

An Overview of Ebola

by

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History

In 1967 in Marburg, Federal Republic of Germany, Frankfurt, Federal Republic of Germany, and Belgrade, Yugoslavia a virus was detected in thirty-one people resembling a rhabdovirus. (1,2) Upon closer inspection, it became apparent that this was a new family, the first filovirus.(1,2) This species was named Marburg after the samples that were collected from the Marburg vaccine laboratory where it was first seen.(1,2) It was determined that the virus originated in a shipment of African Green monkeys (vervets) wild caught in Uganda and destined for use in biomedical research. (1,2) The source of the vervets' infection in Uganda was never found.(1,2) No cases were reported for the next eight years. In 1975, in Johannesburg, South Africa, a naturally occurring acquired human infection was detected in a man who had traveled from Zimbabwe.(1,2) Two of his caretakers became ill as well and indicated Marburg virus.(1,2) Once again, the source of the filovirus was not found.(1,2)

In 1976, a hemorrhagic fever was detected in Nzara, Sudan from individuals working in

the same small room in a cotton factory and also in a hospital located in Yambuka, Zaire.(1,2) The Mridi hospital in Sudan used to treat infected individuals from Nzara cotton factory was decimated as the hemorrhagic fever passed from patient to patient and to healthcare workers by unsterilized needles, syringes, and contact with bodily fluids.(1,2) This virus had a similar genome organization to Marburg but differed from Marburg by one additional protein and its nucleotide sequence.(3,4) The virus was also a filovirus but a new species hence, it was named Ebola after a local river common to Nzara and Yambuka.(1,2) Later, it was found that the Sudan and Zaire filoviruses were actually two different strands of Ebola with different RNA.(2) Again in 1976, it reemerged in the same Nzara factory.(2)

After the discovery of Ebola in 1976, many outbreaks originating from Africa and the Phillipines have been recorded in humans and non-human primates, including a 1989-1990 out break in Reston, Virginia in cynomolgous macaques, 1990-1992 four Ebola cases in the United States and Italy in

non-human primates, 1992-1994 Cote d'Ivoire epidemic in chimpanzees, 1994 transmission from a chimpanzee to a human in Cote d'Ivoire, a 1995 epidemic in the Democratic Republic of the Congo in humans, 1996 human infection in Mayibout and Booue Gabon, 1998-1999 reemergence of the epidemic in the human population of the Democratic Republic of the Congo, 2000-2001 human outbreak in Uganda, 2001-2002 human epidemic Gabon, and as recently as March 2003, in the Democratic Republic of the Congo, in with one hundred fatalities.(6-12)

Morphology and Replication

Ebola is an enveloped, non-segmented, negative-strand RNA virus said to resemble a shepard's crook due to its filamentous appearance.(3,4,11) Ebola has a linear seven viral genes genome which resembles rhabdoviruses and paramyxoviruses.(3,4)

The long filamentous threads are 800-14,000 nm long and have a constant diameter of 80 nm.(3,4) The Ebola virion has a central ribonucleoprotein core linked by two matrix proteins to a glycoprotein bearing lipid bilayer which forms spikes on the virion surface.(3,4) This glycoprotein envelope has been identified as the main determinant during infection.(3,4,11) The gene encoding for the viral glycoprotein in Ebola generates two protein products, one for viral membranes which inhibits the host's neutrophils and one that is cytotoxic to the host's endothelial cells.(13) Ebola has been categorized into 4 subtypes, known as Sudan, Zaire, Reston, and Cote d'Ivoire with 13 unique strains within the subtypes.(11)

