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DISEASE BRIEFING: EBOLA HEMORRHAGIC FEVER

AN ABBREVIATED ENTRY

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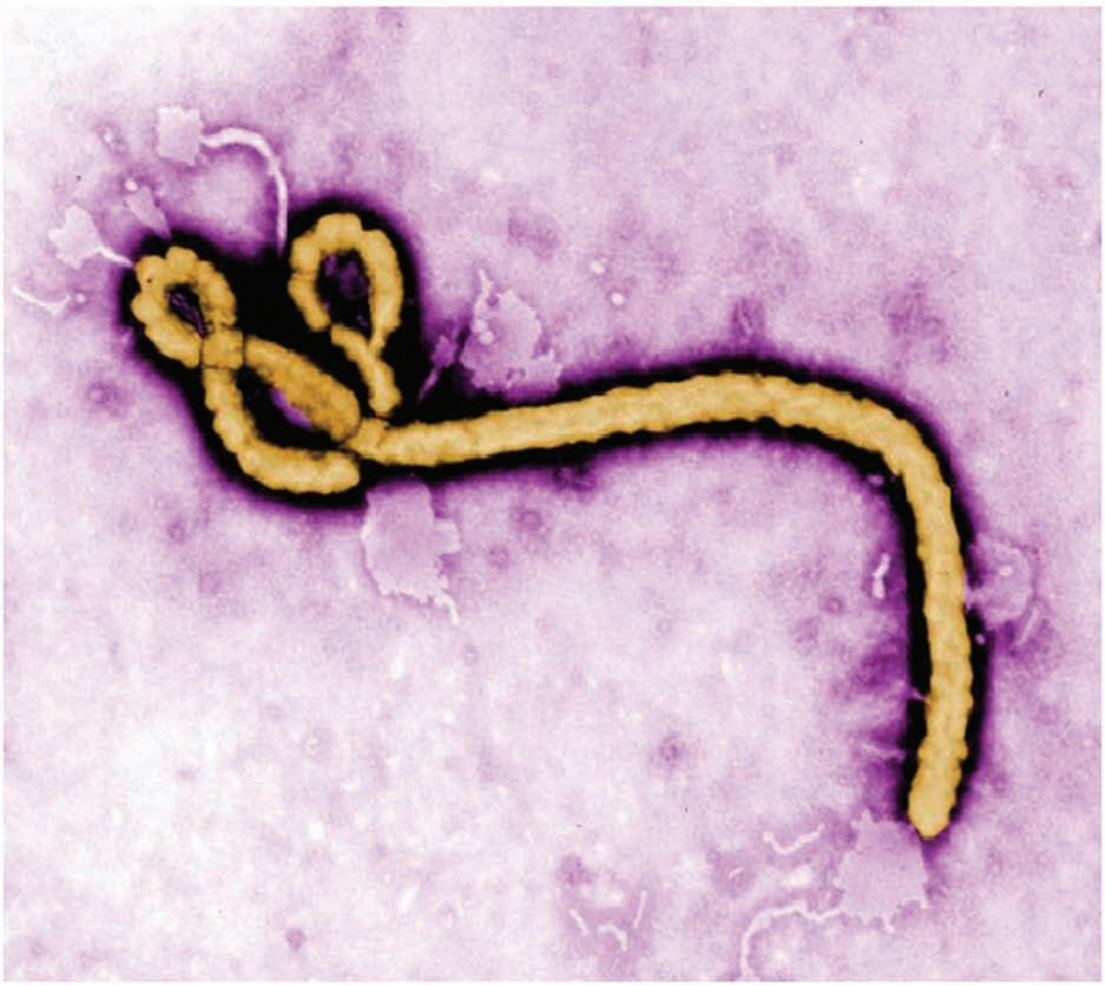
IN THIS ISSUE

FACTS ABOUT EBOLA HEMORRHAGIC FEVER

Ebola hemorrhagic fever is a severe hemorrhagic illness caused by infection with one of several Ebola viruses. Ebola is an extremely virulent pathogen and represents a major public health threat in equatorial Africa (Choi, J.H. et al (2013)). Several outbreaks in humans and nonhuman primates have been registered in the decades since the virus was first identified in the mid-1970s, most recently in Uganda and the Democratic Republic of Congo during the summer of 2012 (Mbonye, A. et al (2012); Unknown Author (2012); Li, Y.H. et al (2013)) and in Guinea and Liberia in the spring of 2014 (Baize, S. et al (2014)).

Infection with Ebola virus causes profound immune suppression and a systemic inflammatory response that culminates in potentially fatal damage to the vascular, coagulation and immune systems (Feldmann, H. et al (2011)). The case fatality rate of the disease is as high as 90%, leading to concern that Ebola virus could be used as an agent of biological warfare (Madrid, P.B. et al (2013)). There is currently no treatment or vaccine available for either humans or animals (Choi, J.H. et al (2013)).



Created by CDC microbiologist Frederick A. Murphy, this colorized transmission electron micrograph (TEM) reveals some of the ultrastructural morphology displayed by an Ebola virus virion. Photo courtesy of Centers for Disease Control and Prevention, Public Health Image Library.

EBOLA VIRUS

THE EBOLA VIRUS

Viral hemorrhagic fevers are a diverse group of life-threatening animal and human diseases caused by RNA viruses belonging to four discrete families: Arenaviridae, Filoviridae, Bunyaviridae and Flaviviridae (Ippolito, G. et al (2012)).

The Ebola viruses and the related Marburg virus (Lake Victoria Marburgvirus) belong to the Filoviridae family, order Mononegavirales. Like other filoviruses, Ebola is an enveloped, non-segmented, single-stranded, negative-sense RNA virus. Ebolavirus (EBOV) particles are filamentous with a uniform diameter (80 nm) but vary in length, reaching up to 14,000 nm (Feldmann, H. et al (2011); Leroy, E.M. et al (2011)).

