The Rhesus Monkey as a Robust Model of Ebola Virus Disease

Writer/Author: Joe Shaw

Addresses:

Key words and abbreviations: EBOV; Ebola virus; ebolavirus; EHF; EVD; Filoviridae; filovirus; Zaire ebolavirus; ZEBOV

ABSTRACT

Ebola virus disease is a presumed zoonotic disease that often results in death; several ebolaviruses can cause Ebola virus disease, but Ebola virus (EBOV, formerly Zaire ebolavirus) is the most deadly. Multiple sporadic outbreaks of EBOV infection have occurred in sub-Saharan Africa since scientists first observed the disease in 1976. Case fatality rates in large outbreaks of EBOV infection have varied from about 50% to 90%. EBOV is transmitted by exposure to infected persons or body fluids, by mechanical means (e.g., use of contaminated needles), or by exposure to bush meat. In the laboratory, rodents and nonhuman primates are infected successfully with EBOV by conjunctival, intramuscular, intraperitoneal, subcutaneous, oral, and aerosol means. Rhesus monkeys are frequently used to study EBOV infection, and they respond to EBOV infection much the same as humans. This review compares and contrasts the signs of EBOV infection in rhesus monkeys with symptoms of EBOV infection in humans.

INTRODUCTION

Ebola virus (EBOV), formerly Zaire ebolavirus [1], causes Ebola virus disease (EVD). A presumed zoonotic disease, EVD often presents with nonspecific signs and symptoms including fever and headache; later clinical manifestations may include rash, hemorrhage, and shock [2, 3]. Like other filoviruses, EBOV is a single-stranded, negative-sense, monopartite RNA virus of the *Filoviridae* and is related to Marburg virus, another hemorrhagic fever-causing virus. Multiple, sporadic EVD disease epidemics have occurred in sub-Saharan Africa with lethality varying from about 50% to 90% in large outbreaks [4-6]. Since its recognition by scientists in the late 1970s [6], EVD has generally been restricted to Africa [7]. EBOV is transmitted by exposure to contaminated body fluid, secretions, infected tissues, or perhaps exposure to bush meat in natural settings [8, 9]; laboratory accidents and use of contaminated needles have also caused infections [6, 10-12]. During the 1976 outbreak of EVD in Zaire, 100% (85/85) infections were lethal when caused by injection [13].

Previous laboratory animal work has shown that EBOV infection results in altered expression of disease- and immune-related proteins such as cytokines [14-16] and proteins involved in coagulation [15]. *In vitro* infection of monocytes or macrophages by filoviruses induces tissue factor expression and elicits induction of proinflammatory cytokines, which are likely responsible, at least partially, for the apoptosis of lymphocytes observed *in vivo* [17].

Several types of nonhuman primates have been used to study EVD, including grivets (*Chlorocebus aethiops*) [18-20], cynomolgus monkeys (*Macaca fascicularis*) [21, 22], rhesus monkeys (*Macaca mulatta*) [18, 19, 23], common marmosets (*Callithrix jacchus*) [24], and hamadryad baboons (*Papio hamadryas*) [20, 25]. Similar pathological features of EBOV infection have been documented among these animals; however, a some features differ. Most notably, grivets do not present with the maculopapular rash that is characteristic of disease in macaques and baboons [18, 19] and is a prominent feature of human disease [10, 12, 26]. The death rate for rhesus monkeys is high [23, 27-31] when challenged with a target dose of 1000 plaque-forming units (PFU).

Multiple epizootics among western gorillas (*Gorilla gorilla*) and common chimpanzees (*Pan troglodytes*) in sub-Saharan Africa have been ascribed to EBOV [8, 9, 32-36]. Some researchers suggest EBOV so endangers western gorillas that the combined pressures of hunting, loss of habitat, and EBOV infections may push these apes to near extinction [33, 35-39]. EBOV infection of great apes is particularly serious because multiple infections in humans have been associated with their butchering or consumption, and great apes have been implicated as a primary source of some human EVD epidemics [8, 36, 37, 40, 41].

