provide post-exposure protection of animals infected with *EBOV* and *MARV*.[136—138]

A third approach to the specific management of filoviral infections explores the potential of postexposure prophylaxis. Such regimens would benefit exposed healthcare workers and other social contacts, and laboratory staff experiencing accidental exposures. Protective immunity towards filoviruses does exist, as demonstrated in the possible benefits of convalescent plasma and animal studies, in which humoral immunity (i.e., IgG) protects against *EBOV* infection.[139] Animals studies also confirm that passive immunization by neutralizing monoclonal antibodies is protective in primates.[140] Previously examined filovirus vaccine candidates that elicit protective humoral immunity experimentally include *EBOV*-like particles containing GP, NP, and VP40; replication-deficient *EBOV* mutants that lack the VP30 gene; and *EBOV* GP-containing fragment or fusion

protein.[141–144] A phase 1 clinical trial has tested DNA vaccines encoding the glycoproteins of *EBOV* and *MARV*.[145]

One of the most promising vaccine candidates for filoviruses is the recombinant vesicular stomatitis virus-based vaccine system that expresses filoviral GP. This system elicits protective immunity against *ZEBOV, SEBOV, BEBOV,* and *MARV*; it also offers substantial postexposure prophylaxis in primate and murine models.[146–152] The vaccine was used for postexposure prophylaxis in a laboratory staff person who sustained a needle stick injury with ZEBOV in 2009.[153] A similar vaccine system used replication-defective recombinant adenovirus that expressed EBOV GP was protective in animal models.[154,155] Phase 1 clinical trials of the vesicular stomatitis virus-based and adenovirus-based vaccines began in late 2014.[156]

Ebola virus disease as a problem in nonendemic countries: issues on prevention and control

The containment of FHF outbreaks in endemic countries requires substantial resources in coordination between the public health system and other authorities of the countries, surveillance of the disease, education and engagement of local citizens, isolation and treatment facilities, and laboratory support.[157,158] These requirements are often beyond the capability of endemic countries. Significant international assistance is usually needed to contain major outbreaks. The discussion on these public health issues is beyond the scope of this article. For health authorities in nonendemic countries, a preparedness plan for emerging infections is an indispensable component of the public health system. The development and adoption of preparedness and response plans for emerging infectious diseases are first fostered in anticipation of pandemic influenza. The outbreaks of severe acute respiratory syndrome (SARS), pandemic influenza A (i.e., H1N1), Middle East respiratory syndrome coronavirus (MERS-CoV), and, more recently, avian influenza A (i.e., H7N9) and EVD underscore the importance of such pre-emptive plans in preventing or mitigating the effects of disease transmission.[159,160] The details may vary, depending on the nature of the pathogens; however, key components in public health response include risk assessment; communication and education; establishing a rapid response team and management team to coordinate responses between different government ministries; monitoring and surveillance; providing adequate facilities and supplies for quarantine and infection control; developing laboratory diagnostics; and where appropriate, antimicrobial and vaccination policies, and/or stockpiling.[159,161]

For clinicians in nonendemic countries, FHF will mostly be encountered in travelers returning from endemic areas. The 2003 SARS epidemic and 2009 influenza A (H1N1) pandemic demonstrated the efficiency of international travel—especially air travel—in the global spread of infectious diseases.[162,163] As a continent, Africa has a relatively small number of international travelers (only Oceania has a smaller number of international tourist arrivals), although the exportation of FHF to nonendemic countries is well documented in the present epidemic.[164] It is tempting to consider establishing travel restrictions to limit the spread of EVD outside of Africa; however, the actual benefit of such policies is limited.[165] At the time of this writing, the WHO has issued no travel restrictions to the affected countries. However, it is prudent to issue health warnings to potential visitors to affected countries. Pretravel education on transmission routes, precautionary measures, self-monitoring of signs and symptoms, and availability of medical care in destination countries should be provided. As in other subspecialties of travel medicine, the visiting friends and relatives (VFRs) are at particularly high risk of contracting travel-related infections.[166] Despite the fact that publicity and health alerts are often announced to the general public, VFRs do not always receive the necessary information on the risks because of cultural differences, language barriers, or different perceptions. Hence, efforts must be targeted to VFRs (especially African communities in this context) to reduce the risk of disease importation.

Remote infrared thermal scanners have been used as a means of fever screening at airports in some countries. The practice first gained popularity during the 2003 SARS epidemic, and was subsequently evaluated in the 2009 influenza A (H1N1) pandemic.[167–170] To a lesser extent, this method has also been used for the screening of other febrile illness such as dengue fever.[171] Infrared thermal scanning is relatively popular in Asian countries such as Taiwan, Japan, Korea, and Hong Kong, and it is usually used in conjunction with health questionnaires for self-reported symptoms such as fever and travel history. The tympanic temperature would be measured for suspected cases. The sensitivity, specificity, and positive predictive value of thermal scanning are affected by a variety of factors such as the instruments used, the threshold temperature, the part of the body being measured, the distance of the instruments from the individual, and the previous use of antipyretic agents. Patients in the incubation period of an infection obviously will not be detected by this screening method. Despite the relatively low sensitivity, specificity, and positive predictive values of infrared thermal scanning, some investigators consider it a useful adjunctive measure for border screening, although it cannot be relied on as the sole method for screening.[167–170]