The 18.9-kb viral genome consists of eight major subgenomic mRNAs encoding for seven structural proteins organized in the following fashion: 3′ leader - nucleoprotein (NP) - virion protein (VP) 35 - VP40 - glycoprotein (GP) - VP30 - VP24 - RNA-dependent RNA polymerase (L)-5′ trailer, and one nonstructural protein (sGP) (Feldmann, H. et al (2011); Leroy, E.M. et al (2011)). The ribonucleoprotein complex, which is involved in viral transcription and replication, is composed of a genomic RNA molecule encapsulated by NP linked to VP30 and VP35 and the RNA-dependent RNA polymerase (Leroy, E.M. et al (2011)). The trimeric glycoprotein GP forms surface spikes on the virion envelope that mediate cellular attachment and entry; GP acts as a shield, impeding antiviral immunity, and is believed to be a major determinant of pathogenicity (Reynard, O. et al (2009)). VP40, the most abundant protein, is associated with the inner surface and drives the process of viral budding (Harty, R.N. (2009); Silva, L.P. et al (2012)). VP24, VP35 and NP are required for the formation of the nucleocapsid (Beniac, D.R. et al (2012)) and are important determinants of pathogenicity (de Wit, E. et al (2011)). VP35 acts as a type I interferon antagonist, while VP24 interferes with interferon signaling (Feldmann, H. et al (2011)). More recently, VP35 has also been shown to bind to and mask the viral RNA, preventing the host immune system from attacking it (Leung, D.W. et al (2010)). The three-dimensional structure and organization of EBOV have been determined using cryo-electron microscopy and cryo-electron tomography (Beniac, D.R. et al (2012)).

Five distinct Ebola viruses have been described. Four of these are native to Africa and are pathogenic to humans as well as nonhuman primates: Sudan Ebola virus (SEBOV, discovered in 1976), Zaire Ebola virus (ZEBOV, discovered in 1976), Côte d'Ivoire Ebola virus (CIEBOV, discovered in 1994) (Feldmann, H. et al (2011)) and Bundibugyo Ebola virus (BEBOV, discovered in 2007) (MacNeil, A. et al (2010); Wamala, J.F. et al (2010)). A fifth virus, Reston Ebola virus (REBOV, discovered in 1989), is found only in the Philippines and to date appears to cause disease only in nonhuman primates and domestic pigs (Miranda, M.E. et al (2011)). Virulence differs among the different virus strains, ZEBOV being associated with the highest case-fatality rate (Feldmann, H. et al (2011)). In addition to their pathogenicity in humans, Ebola viruses are also a significant cause of morbidity and mortality in great apes (Leroy, E.M. et al (2011)).

IMPORTANT RNA VIRUSES AND THE DISEASES THEY PRODUCE IN HUMANS

FAMILY/CHARACTERISTICS	VIRUSES	DISEASES
Orthomyxoviruses (Orthomyxoviridae) Single-stranded RNA, enveloped (No DNA step in replication; negative-sense genome; segmented genome)	Influenza A and B virus	Upper respiratory infection, croup
Paramyxoviruses (Paramyxoviridae) Single-stranded RNA, enveloped (No DNA step in replication; negative-sense genome; nonsegmented genome)	Parainfluenza 1-3 virus	Upper respiratory infection, croup
	Respiratory syncytial virus	Upper respiratory infection, croup
	Measles virus	Measles
	Mumps	Aseptic meningitis
Coronaviruses (Coronaviridae) Single-stranded RNA,enveloped (No DNA step in replication; positive-sense genome)	Human coronaviruses	Upper respiratory infection
Rhabdoviruses (Rhabdoviridae) Single-stranded RNA, enveloped (No DNA step in replication; negative-sense genome; nonsegmented genome)	Rabies virus	Rabies
Picornaviruses (Picornaviridae) Single-stranded RNA, nonenveloped	Rhinoviruses	Common cold
	Hepatitis A virus	Hepatitis
	Enteroviruses: • Polioviruses • Coxsackie A24 viruses Coxsackie B viruses • Coxsackie B1-5 viruses • Coxsackie A9 viruses Echoviruses	Paralysis Acute hemorrhagic conjunctivitis Myocarditis, pericarditis Aseptic meningitis Aseptic meningitis Aseptic meningitis, encephalitis
Caliciviruses (Calciviridae) Single-stranded RNA, nonenveloped	Norwalk virus	Gastroenteritis
	Hepatitis E virus	Hepatitis
Togaviruses (Togaviridae) Single-stranded RNA, enveloped (No DNA step in replication; positive-sense genome)	Alphaviruses (Group A arboviruses)	Encephalitis, hemorrhagic fever
	Rubivirus	Rubella
Flaviviruses (Flaviviridae) Single-stranded RNA, enveloped (No DNA step in replication; positive-sense genome)	Group B arboviruses	Encephalitis, hemorrhagic fever
	Hepatitis C virus	Hepatitis
	Dengue virus	Dengue fever
Bunyaviruses (Bunyaviridae) Single-stranded RNA, enveloped (No DNA step in replication; negative-sense genome; segmented genome)	Some arboviruses	Encephalitis, hemorrhagic fevers
	Hantavirus	Fever, renal involvement
Reoviruses (Reoviridae) Double-stranded RNA, nonenveloped	Human rotaviruses	Gastroenteritis

IMPORTANT RNA VIRUSES AND THE DISEASES THEY PRODUCE IN HUMANS, CONTINUED

FAMILY/CHARACTERISTICS	VIRUSES	DISEASES
Arenaviruses (Arenaviridae) Single-stranded RNA, enveloped (No DNA step in replication; negative-sense genome; segmented genome)	Lymphocytic choriomeningitis (LCM virus)	Meningitis
	Lassa virus	Hemorrhagic fever
Retroviruses (Retroviridae) Single-stranded RNA, enveloped (DNA step in replication)	HTLV-I, HTLV-II	T cell leukemia, lymphoma, paresis
	HIV-1, HIV-2	AIDS
Filoviruses (Filoviridae) Single-stranded RNA, enveloped (No DNA step in replication; negative-sense genome; nonsegmented genome)	Marburg virus	Marburg disease
	Ebola virus	Ebola hemorrhagic fever