We must deal with EBOV aggressively because it 1) has the potential to cause public panic [42, 43], 2) has a low infectious dose [44], 3) may be transmitted from person to person by close contact [13], 4) may be delivered by aerosols [23, 45, 46], which are considered a likely means of release in a biological terrorism or biowarfare event [47-49], 5) can infect domestic farm animals [46] , 6) may possibly already be weaponized [50-52], and 7) has no licensed vaccines or therapeutics available for prevention or treatment of humans or animals [53].

There are several ebolaviruses including Sudan virus, Taï Forest virus, Bundibugyo virus, and Reston virus, but Ebola virus (EBOV) has killed the most people [54]. This review examines the rhesus monkey as a model for EBOV infection, comparing and contrasting findings in that nonhuman primate with those of humans with EVD.

EBOV INFECTION

Route of Infection

Rhesus monkeys have been infected in the laboratory with EBOV by various means: aerosol, intramuscular, intraperitoneal, ocular, and oral (Table 1). However, in one reported case, two control animals were infected even though exposure was unintended, and the route of exposure

could not be documented [55]. Unlike laboratory animals, the route of infection for humans is usually unknown but is thought to occur through: 1) exposure to infected persons or corpses [6, 40, 56], 2) use of unclean needles or needle sticks [6, 10, 11], or 3) exposure to bush meat or bats [40, 57].

Incubation Period

The first indication of EBOV infection in rhesus monkeys is fever, which may occur as early as days two or three post-exposure (PE) [58] but may occur as late as day six post-exposure [23, 31]. Many human cases of EVD are reported after the onset of symptoms following an unknown incubation period. Moreover, the infectious dose and route of exposure are usually unknown for human cases, with the exception of needle sticks or other nosocomial exposures, hindering the estimation of a precise incubation period. Previous reports have indicated incubation times from as short as two days to as long as 25 days [6, 57, 59-61]. In two reports of disease acquired via needle stick events, symptoms appeared six days after exposure [10, 11].

Symptoms and Signs

Rhesus monkeys challenged with EBOV in the laboratory develop fever as the earliest clinical manifestation of disease between days two and seven PE [18, 19, 58, 62], and body temperatures may rise to 40.6°C [63]. In humans with EVD, fever is generally noted as one of the first indications of disease, but other symptoms such as nausea, myalgia, gastrointestinal disorders, or other influenza-like symptoms may occur with fever. Body temperature may rise to 41°C in humans [12]. Body temperatures of both rhesus monkeys and humans may decrease to normal or subnormal near death [26, 64].

Signs of EVD in rhesus monkeys include anorexia, bleeding, diarrhea, dehydration, epistaxis, hunched posture, ruffled fur, listlessness, neurological abnormalities, rash, and weight loss [19, 23, 65]. Humans with EVD may experience many of the following: abdominal pain, anorexia, bleeding, coagulation abnormalities, dehydration, diarrhea, hiccups, listlessness, myalgia, nausea, rash, vomiting, or weight loss [6, 12, 26, 59, 66].

A maculopapular rash is a prominent feature of EBOV infection in rhesus monkeys and humans alike [10-12, 18, 27, 28, 66] (Table 1); it is evident one to five days after onset of fever in both rhesus monkeys and humans. The rash may occur on face, arms, legs, inguinal areas, or groin; however, it does not occur in all monkeys or patients. The end-stage manifestations of disease parallel the progression from a systemic inflammatory response syndrome state to a septic shock-like state culminating in a multi-organ dysfunction syndrome typically associated with bacterial sepsis [67-69].

Lethality

Rhesus monkeys infected with EBOV may die as early as days five to six after infection [70, 71]. Infection with EBOV is essentially 100% lethal for rhesus monkeys irrespective of route (intramuscular, intraperitoneal, conjunctival, oral, or aerosol), but there are exceptions. In one instance, ~14 PFU delivered by aerosol was lethal [72], but in another study, three of nine

animals survived following intramuscular challenge with ~50 PFU [73]. In still another study, one of six animals survived challenge with 200,000 guinea pig infectious units [28]. It is difficult to retrospectively compare studies because the background of inocula has varied between them; virus may have been mouse-adapted [74], passaged through guinea pigs [75] or through Vero E6 cells [64], and the number of passages may have varied.