Contact tracing must be promptly initiated for any potential contacts of returned travelers diagnosed with a communicable infectious disease such as EVD. Detailed guidance on contact tracing for patients with EVD and other forms of viral hemorrhagic fevers have been published.[172,173] All suspected contacts must undergo individual risk assessments, based on the travel history, the epidemic situation in the affected countries, and the nature of potential exposure before and during travel.[173] Detailed instructions and information must be provided to the contacts, who will then be monitored for fever and the development of symptoms. The monitoring may be performed in the community or within healthcare facilities, during which some limitations in—or at least, advice against—the freedom to travel within the community or country may be necessary and interference of daily activities may be inevitable.[174] Close and empathetic liaison between the contacts and health departments is essential to ensure compliance with monitoring and to minimize psychological impacts. A preparedness plan for contact tracing, disease surveillance, clinical management,

isolation precautions, and outbreak management must be in place to manage such incidents in nonendemic countries.

Hemorrhagic fever remains an uncommon cause of fever in returned travelers, although it is severe clinically and has substantial public health implications. The risk of FHF in returned travelers is low. Ten cases of Marburg hemorrhagic fever were reported from 1967 to 2012, and all patients had a travel history to Africa.[175] Cases of imported EVD (excluding the nonpathogenic *REBOV*) were described in South Africa in 1996 (*ZEBOV*) and in Switzerland in 1994 (*TAFV*).[176] Other causes of fever in such settings must be excluded by appropriate laboratory investigations. These include (depending on the itinerary and exposure history) other causes of fever with or without hemorrhagic presentations such as meningococcal infections, severe sepsis due to other bacterial infections (including rare infections such as anthrax and plague as guided by the clinical picture and exposure history), leptospirosis, typhoid and other causes of enteric fever, rickettsioses, malaria, trypanosomiasis, visceral leishmaniasis, dengue fever, and yellow fever. In particular, malaria (which is a very common treatable cause of fever in returned travelers and is endemic in sub-Saharan Africa) must be excluded by appropriate testing. Depending on the travel destination, other causes of viral hemorrhagic fever have to be considered such as Lassa fever, *Hantavirus* infection, Crimean-Congo hemorrhagic fever, and Rift Valley fever. The travel history should also include human contacts with sick individuals in the community (e.g., attending local funerals) or in healthcare facilities (as in the case of volunteer workers).[84]

Another important exposure history is interaction with bats and other wild animals (especially primates). For example, a history of spelunking has resulted in FHF among local citizens and foreign visitors. Cases of Marburg hemorrhagic fever have occurred after exposure of visitors to bats in caves in Kenya and Uganda.[177,178]

When FHF is suspected based on travel and/or exposure histories, pre-emptive isolation is essential to minimize the risk of subsequent interpersonal spread, until the diagnosis is excluded. The routes of transmission of filoviruses are well documented. Transmission *can* be interrupted, provided that proper infection control and public health measures are implemented. Detailed infection control guidelines on caring for patients with FHF have been published.[57,179–182] In essence, the principles of isolation and use of personal protective equipment are not significantly different from standard precautions and transmission-based precautions (i.e., contact, droplet, and airborne) that are universally practiced in healthcare facilities. However, numerous studies and reviews have confirmed that the compliance with the choice and use of personal protective equipment among healthcare workers are almost always suboptimal.[183–185] Essential factors that ensure the optimal implementation of infection control protocols are adequate training of healthcare workers on isolation procedures and on the appropriate use of personal protective equipment, preferably by interactive training with clear instructions; adequate manpower and organizational support; and the availability of timely and adequate guidance and support.[186–188] Such training should not be limited to staff working in clinical areas; it must also include other healthcare workers such as laboratory workers and paramedical personnel. The key elements of infection control consist of patient placement (e.g., isolation facilities), strict adherence to hand hygiene (e.g., the WHO's "five moments for hand hygiene"), proper use of personal protective equipment such as gloves (e.g., double gloves in special circumstances), waterproof gowns, respiratory protection (e.g., surgical masks, respirators during aerosol-generating procedures), eye protection, rubber boots, and safe handling of sharp objects.[55] Support staff engaged in environmental disinfection, funerals, and burial services must also be adequately trained on the proper use of personal protective equipment and chemical disinfectants, and on the handling of human remains to ensure adequate disinfection and minimize the risk of accidental exposure to the virus.[55,189,190]

Conclusion

Filoviral hemorrhagic fevers are uncommon, but they pose real clinical and public health threats to countries outside endemic areas. The routes of transmission, clinical manifestations, and infection control measures are well documented. Prevention of secondary transmission is possible, provided that cases are promptly identified, and standard isolation and precautionary measures are strictly followed. As in cases of pandemic influenza or other epidemic-prone infections, a preparedness plan is essential to cope with the importation of the diseases and limit their subsequent spread. To minimize the risk of disease importation, it is essential to have a concerted program to engage VFRs and other at-risk travelers by community health education and advisories. The current EVD outbreak provides an opportunity to evaluate new therapeutic and prophylactic strategies, whereas their actual value of these strategies in controlling the current and future epidemics require detailed clinical evaluations.

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