TRANSMISSION AND LIFE CYCLE

Filoviruses are zoonotic, and fruit bats are widely considered to be reservoir species and primary source of infection. Bats of the Pteropodidae family appear to be a natural reservoir for Zaire Ebola virus (Ebola hemorrhagic fever. WHO Fact Sheet no. 103 (World Health Organization, August 2012)), and naturally infected fruit bats have been identified in the endemic region. Other potential reservoirs and vectors may also exist, however (Feldmann, H. et al (2011); de Wit, E. et al (2011)). In the case of REBOV, swine have been identified as a natural host species (Barrette, R.W. et al (2009)), and *Rousettus amplexicaudatus* fruit bats may be a natural reservoir species (Taniguchi, S. et al (2011)).

Ebola virus is often transmitted to humans from the carcasses of infected animals. In the endemic region of Africa, chimpanzees and other primates are often used as food. Once the virus has infected one human it can be transmitted to others, usually through mucosal surfaces or breaks in the skin, when an individual comes into direct contact with body fluids (blood, semen, genital secretions), skin or nasal secretions from infected individuals or cadavers. Person-to-person transmission of EBOV is relatively inefficient, as seen by a secondary attack rate of approximately 10%. Aerosols and droplets are not considered important routes of transmission, although they may contribute (Feldmann, H. et al (1996)). Transmission may also occur in the healthcare or laboratory setting. During the seminal outbreaks in Sudan and Zaire in 1976, reuse of contaminated needles contributed significantly to the spread of the disease (Feldmann, H. et al (2011)). Transmission from infected mothers to infants via breast milk has been hypothesized (Feldmann, H. et al (2011)).

PATHOGENESIS, MORBIDITY AND MORTALITY

The virus is trophic for a range of cell types, but infects and replicates preferentially in monocytes, macrophages and dendritic cells. These cells also play an important role in the subsequent dissemination of the virus throughout the body via the lymphatic system and blood (Feldmann, H. et al (2011); Leroy, E.M. et al (2011)). Viral replication is intense, mostly occurring in secondary lymphoid organs and the liver. The virus subsequently spreads to hepatocytes, endothelial cells, fibroblasts and epithelial cells. In spite of the intensity and extent of viral replication in organs such as the liver, the resulting damage does not appear to be sufficient to result in death or other severe manifestations of Ebola hemorrhagic fever, suggesting that the host response must also contribute to pathogenesis. The exaggerated release of cytokines and other inflammatory mediators results in a cytokine storm with detrimental consequences ranging from vascular leakage to T-cell apoptosis (Leroy, E.M. et al (2011)). An early, robust and balanced immune response, characterized by IgM response within two days of symptom onset and IgG response within 5-8 days of symptom onset, is associated with a more favorable outcome (Hoenen, T. et al (2012)).

Following an incubation period, Ebola hemorrhagic fever emerges abruptly and is characterized by early symptoms of fever, chills, malaise and myalgia. These may be followed by multisystem involvement manifesting as prostration, anorexia, nausea/vomiting, abdominal pain, diarrhea, chest pain, cough, shortness of breath, headache, confusion, coma, edema or postural hypotension. Hemorrhagic symptoms manifest at the peak of illness and include petechiae, ecchymoses, uncontrolled bleeding or oozing from venopuncture sites and mucosal hemorrhage; these may be severe, although fewer than half of all patients have hemorrhagic manifestations. Massive blood loss, if it does occur, usually affects the gastrointestinal tract. Late-stage symptoms include shock, convulsions, profound metabolic disturbances, diffuse coagulopathy and multiorgan failure, manifesting as a syndrome that is similar in some ways to septic shock (see Sepsis and Septic Shock). Symptoms manifest earlier and disease progresses more rapidly in patients with fatal disease, with death occurring 6-10 days after onset of symptoms (Feldmann, H. et al (2011); de Wit, E. et al (2011); Hoenen, T. et al (2012)).

The route of infection may influence both the course of disease and outcome. The incubation period following transmission via contact exposure is reported to be longer than for parenteral infection (mean 9.5 vs. 6.3 days) (Feldmann, H. et al (2011)). Viral load is linked to outcome, with data in humans and nonhuman primates showing improved likelihood of survival when viremia is lower than 1x10(4.5) pfu/mL of blood (Feldmann, H. et al (2011)). Outcome is also closely associated to the infecting strain. The case-fatality rate ranges from 60-90% for Zaire Ebola virus species to 40-60% for Sudan Ebola (Feldmann, H. et al (2011); Leroy, E.M. et al (2011)) and approximately 40% for Bundibugyo Ebola (MacNeil, A. et al (2010)). Only one person has been infected with the Côte d'Ivoire strain and survived the illness (de Wit, E. et al (2011); Leroy, E.M. et al (2011)). No human cases of illness caused by Reston Ebola virus infection have been reported. In February 2009, WHO reported that four people working with infected pigs in the Philippines had tested positive for REBOV antibodies, but did not show symptomatic disease (Unknown Author (2009); Ebola Reston in pigs and humans in the Philippines (World Health Organization, February 3, 2009)). Although the REBOV strain appears at this time to be less pathogenic to humans, it should be noted that all subjects analyzed were healthy adult males. There are no data available at this time for potentially more vulnerable groups such as children, pregnant women or immunosuppressed patients (Ebola hemorrhagic fever. WHO Fact Sheet no. 103 (World Health Organization, August 2012)).

Among survivors, sequelae may persist long after recovery from acute illness and include myelitis, recurrent hepatitis, psychosis, uveitis (Feldmann, H. et al (2011)) and prolonged poor health, as well as psychosocial sequelae such as fear and rejection (Macneil, A. et al (2012)). High antibody titers may be detected for years after infection (Leroy, E.M. et al (2011)).