The death rate for humans in EVD outbreaks has varied from about 50% to as high as 90% [54]. Laboratory accidents (needle sticks) may result in death [10], but in at least one needle-stick case the patient developed symptoms but survived [11]. Importantly, in the 1976 EVD outbreak, the case fatality was 100% (85 of 85) for documented exposures via needle sticks or injections, compared to approximately 80% (119 of 149) for infection by other means [6] (Table 1).

Viremia

EBOV infection of rhesus monkeys generally results in high levels of circulating virions; peak levels have been reported to range from 1E+05 PFU/mL to over 1E+07 PFU/mL [19, 28, 76]. Only one report describes similar levels of circulating virus in humans at 3E+06 PFU/mL [6]. Two other studies tried to determine viral load with the plaque assay, but they both had difficulty isolating virus from serum [12, 77]. The samples from the Kikwit 1995 outbreak in particular had levels of circulating antigen at dilutions of 1:500 making it difficult to believe a viral load of only 31 PFU/mL that was obtained in the same study [77]. In addition, non-quantitative, reverse transcriptase PCR assays easily detect virus in the blood or plasma of patients [10, 40, 41] (Table 1).

Hematology

An increase in absolute numbers of neutrophils has been reported in rhesus monkeys exposed to EBOV, and relative neutrophil levels rose to more than 90% of the total leucocytes [28]. In contrast, numbers of circulating lymphocytes and platelets decrease precipitously in rhesus monkeys infected with EBOV [27, 74] with platelets falling to 24% of pre-infection levels by day seven [74]. Lymphocyte levels fell as much as 22% by day seven with a larger drop seen in $CD4^+$ cells than in $CD8^+$ cells, 22% versus 10% decreases respectively [27]. Similarly in humans, there is one report of increased neutrophils in a patient with EVD, but there are conflicting reports of leucocyte counts [10, 59] in patients with EVD. The total leucocyte count fell to 1450 cells/mm^3 in one surviving patient with EVD before he started to recover [12]; however, two patients who succumbed to EVD had normal, 7600-8900 cells/mL, or higher than normal, 9400-12,300 cells/mm^3, leukocyte counts [6]. Platelet counts in human cases also show conflicting results with one patient showing severely decreased numbers at 30×10^9 platelets/L on day eight after first symptoms appeared [59] and other fatal cases maintained normal counts at 150-253 $\times 10^9$ platelets/L despite obvious hemorrhaging [6] (Table 2).

Liver Enzymes

The concentrations of multiple liver enzymes rise dramatically from baseline in the blood of rhesus monkeys infected with EBOV. Alkaline phosphatase levels can be increased more than

five-fold by day six PE [31]; in humans the alkaline phosphatase concentrations are less consistently higher than normal with only about 60% of patients in one study showing higher than normal values [40]. Alanine aminotransferase and aspartate aminotransferase concentrations are typically greater than five-fold above baseline by day six PE in rhesus monkeys [27, 30]. In humans, alanine aminotransferase and aspartate aminotransferase concentrations are all consistently higher than normal [40]. Increases in γ-glutamyltransferase concentration tend to be more modest in rhesus monkeys so that they are only two to three times higher than normal levels as early as day seven post-infection [27]; in humans, only 50% of patients were reported to have increased γ-glutamyltransferase concentration [40]. Despite the varying concentrations of liver enzymes within the serum, nearly 100% of all rhesus monkeys and patients with EVD have liver dysfunction (Table 3).

Kidney Function

Infected monkeys may have increased levels of circulating creatinine and serum/blood urea nitrogen [30, 65]; however, not all monkeys have kidney impairment before succumbing to EBOV infection. As in the animal studies, only a portion (23%) of infected humans has decreased renal function [40]. Increased creatinine and serum/blood urea nitrogen concentrations have also been reported in patients with EVD [10, 40] (Table 3).