Replication of Ebola is similar to replication of rhabdovirus and paramyxovirus.(3,4) Replication of the virus takes approximately twelve hours occurring in the host's mononuclear phagocytes.(11) First, the virion attaches to the host's cell surface through the host's alpha folate receptor,

apparently egressing on a cholesterol rich lipid raft.(3,4,11) This begins the receptor-mediated endocytosis which is followed by the fusion of the viral envelope with the cellular endosomal membrane.(3,4) This fusion allows the release of the virus's central nucleoprotein into the host's cytoplasm and the transcription of the viral RNA.(3,4) The first two viral proteins transcribed induce production of the antigenome, which provides the template for genome synthesis.(3,4)

Disease Process

Ebola virus infection occurs in monocytes, macrophages, and regional lymph nodes of the host.(3,4) Mobile macrophages with virus particles infect the liver and spleen.(3,4) Cytokine and chemokine release increases the permeability of the endothelial lining of blood vessels.(3,4,11) This cytokine release causes the development of disseminated intravascular coagulation.(3,4) Lysis, not infection, of lymphocytes in the spleen, thymus, and lymph system occurs during the late stages of Ebola.(3,4) Higher concentrations of the virus and more damage to organs are found in the liver, kidney, spleen, and lungs from Ebola induced proinflammatory cytokines. (11) In some patients, particularly in those with a lack of immune response, this leads to multi-organ failure.(11)

The incubation period of Ebola appears to be approximately six days although it may range from 3-16 days.(1-4,11, 14) Symptoms include sudden onset of fever, malaise, prostration, sore throat, chest pain, abdominal pain, diarrhea, headache, fever, conjunctival injection, pharyngitis, erythematous macular rash, chills, petechiae, ecchymoses, conjunctival hemorrhages and oozing from venipuncture sites.(2-4,11) The most compelling sign of Ebola appears to be the presence of the rash.(3,4) The rash appears on the lateral trunk, groin, and

axillary areas and spreads to the entire body, excluding the face, in a matter of hours.(14) Contrary to the ideas in the media, massive bleeding is rare.(3,4) Laboratory indications include lymphopenia, neutrophilia, thrombocytopenia, prolongation of coagulation time, and increased aspartate aminotransferase.(3,4)

Spontaneous abortion in pregnant women is common and equally high in all trimesters.(15) It is assumed intravascular coagulopathy and Ebola infection of fetus cause the spontaneous abortions.(15) Similar to Malaria, Ebola may be more severe in pregnant women and live born infants die within nineteen days of birth.(3,4,15)

Typically, within ten days of the onset of symptoms there will be improvement or death from diffuse or extensive hemorrhage this involving the liver, lymphoid, kidneys and vascular endothelial cells, coma, convulsions and shock due to the multi-organ failure.(2-4,11)

Survivors vs. Non-Survivors

Survival is best predicted if the patient is still alive 10days after onset of symptoms.(3,4) Survivors of the 1995 Democratic Republic of Congo epidemic showed increased coughing and increased hemoptysis compared to non-survivors.(14) Survivors also display more myalgia, arthralgia, and early onset of bilateral conjunctival injection.(14) Survivors appear to have early increasing levels of IgG, clearance of circulating viral antigen, and activation of cytotoxic T cells.(10) Post recovery, survivors have been found to suffer from uveitis, ocular pain, photophobia, hyper-lacrimation, fever, arthralgia, malalgia, headaches, fatigue, bulimia, amenorrhea, hearing loss, and tinnitus.(14)

Death occurs in those with impaired humoral response, no IgG response, early activation of T cells, and DNA

fragmentation in leukocytes. (14) It is hypothesized that the DNA fragmentation of leukocytes causes the massive intravascular apoptosis.(10) Other symptoms positive for fatalities include bleeding of mucosa, anuria, hiccups, and tachypnea.(14) Tachypnea seemed to be most indicative of death.(14) Not recorded previously was hyperventilation seen in dying patients in 1995 Democratic Republic of Congo Ebola epidemic.(14)

Treatment

Currently there is no treatment for Ebola virus infection.(3,4) Treatment has been implemented with interferon alpha, transfusions and dialysis, and equine anti-Ebola immunoglobulin but without definitive results due to the late stage in the disease process it was implemented.(3,4)