RISK FACTORS

With the exception of periodic outbreaks, EBOV does not typically persist in the human population. Thus the introduction of EBOV from the reservoir species to humans appears to involve one or more stochastic events: typically transmission of the virus from the reservoir species (bats) to the incidental nonhuman host species (chimpanzees, gorillas), followed by human contact with the infected animal, primarily during the hunting and preparation of bush meat (Macneil, A. et al (2012)), although direct transmission from bats to humans is also possible (Hoenen, T. et al (2012)). The observation of REBOV infection in domestic pigs in the Philippines and the subsequent demonstration in laboratory studies that ZEBOV can also be transmitted among swine (Kobinger, G.P. et al (2011)) has led to concern that humans could theoretically become infected through the consumption of food and other products obtained from infected pigs (Hoenen, T. et al (2012)), although no cases of this type of animal-to-human transmission have been observed in the field.

Risk factors for human-to-human transmission of EBOV include close contact with sick individuals, primarily in the family or health care setting, and contact with dead bodies during preparation and burial. Health care workers in low-resource settings are at very high risk of infection (Macneil, A. et al (2012)).

EPIDEMIOLOGY

The first cases of Ebola hemorrhagic fever were reported in 1976, when two nearly simultaneous outbreaks occurred in northern Zaire (now the Democratic Republic of Congo) and southern Sudan. The causative agents of the outbreaks were identified as two different species of a novel filovirus, which was named "Ebola" after a river in northern Zaire.

Confirmed cases of Ebola hemorrhagic fever have since been reported in the Congo, Côte d'Ivoire, Democratic Republic of Congo, Gabon, Sudan and Uganda (Geographic distribution of Ebola hemorrhagic fever outbreaks and fruit bats of Pteropodidae family (World Health Organization, 2009)), as shown in the table below. The largest outbreak to date took place in Uganda between October 2000 and February 2001, during which time 425 people developed clinical illness and 224 (53%) died (Okware, S.I. et al (2002)).

In July 2012, a new index case of Ebola was confirmed by the World Health Organization in Uganda. As of August 17, WHO had reported a cumulative number of 24 probable and confirmed cases of Ebola hemorrhagic fever in Uganda, including 16 deaths (Ebola in Uganda - update (World Health Organization, August 17, 2012)), and stated that the outbreak there was under control. However, also in August 2012, 15 suspected new cases of Ebola and 10 deaths were reported in the Democratic Republic of Congo. By September 15, the number of laboratory-confirmed and probable cases of Ebola hemorrhagic fever in the Congo had reached 46, 19 of which were fatal (Ebola outbreak in Democratic Republic of Congo, update (World Health Organization, September 18, 2012)). All told, three separate outbreaks of Ebola were recorded in 2012, as shown in the table below.

In March 2014, WHO was notified of a new outbreak of Ebola virus disease in Guinea (Baize, S. et al (2014)), which later spread to Liberia. This marked the first time that the disease had been detected in West Africa, according to WHO. The strain implicated in the outbreak has 98% homology to the Zaire ebolavirus (ZEBOV) strain last detected in 2009. As of May 10, 2014, a cumulative total of 233 clinically compatible cases of Ebola hemorrhagic fever in West Africa, including 157 deaths, had been officially reported to the World Health Organization (see WHO Global Alert and Response: Ebola virus disease (EVD) and Ebola hemorrhagic fever - Guinea outbreak (Centers for Disease Control and Prevention) for up-to-date information).

CHRONOLOGY OF MAJOR EBOLA HEMORRHAGIC FEVER OUTBREAKS (AS OF MARCH 2014)

YEAR	COUNTRY	VIRUS SUBTYPE	CASES	DEATHS	CASE FATALITY
2012	Democratic Republic of Congo	BEBOV	57	29	51%
2012	Uganda	SEBOV	7	4	57%
2012	Uganda	SEBOV	24	17	71%
2011	Uganda	SEBOV	1	1	100%
2008	Democratic Republic of Congo	ZEBOV	32	14	44%
2007	Uganda	BEBOV	149	37	25%
2007	Democratic Republic of Congo	ZEBOV	264	187	71%
2005	Congo	ZEBOV	12	10	83%
2004	Sudan	SEBOV	17	7	41%
2003 (Nov-Dec)	Congo	ZEBOV	35	29	83%
2003 (Jan-Apr)	Congo	ZEBOV	143	128	90%
2001-2002	Congo	ZEBOV	59	44	75%
2001-2002	Gabon	ZEBOV	65	53	82%
2000	Uganda	SEBOV	425	224	53%
1996	South Africa (ex Gabon)	ZEBOV	1	1	100%
1996 (Jul-Dec)	Gabon	ZEBOV	60	45	75%
1996 (Jan-Apr)	Gabon	ZEBOV	31	21	68%
1995	Democratic Republic of Congo	ZEBOV	315	254	81%
1994	Côte d'Ivoire	CIEBOV	1	0	0%
1994	Gabon	ZEBOV	52	31	60%
1979	Sudan	SEBOV	34	22	65%
1977	Democratic Republic of Congo	ZEBOV	1	1	100%
1976	Sudan	SEBOV	284	151	53%
1976	Democratic Repubic of Congo	ZEBOV	318	280	88%

ABBREVIATIONS USED: SEBOV, SUDAN EBOLA VIRUS; ZEBOV, ZAIRE EBOLA VIRUS; BEBOV, BUNDIBUGYO EBOLA VIRUS; CIEBOV, CÔTE D'IVOIRE EBOLA VIRUS.

Source: Ebola haemorrhagic fever. Fact sheet No. 103 (World Health Organization, updated March 2014). Available at http://www.who.int/mediacentre/factsheets/fs103/en/index.html.

For more epidemiology information, consult the Incidence and Prevalence Database (IPD): IPD: Ebola virus.