Cytokines

Concentrations of inflammatory cytokines increase dramatically in rhesus monkeys infected with EBOV including IL-6, IL-10, MCP-1, MIP1α, and MIP1β [31]. Increases are typically by 100-fold or more and occur by days six to eight PE. Humans also have dramatic concentration increases of cytokines including: IL-1 RA, IL-8, IL-15, MCP-1, MIP1α, and MIP1β [78]. The increases in humans occurred one to five days after onset of symptoms. Increases were often lower in patients surviving EVD than in terminal patients [78]. IL-6 serum concentrations in human patients are inconsistent. Most tested samples from the 1996 Gabon EVD outbreaks (both Mayibout II and Booué) and the 2003 Republic of the Congo EVD outbreak had increased serum IL-6 concentrations [78]. However, samples from the 1995 Kikwit EVD outbreak were stratified based on fatal cases and survivors [79]; fatal cases had lower concentrations of IL-6 (35 pg/mL) when compared to uninfected control patients (109 pg/mL), and survivors of EBOV infection had higher than normal concentrations of IL-6 (229 pg/mL). Another study using samples from the two Gabon EVD outbreaks in 1996 found that both survivors and non-survivors had increased concentrations of IL-6 compared to normal levels (< 10 pg/mL), but survivors had higher concentrations of IL-6 when compared to the fatal cases, 490 pg/mL and 190 pg/mL respectively [80]. All studies with rhesus monkeys have revealed increased concentrations of IL-6 when compared to baseline [31, 70]. The Kikwit human serum samples showed stratification of IL-10 concentrations as well. In case of fatalities, higher concentrations of serum IL-10 (195 pg/mL) were found when compared to uninfected control patient samples (50 pg/mL) and the survivors of EBOV infection (45 pg/mL) [79]. Each rhesus monkey tested for IL-10 concentrations had higher than baseline concentrations [31]. It is important to note that all of the

rhesus monkey studies involving cytokine research used a uniformly lethal dose of EBOV (1,000 PFU), so there are no surviving animals to compare with the fatalities [31, 70] (Table 4).

Coagulation

Decreased concentrations of circulating activated protein C have been reported for rhesus monkeys infected with EBOV by as early as day two PE [81]; in contrast, concentrations of D-dimers were reported to increase [31, 82]. Other reports describe increases in partial thrombin and activated partial thromboplastin times in monkeys infected with EBOV, decreased concentrations of Factor I, and lack of platelet aggregation over the course of the disease [65, 74]. Another report describes increased activity of tissue factor accompanied by lowered concentration of tissue factor pathway inhibitors/Factor Xa complexes [82]. No similar changes have been reported in humans with EVD but, elevated concentrations of D-dimers have been reported in humans infected with another ebolavirus, Sudan virus [83]. Coagulation abnormalities were observed in the 1976 outbreak of EVD in Zaire [2], and hemorrhage is often a feature of EVD [6, 10, 26, 40, 59, 66, 84].

Pathology

Pathological findings in rhesus monkeys are extensive because many animals have been studied, and they generally reproduce the damage observed in humans with EVD [85]. Splenomegaly is noted in both rhesus monkeys and humans infected with EBOV [3, 18, 86], and the spleens typically show extensive infection in infected animals (Figure 1A) [31, 58]. One of the earliest reports of rhesus monkey infection noted severe spleen destruction by EBOV with few spleen cells remaining recognizable in necropsy samples (Figure 1B) [75]. Both infected humans and rhesus monkey often have lymphadenopathy [87, 88]. Rhesus monkeys show marked signs of infection of dendritic and other immune cells within lymph nodes (Figure 2) [89].

There is limited information available for humans with EVD because few patients have been studied and the disease often occurs in remote locations. Humans with EVD have hepatic abnormalities including hepatomegaly and fatty degeneration of the liver. Hepatocytes form necrotic foci and Kupffer cells are present throughout the lobules. Liver cells display acidophilic necrosis, and virions can be observed in the sinusoids [90] (Figure 3A). Rhesus monkeys infected with EBOV show a similar liver involvement with specific EBOV staining evident in hepatocytes [31] (Figure 3B). There is one report of viral antigen detected in a biopsy from the eyelid of an EVD survivor [91].