Risk

Anyone coming in contact with bodily fluid including feces, urine, vomit, sweat, and blood, of an Ebola infected patient is at risk for contracting the virus.(2-4,11) Typically these are family caregivers, healthcare workers, and burial preparers. Patients in the late stage of infection and the recently deceased are at the greatest risk for spreading the virus.(3,4) There has been evidence of aerosolized Ebola virus spread in the laboratory and also between non-human primates but not between infected humans. (3,4,7,11)

Containment

Containment of an Ebola epidemic is easily achieved by instituting nurse barrier methods, isolation of the infected, and sterilization of needles and syringes. (3-4,11) A clear example of this is Mosongo General Hospital in the Democratic Republic of the Congo that was able to keep 300 patients in their hospital and continue activities including surgeries without spread

of the virus due to controlling the outbreak with early recognition, isolation, good sterilization methods, and appropriate nurse barrier methods.(9) The Ebola virus may be killed by UV light and household bleach however, it can survive at room temperature in liquid or dried blood for days.(3,4)

Vaccine Research

Currently guinea pigs, non-human primates, and mice are used as models for human infection.(3,4,11) Barreintos, et al. found that cyanovirin-N binds with high specificity to viral surface glycoprotein GP_{1,2} and inhibits infectivity of Ebola.(16) Evidence of this positive inhibition provides a hopeful new avenue for new research.(16) DNA vaccines that are incorporated into the injection site and get transcribed, translated, and post translationally modified stimulate immune response and enhance antigen recognition have also had positive results in animal models.(11,13) However, the efficacy seen in mice does not translate for a rapid vaccine as the mouse is not the best model for human infection.(17).

Subunit vaccines vectored by adenovirus using a DNA booster have shown some efficacy in non-human primates.(11) Venezuelan equine encephalitis virus replicons used in subunit vaccines replicate the vector structural proteins of the virus spike have shown some efficacy in non-human primates as well.(11) Macaques immunized with adenoviral vector encoding the Ebola glycoprotein has shown a resistance up to 28 days post Ebola challenge.(18) This shows great promise for a future vaccine.(18)

Other avenues for future research focus on 1) prevention virion binding, 2) blocking the virus receptor in the host, 3) preventing membrane fusion between the virus and host, 4) interfering with transcriptions and genome replication of the virus, 5) inhibiting cellular S-adenosylhomocysteine hydrolase,

and 6) enhancing innate antiviral mechanisms, 7) interfering with viral maturation assembly, budding and release.(3,4) Currently, the most rapidly growing area of research is in the enhancement of innate antiviral mechanisms.(3,4)

Several problems in the pursuit to develop of Ebola vaccines are that the infections occur in Africa, not in areas with the economic and technical resources for research; very little is known about the virus; animal models are not similar enough to the humans; and Ebola is so lethal, that research must be done in Biohazard Safety Level 4 laboratories of which there are few.(3,4)

Tests for Ebola

Currently there are several tests for Ebola in use. The easiest to use is immunochemistry testing of formalin fixed postmortem skin that may be used in field and in the hospitals in Africa.(3,4,7,19) This testing does not require the Biohazard Safety Level 4 laboratories only found in industrialized countries.(3,4) ELISA, antigen-capture enzyme-linked immunosorbent assay, is IgG specific detecting the binding of the antibodies to the antigens and has detected Ebola after 10 years as well as the ability to detect IgM while sick.(3,4,11,20) ELISA has the possibility for field use, but currently it requires 7-14 days incubation and a Biohazard Safety Level 4 laboratory.(3,4,11,7)

Plaque reduction assays are also available which test the antibodies ability to inhibit plaque formation on cell monolayer.(11) T cell proliferation assays test the ability of the antibodies to induce T cell proliferation.(11) Cytotoxicity assays measure lysis of target cells with the Ebola antigen.(11)