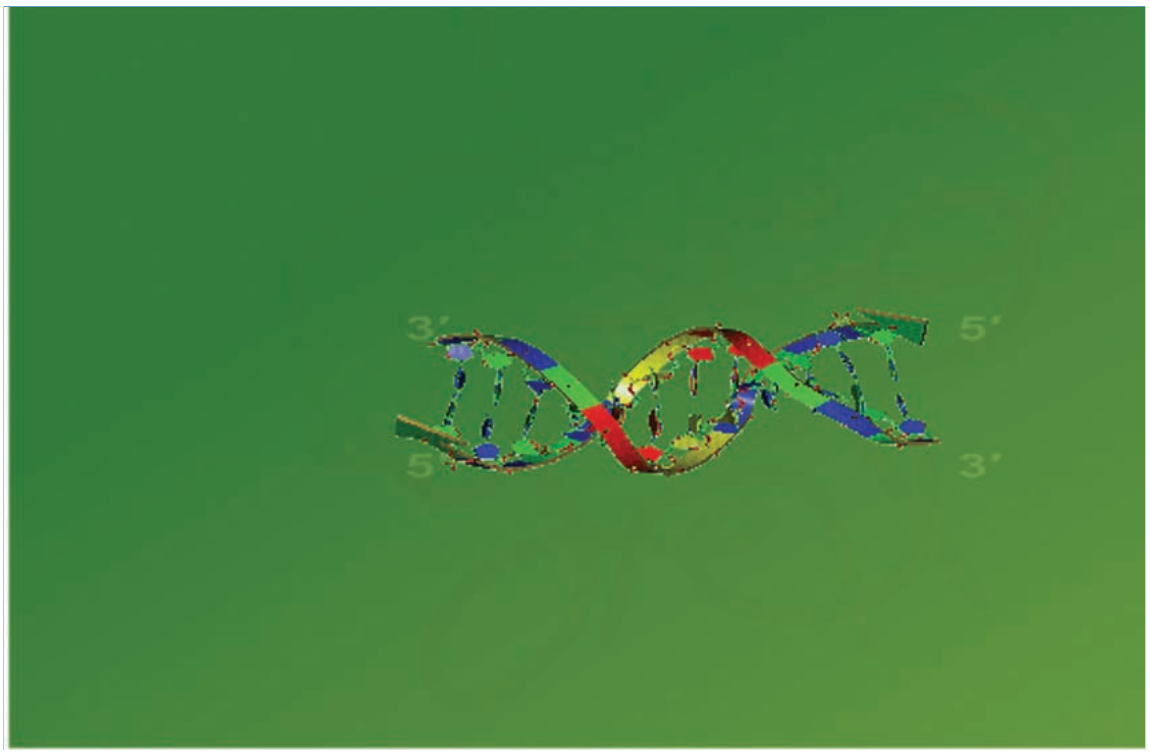
COST

Ebola hemorrhagic fever is a relatively rare disease and as such incurs a relatively low direct cost to society. However the long-term, indirect cost to an affected community can be significant. For example, in a community serviced by few health care workers, the illness or death of a nurse or doctor may temporarily leave residents without any medical care at all. Furthermore, when a health center with limited resources must care for a patient with Ebola, standard medical care and attention for patients with other diseases may not be available (Macneil, A. et al (2012); Hoenen, T. et al (2012)).

DIAGNOSIS

Ebola virus infections can only be diagnosed definitively in the laboratory. A number of different tests have been used to identify the virus, including:

- enzyme-linked immunosorbent assay (ELISA) for immunoglobulin G and M (Nakayama, E. et al (2010));
- antigen detection tests;
- serum neutralization test;
- reverse transcriptase polymerase chain reaction (RT-PCR) assay (Wang, Y.P. et al (2011));
- electron microscopy of clinical specimens (Wang, Y.P. et al (2011));
- virus isolation by cell culture (Wang, Y.P. et al (2011)).



POLYMERASE CHAIN REACTION (PCR)

Clinical specimens should be handled according to WHO guidelines (see Interim infection control recommendations for care of patients with suspected or confirmed filovirus (Ebola, Marburg) hemorrhagic fever (World Health Organization, March 2008)) and analyzed in a biosafety level 4 (BSL-4) laboratory (Leroy, E.M. et al (2011)).

DIFFERENTIAL DIAGNOSIS

Particularly in the early stages of an outbreak, the diagnosis of Ebola hemorrhagic fever may be hindered by the similarity of its symptoms to those of other diseases that are frequently encountered in the affected region. These diseases — which should be considered in the differential diagnosis of Ebola — include Marburg virus and other viral hemorrhagic fevers, Malaria, typhoid fever, shigellosis, cholera, rickettsiosis, meningococcal septicemia, plague, leptospirosis, Anthrax, typhus, yellow fever, Chikungunya fever and fulminant viral hepatitis (Feldmann, H. et al (2011); Ebola hemorrhagic fever. WHO Fact Sheet no. 103 (World Health Organization, August 2012)).

PREVENTION

Especially during outbreaks, proper preventive measures should be taken to reduce the risk of disease transmission. These include educational public health messages regarding proper handling of potentially infected animals, reducing contact with infected patients and proper burial measures for people suspected to have died from Ebola. Health care workers, who are at risk of contracting the illness through contact with patients, should wear gloves and other appropriate personal protective equipment. Laboratory workers handling samples obtained from suspected Ebola victims should also take the proper precautions.

VACCINES

Because Ebola hemorrhagic fever is a relatively rare disease primarily affecting underdeveloped countries, the development of a vaccine was long considered unnecessary. However several factors have changed this outlook, most significantly the potential for use of the virus as a weapon of bioterrorism (Geisbert, T.W. et al (2010)). As a result, various pre- and postexposure vaccines have been developed and evaluated in recent years. Conventional inactivated viral vaccines were the first vaccines studied, but were not effective in nonhuman primate models (Richardson, J.S. et al (2010)). Greater efficacy has been reported for postexposure vaccines based on vesicular stomatitis virus (VSV), as well as preexposure vaccines based on recombinant adenovirus type 5, human parainfluenza virus type 3 (Falzarano, D. et al (2011); Richardson, J.S. et al (2010)) and virus-like particle vaccines (Warfield, K.L. et al (2007); Warfield, K.L. et al (2011)). A DNA vaccine expressing envelope glycoproteins (GP) from the Zaire and Sudan species as well as the nucleoprotein (NP) was tested in a phase I clinical study in healthy adult volunteers (Martin, J.E. et al (2006)).