Lung involvement is not always noted in human cases, however, rhesus monkey studies often uncovered evidence of lung infection [23, 58, 75]. Rhesus monkeys infected by the aerosol route do show extensive evidence of lung infection [23, 64] (Figure 4). Tachypnea is common in terminally ill patients, but this can be attributed to the progressive illness and shock without evidence of severe lung infection.

CONCLUSIONS

EBOV infection is often fatal and occurs sporadically in remote regions of sub-Saharan Africa. Due to the irregular and reclusive nature of outbreaks, there is limited information characterizing the disease course in humans, and a suitable animal model is needed for use in developing prophylactics and therapeutics. The information herein demonstrates that the rhesus monkey model of EBOV infection recapitulates what is known of the human disease irrespective of route of infection: aerosol, conjunctival, oral, intramuscular, intraperitoneal, or even by unknown means (Table 1, Table 2, Table 3, and Table 4).

Other animals including rodents have been used to model EVD. These models differ from the rhesus model in certain ways. For instance, rodent models may require newborn animals [92], immune compromised animals [93], or rodent-adapted viruses [74, 94-96]. EBOV-infected grivets do not develop a rash [18, 19, 97], which is a prominent feature of disease in rhesus monkey and humans (Table 1). EBOV infection has been studied in baboons [20], but such studies are few [98] and do not offer the wealth of data that is available from studies of rhesus monkeys.

Cynomolgus monkeys develop signs and symptoms in general agreement with those observed in rhesus monkeys but maximum body temperature in rhesus monkeys is higher than in cynomolgus macaques [21, 63, 72] and is closer to maximum body temperature observed in humans with EVD [12]. Nevertheless, the rhesus model has been used for testing of antiviral PE therapeutics and less frequently vaccines [27, 30, 31, 45, 82, 99, 100], whereas cynomolgus monkeys have been used primarily for vaccine development and a few antibody therapeutic studies [101-108].

Often the human data that has been collected or reported is anecdotal in nature and is not stratified or linked to the stage of disease. Other potentially confounding factors include, but are not limited to age, concomitant infections, health status, mode of infection, use of supportive care measures, or disease outcome. These factors limit the observable features of the disease in many cases, or limit observations to sometimes unknown,times after infection. In addition, studies of humans with EVD typically occur in remote areas where laboratory equipment may be in short supply and where many physiological parameters cannot easily be measured.

Nonetheless, the findings in rhesus monkeys, infected by any means, reflect what has been reported for humans with EVD (even while there are differences among the human manifestations) and suggest the rhesus monkey model of EVD mimics the human disease. Thus, the rhesus monkey model is an appropriate model for studying disease pathogenesis as well as testing of candidate medical countermeasures for EVD.