Reservoirs

All areas endemic to Ebola virus infection have had presence of disturbed forest and communicating activities of infected people between forest and urban areas as a common characteristic.(21) So far thousands of arthropods, mammals including monkeys, dogs, bats, apes, goats, sheep, pigs, cattle, mongoose, antelope, squirrels, rodents, reptiles (including tortoises and snakes), amphibians, and birds have been collected and tested however none have shown to be the vector of Ebola.(21-24) Ebola strains have been seen to naturally occur in non-human primates including gorillas, vervets, cynomolgous macaques, and chimpanzees.(5-7) None of these animals are assumed to be the natural reservoir for Ebola because they all succumb to a similar death as seen with humans in the similar rapid period of time.(5-7) Interestingly, bats can harbor the virus for four weeks with no mortality so they are currently the greatest possibility.(11,22,23) Monath has suggested that the virus vector may actually be from plants or arthropods, specifically arthropods who eat primarily plants. (22)

Bioterrorism

Filoviruses are classified as a Category A biowarfare agent by the Centers for Disease Control and Prevention.(3) Filovirus are highly virulent, have the ability to become aerosolized and have the capacity to induce fear and anxiety to the American public.(3) The US has not performed biowarfare research on Ebola because it was discovered seven years after the Biological Weapons Convention.(3) However, it is assumed that Russia collected samples of Marburg in 1967 and that a group of Japanese terrorists collected samples from the 1995 outbreak of Ebola in the Democratic Republic of the Congo.(3)

The greatest threat of Ebola as a biological weapon is not the disease itself, but the

extreme fear and panic it will cause.(3) Most of the public believes the fictionalized accounts that Ebola is an airborne pathogen.(3) Fortunately, Ebola is easily contained and therefore has little chance of ever becoming a large epidemic in the United States.(3)

References

1. Peters CJ, Johnson ED, Jahrling PB, Ksiak TG, Rollin PE, White J, Hall W, Trotter, R, Jaax N. 1993. Filoviruses. In *Emerging Viruses* (ed. SS Morse), pp.159-175. Oxford University Press, New York, NY.
2. Murphy FA, Peters CJ. 1998. Ebola Virus: Where does it come from and where is it going? In *Emerging Infections* (ed. RM Krause), pp. 375-410. Academic Press, New York, NY.
3. Bray M. Defense against filoviruses as biological weapons. *Antiviral Research*. 2003;57:53-60.
4. Bray M, Paragas J. Experimental therapy of filovirus infections. *Antiviral Research*. 2002;54:1-17.
5. Formentry P, Hatz C, Le Guenno B, Stoll A, Rogenmoser P, Widmer A. Human infection due to Ebola virus, subtype Cote d'Ivoire: Clinical and biological presentation. *J Infect Dis*. 1999;179(Suppl 1):S48-S53.
6. Formentry P, Boesch C, Wyers M, Steiner C, Donati F, Dind F, Walker F, Le Guenno B. Ebola virus outbreak among wild chimpanzees living in a rain forest of Cote d'Ivoire. *J Infect Dis*. 1999;179(Suppl 1):S120-S126.
7. Rollin PE, Williams RJ, Bressler DS, Pearson S, Cottingham M, Pucak G. et.al. Ebola (Subtype Reston) virus among quarantined non-human primates recently imported from the Phillipines to the United States. *J Infect Dis*. 1999;179(Suppl 1):S108-S114.