The greatest success has been achieved using the recombinant VSV-based Ebola vaccine. In addition to promising results obtained in guinea pigs, mice and rhesus macaques (Feldmann, H. et al (2007)), the vaccine was also successfully administered to an individual who suffered an accidental laboratory exposure. The subject, who was given the vaccine 48 hours after the accident, developed a fever but had no other signs of disease, and the virus remained undetectable in serum and peripheral blood during a three-week observation period (Günther, S. et al (2011)). Although this incident appears to demonstrate efficacy, it was never confirmed that the individual had actually been infected with EBOV (Falzarano, D. et al (2011)). Nonetheless, in the absence of any effective treatment for Ebola hemorrhagic fever, a postexposure vaccine is considered the best alternative to protect laboratory and health care personnel working with the virus (Falzarano, D. et al (2011)).

In order to provide optimum protection against all strains of Ebola as well as the related Marburg virus, an eventual vaccine would ideally need to contain at least three components (Feldmann, H. et al (2011)). Furthermore, given the remoteness of the endemic region, a single-dose vaccine is most desirable (Geisbert, T.W. et al (2010); Hoenen, T. et al (2012)). Finally, the route of vaccine administration is an important consideration. Although intramuscular injection is the most widely

used route of administration for current vaccines, a product that must be administered with a needle presents significant safety risks in an outbreak situation. Thus mucosal immunization (e.g., intranasal or oral) has been proposed as an attractive alternative, and needle-free delivery systems are being pursued (Richardson, J.S. et al (2010)). In general, a postexposure prophylactic vaccine that could be routinely administered to health care professionals in the endemic zone, with mass vaccination of the regional population in the event of an outbreak (i.e., ring vaccination), is conceivably the most practical approach (Macneil, A. et al (2012); Hoenen, T. et al (2012)).

The FDA has passed the Animal Efficacy Rule (see Guidance for industry: Animal models — Essential elements to address efficacy under the animal rule. Draft guidance (Food and Drug Administration, January 2009)) which enables regulatory approval of drugs and vaccines which cannot ethically or feasibly be tested in humans — including vaccines to prevent infection by agents of bioterrorism — on the basis of demonstrated efficacy in animal models. Although this procedure would probably be appropriate for development of an Ebola vaccine, reliable correlates of immune protection must first be identified. To date, the identification of reliable immune correlates in naturally infected human survivors has been hindered by the high mortality rate associated with the virus as well as the unpredictable nature of outbreaks (Sullivan, N.J. et al (2009); Richardson, J.S. et al (2010)).

EBOLA VACCINES UNDER ACTIVE DEVELOPMENT

DRUG NAME	ORGANIZATIONS	DESCRIPTION	PHASE
Ad5-optZGP/DEF-201	Defyrus	Combination of DEF-201 with an Ebola vaccine	Preclinical
BNSP-333-GP	US Department of Health & Human Services	Bivalent vaccine consisting of recombinant rabies BNSP333 virus carrying the Zaire ebola virus (ZEBOV) Mayinga strain glycoprotein (GP)	Preclinical
MVA-BN Filo	Bavarian Nordic	Cancer vaccine consisting of an attenuated version of the Modified Vaccinia Ankara (MVA-BN) virus encoding a filovirus antigen	Preclinical
rVSV-Ebola	Profectus BioSciences	Recombinant vesicular stomatitis virus (rVSV) expressing surface glycoproteins from Ebola virus	Preclinical

TREATMENT

At this time there are no safe and effective vaccines, nor are there any effective disease-specific treatments for Ebola hemorrhagic fever (Choi, J.H. et al (2013)). Supportive therapy — which is directed toward maintenance of blood volume and electrolyte balance, pain management and control of secondary infections — is the only available option (Feldmann, H. et al (1996); Richardson, J.S. et al (2010)). Patients should be isolated and all contacts traced, and medical personnel should follow proper procedures including use of adequate barrier nursing techniques and HEPA-filtered respirators (Feldmann, H. et al (1996); Bausch, D.G. et al (2008)).

Various experimental treatment approaches have been proposed and evaluated in rodents and/or nonhuman primates including passively acquired antibodies such as those obtained in the blood or serum of convalescent patients (Jahrling, P.B. et al (2007)), surface glycoprotein (GP)-specific monoclonal antibodies (Qiu, X. et al (2012); Qiu, X. et al (2012); Takada, A. et al (2007)), antisense oligonucleotides (Warren, T.K. et al (2010)), small interfering RNAs (siRNAs) (Geisbert, T.W. et al

(2010)), modulators of the coagulation cascade (Geisbert, T.W. et al (2003); Hensley, L.E. et al (2007)) and inflammatory modulators such as type I interferon (Feldmann, H. et al (2011)). Earlier-stage studies have suggested potential for inhibitors of the endo/lysosomal cholesterol transporter protein Niemann-Pick C1 (NPC1), which interacts with the virus glycoprotein GP and is essential for viral entry (Côté, M. et al (2011); Carette, J.E. et al (2011); Choi, J.H. et al (2013)).

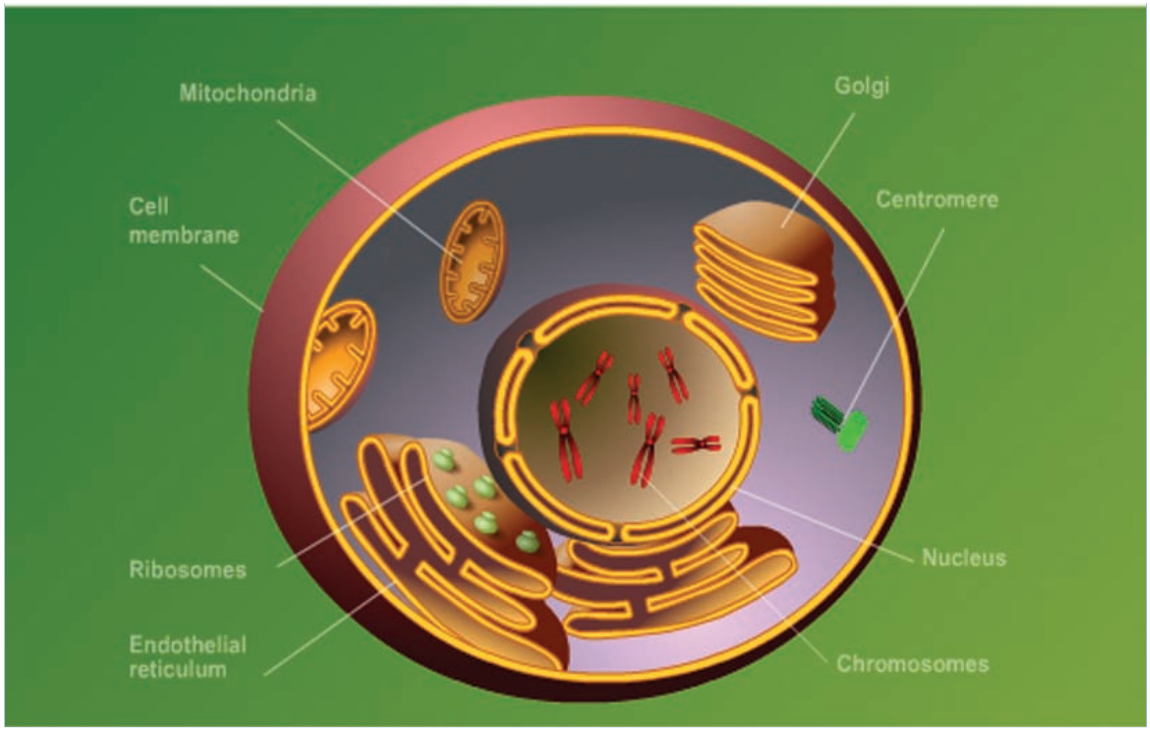
The study of investigational agents is hindered by the need to manipulate EBOV in high-level biosafety labs as well as by ethical constraints, which make the testing of drugs in traditional controlled clinical trials unfeasible. Some researchers have proposed evaluating investigational agents in the field, under outbreak conditions, while recognizing the myriad political, scientific, financial, logistic, ethical and legal challenges that this presents (Bausch, D.G. et al (2008)).

ANTIVIRAL AGENTS

No conventional or licensed antiviral agents have been found effective against EBOV (Bausch, D.G. et al (2008); Kondratowicz, A.S. et al (2012)). Viral load has been linked to survival, with a 2-3 log difference in viral load sometimes accounting for the difference between survival and death. Research efforts are thus being directed to the discovery of new antiviral agents that are capable of reducing viral load, albeit transiently, as well as other agents that directly inhibit the virus (Kondratowicz, A.S. et al (2012)) or viral entry (Choi, J.H. et al (2013)). Antiviral agents are most likely to be effective during the earlier (incubation and precoagulopathy) stages of disease progression (Ippolito, G. et al (2012)).

SMALL INTERFERING RNAS

Inhibition of viral gene expression using small interfering RNAs (siRNAs) is a growing area of antiviral research (Arbuthnot, P. (2010)) and is one of the few promising approaches to the treatment of Ebola at this time.



MECHANISM OF GENE EXPRESSION INHIBITION THROUGH RNA INTERFERENCE

The efficacy of TKM-Ebola (also known as Ebola SNALP, TKM-100201), a stable nucleic acid lipid particles (SNALP)-targeted siRNA directed against the Ebola virus was assessed in a nonhuman primate model of uniformly lethal Zaire Ebola virus (ZEBOV) hemorrhagic fever in a proof-of-concept study in rhesus macaques. TKM-Ebola, consisting of three pooled SNALP-formulated anti-ZEBOV siRNA molecules targeting ZEBOV protein L, matrix protein VP40 and polymerase cofactor VP35, was administered at 2 mg/kg/dose by bolus i.v. infusion to three macaques at 30 minutes and on days 1, 3 and 5 following a ZEBOV challenge. Another group of four macaques received the treatment at 30 minutes and on days 1-6 following the challenge with ZEBOV. Four and seven treatments with TKM-Ebola after ZEBOV exposure correlated with 66% and 100% protection against lethal ZEBOV infection, respectively. The treatment was well tolerated, with only minor changes in liver enzymes possibly related to viral infection reported (Geisbert, T.W. et al (2010)).

In February 2012, Tekmira Pharmaceuticals commenced enrollment in a phase I trial of TKM-Ebola. The single-blind, placebo-controlled study is designed to assess the safety and tolerability of TKM-Ebola and to evaluate the pharmacokinetics and systemic exposure following both a single ascending dose and multiple ascending doses of the product. A maximum of 56 healthy adults will participate in this study in two stages. A single-ascending-dose stage will have up to six cohorts with four subjects in each cohort (three receiving TKM-Ebola and one receiving placebo). A multiple-ascending-dose stage will have up to three cohorts with four subjects per cohort (three receiving TKM-Ebola and one receiving placebo) (NCT01518881, see Unknown Author (2012)).

The product will be developed under the FDA's Animal Efficacy Rule for therapeutics that cannot meet the requirements for traditional approval because human efficacy studies are not feasible. TKM-Ebola is being developed under a contract with the U.S. government's Transformational Medical Technologies Program. However, in August 2012 Tekmira received a temporary stop-work order from the U.S. Department of Defense (DoD) with respect to the TKM-Ebola program. Other contractors have received similar notices due to funding constraints imposed on the DoD.