8. Miranda ME, Ksiazek TG, Retuya TJ, Khan AS, Sanchez A, Fullhorst CF, et al. Epidemiology of Ebola (Subtype Reston) Virus in the Phillipines, 1996. *J Infect Dis.* 1999;179(Suppl 1):S115-S119.
9. Ndambi R, Akamituna P, Bonnet MJ, Tukadila AM, Muyembe-Tamfum JJ, Colebunders R. Epidemiologic and clinical aspects of the Ebola virus epidemic in Mosango, Democratic Republic of the Congo, 1995. *J Infect Dis.* 1999;179(Suppl 1):S8-S10.
10. Baize S, Leroy E, Georges-Courbot MC, Capron M, Lansoud-Soukate J, Debre SP, et al. Defective humoral response and extensive intravascular apoptosis are associated with fatal outcome in Ebola virus-infected patients. *Nature Medicine.* 1999;5:423-426.
11. Hart MK. Vaccine research efforts for floviruses. *Int J Parasitology.* 2003;33:583-595.
12. Frankish H. Death toll continues to climb in Congo Ebola outbreak. *The Lancet.* 2003;361:1020.
13. Nabel GJ. Vaccine for AIDS and Ebola virus infection. *Virus Research.* 2003;92:213-217.
14. Bwaka MA, Bonnet MJ, Calain P, Colebunders R, De Roo A, Guimard Y, et al. Ebola hemorrhagic fever in Kikwit, Democratic Republic of the Congo: Clinical observations in 103 patients. *J Infect Dis.* 1999;179(Suppl 1):S1-S7.
15. Mupapa K, Mukundu W, Bwaka MA, Kipasa M, De Roo A, Kuvula K, et al. Ebola hemorrhagic fever and pregnancy. *J Infect Dis.* 1999;179(Suppl 1):S11-S12.
16. Barrientos LG, O'Keefe BR, Bray M, Sanchez A, Gronenborn AM, Boyd MR. Cyanovirin-N binds to the viral surface glycoprotein GP1,2 and inhibits infectivity of Ebola virus. *Antiviral Research.* 2003;58:47-56.
17. Riemenschneider J, Garrison A, Geisbert J, Jahrling P, Hevey M, Negley D, et al. Comparison of individual DNA vaccines for B. anthracis, Ebola virus, Marburg virus, and Venezuelan equine encephalitis virus. *Vaccine.* 2003;21:4071-4080.
18. Sullivan NJ, Geisbert TTW, Geisbert JB, Xu L, Yang ZY, Roederer M, et al. Accelerated vaccination for Ebola virus hemorrhagic fever in non-human primates. *Nature* 2003;424:681-684.
19. Zaki Sr, Shieh WJ, Greer PW, Goldsmith CS, Ferebee T, Katshitshi J, et al. A novel immunochemical assay for the detection of Ebola virus in skin: Implication for diagnosis, spread, and surveillance of Ebola hemorrhagic fever. *J Infect Dis.* 1999;179(Suppl 1):S36-S47.
20. Ksiazek TG, West CP, Rollin PE, Jahrling PB, Peters CJ. ELISA for the detection of antibodies to Ebola virus. *J Infect Dis.* 1999;179(Suppl 1):S192-S198.
21. Reiter P, Turell M, Cleman R, Miller B, Maupin G, Liz J, et al. Field investigations of an outbreak of Ebola hemorrhagic fever, Kikwit, Democratic Republic of the Congo, 1995: Arthropod studies. *J Infect Dis.* 1999;179(Suppl 1):S148-S154.
22. Monath TP. Ecology of Marburg and Ebola viruses: Speculations and directions for future research. *J Infect Dis.* 1999;179(Suppl 1):S127-S138.
23. Breman JG, Johnson KM, van der Groen G, Robbins CB, Szczeniowski MV, Ruti K, et al. A search for Ebola virus in animals in the Democratic Republic of the Congo and Cameroon: Ecologic, virologic, and serologic surveys, 1979-1980. *J Infect Dis.* 1999;179(Suppl 1):S139-S147.

24. Leirs H, Mills JM, Kerbs JW, Childs JE, Akaibe D, Wilillen N, et. al. Search for the Ebola virus reservoir in Kikwit, Democratic Republic of the Congo: Reflections on a vertebrate collection. *J Infect Dis.* 1999;179(Suppl 1):S155-S163.

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