CURRENT EBOLA HEMORRHAGIC FEVER PIPELINE

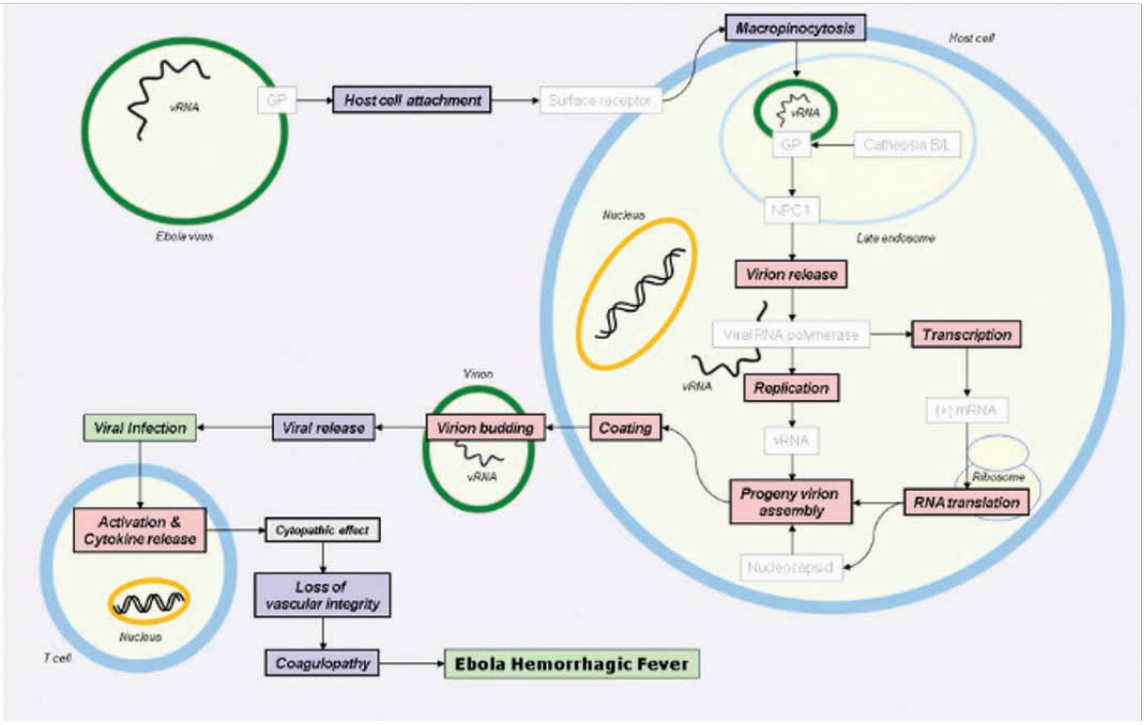
Consult the table below for an overview of all products mentioned in this review, including drugs, biologics and diagnostic agents that have been marketed or are under active development for this indication. The table may also include drugs not covered in the preceding sections because their mechanism of action is unknown or not well characterized.

DRUGS AND BIOLOGICS UNDER ACTIVE INVESTIGATION FOR THE PREVENTION AND TREATMENT OF EBOLA HEMORRHAGIC FEVER

DRUG NAME	ORGANIZATIONS	DESCRIPTION	PHASE
AVI-7537	Sarepta Therapeutics	19-Mer phosphorodiamidate morpholino antisense oligomer (PMO) whose sequence is: 5'-GCCATGGTTTTTTCTCAGG-3'	Phase I
Ebola SNALP	Tekmira	Combination of small interfering RNAs with 2'-O-methyl versions of guanines and uridines (EK-1 mod, VP24-1160 mod and VP35-855 mod) respectively targeting the Zaire Ebola virus L, VP and VP35 genes formulated in stable nucleic acid lipid particles (SNALPs)	Phase I
TKM-100802	Tekmira		Phase I
Ad5-optZGP/DEF-201	Defyrus	Combination of DEF-201 with an Ebola vaccine	Preclinical
BCX-4430	BioCryst	(2S,3S,4R,5R)-2-(4-Amino-5H-pyrrolo[3,2-d]pyrimidin-7-yl)-5-(hydroxymethyl) pyrrolidine-3,4-diol	Preclinical
BNSP-333-GP	US Department of Health & Human Services	Bivalent vaccine consisting of recombinant rabies BNSP333 virus carrying the Zaire ebola virus (ZEBOV) Mayinga strain glycoprotein (GP)	Preclinical
MB-003	Mapp Biopharmaceutical; US Army Med Res Inst Infectious Diseases; Kentucky BioProcessing (KBP)	Mixture of deimmunized mouse-human chimeric monoclonal antibodies (h-13F6, c13C6 and c6D8) targeting non-overlapping glycoprotein (GP) epitopes of Ebola virus (EBOV); produced via *Nicotiana benthamiana* (deltaXTFT)-based rapid antibody manufacturing platform (RAMP)	Preclinical
MVA-BN Filo	Bavarian Nordic	Cancer vaccine consisting of an attenuated version of the Modified Vaccinia Ankara (MVA-BN) virus encoding a filovirus antigen	Preclinical
rVSV-Ebola	Profectus BioSciences	Recombinant vesicular stomatitis virus (rVSV) expressing surface glycoproteins from Ebola virus	Preclinical

TARGETS FOR THERAPEUTIC INTERVENTION

For an overview of validated therapeutic targets for this indication, consult the targetscape below. The targetscape shows an overall cellular and molecular landscape or comprehensive network of connections among the current therapeutic targets for the treatment of the condition and their biological actions. An arrow indicates a positive effect; a dash indicates a negative effect. Gray or lighter symbols are targets that are not validated. For in-depth information on a specific target or mechanism of action, see the corresponding section in this report.



EBOLA HEMORRHAGIC FEVER TARGETSCAPE

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