REFERENCES

1. Kuhn J. H., B.S., Ebihara H., Geisbert T. W., Jahrling P. B., Kawaoka Y., Netesov S. V., Nichol S. T., Peters C. J., Volchkov V. E., Ksiazek T. G. , *Family Filoviridae*, in *Virus Taxonomy - Ninth Report of the International Committee on Taxonomy of Viruses*, A.M.J. King Andrew M. Q., Carstens Eric B., Lefkowitz Elliot J, Editor. 2011, Elsevier/Academic Press: London, United Kingdom. p. 665-671.
2. Isaacson, M., et al., *Clinical aspects of Ebola virus disease at the Ngaliema hospital, Kinshasa, Zaire, 1976*, in *Ebola Virus Haemorrhagic Fever*, S.R. Pattyn, Editor. 1978, Elsevier/North-Holland Biomedical Press: New York. p. 15-20.
3. Piot, P., Bureau, G, Breman D, et al., *Clinical Aspects of Ebola Virus Infection in Yambuku Area, Zaire, 1976*, in *Ebola Virus Haemorrhagic Fever*, S. Pattyn, Editor. 1978, Elsevier/North Holland: Amsterdam: Amsterdam. p. 7-14
4. Hartman, A.L., J.S. Towner, and S.T. Nichol, *Ebola and marburg hemorrhagic fever.* Clin Lab Med, 2010. **30**(1): p. 161-77.
5. Pourrut, X., et al., *The natural history of Ebola virus in Africa.* Microbes Infect, 2005. **7**(7-8): p. 1005-14.
6. WHO, *Ebola haemorrhagic fever in Zaire, 1976.* Bull World Health Organ, 1978. **56**(2): p. 271-93.
7. Sarwar, U.N., S. Sitar, and J.E. Ledgerwood, *Filovirus emergence and vaccine development: A perspective for health care practitioners in travel medicine.* Travel Med Infect Dis, 2010. **9**(3): p. 126-34.
8. Leroy, E.M., et al., *Multiple Ebola virus transmission events and rapid decline of central African wildlife.* Science, 2004. **303**(5656): p. 387-90.
9. Rizkalla, C., Blanco-Silva, F, Gruver, S, *Modelling the Effect of Ebola and bushmeat hunting on western lowland Gorillas.* EcoHealth, 2007. **4**(2): p. 151-155.
10. Akinfeeva L., Aksionova O., Vasilevich I., *Translated [A case of Ebola hemorrhagic fever].* Infektsionnie Bolezni [Russian], 2005. **3**: p. 85-88.
11. Emond, R.T., et al., *A case of Ebola virus infection.* Br Med J, 1977. **2**(6086): p. 541-4.
12. Heymann, D.L., et al., *Ebola hemorrhagic fever: Tandala, Zaire, 1977-1978.* J Infect Dis, 1980. **142**(3): p. 372-6.
13. Breman, J.G.P., P., Johnson, K.M., White, M.K., Mbuyi, M., Sureau, P., Heyman, D.L. , *The Epidemiology of Ebola Haemorrhagic Fever in Zaire, 1976*, in *Ebola Virus Hemorrhagic Fever*, P. S.R., Editor. 1978, NY: Elsevier /North-Holland Biomedical Press: New York, NY.
14. Wauquier, N., et al., *Association of KIR2DS1 and KIR2DS3 with fatal outcome in Ebola virus infection.* Immunogenetics, 2010. **62**(11-12): p. 767-71.
15. Kash JC, M.E., Carter V, Grosch M, Perwitasari O, Proll SC, Thomas MJ, Weber F, Klenk HD, Katze MG, *Global suppression of the host antiviral response by Ebola- and Marburgviruses: increased antagonism of the type I interferon response is associated with enhanced virulence.* J Virol, 2006. **80**(6): p. 3009-20.
16. Harcourt, B.H., A. Sanchez, and M.K. Offermann, *Ebola virus selectively inhibits responses to interferons, but not to interleukin-1beta, in endothelial cells.* J Virol, 1999. **73**(4): p. 3491-6.
17. Mahanty, S., et al., *Cutting edge: impairment of dendritic cells and adaptive immunity by Ebola and Lassa viruses.* J Immunol, 2003. **170**(6): p. 2797-801.

18. Baskerville, A., et al., *The pathology of experimental Ebola virus infection in monkeys.* J Pathol, 1978. **125**(3): p. 131-8.
19. Bowen, E.T., et al., *Ebola haemorrhagic fever: experimental infection of monkeys.* Trans R Soc Trop Med Hyg, 1978. **72**(2): p. 188-91.
20. Ryabchikova, E.I., L.V. Kolesnikova, and S.V. Luchko, *An analysis of features of pathogenesis in two animal models of Ebola virus infection.* J Infect Dis, 1999. **179 Suppl 1**: p. S199-202.
21. Geisbert, T.W., et al., *Pathogenesis of Ebola hemorrhagic fever in cynomolgus macaques: evidence that dendritic cells are early and sustained targets of infection.* Am J Pathol, 2003. **163**(6): p. 2347-70.
22. Geisbert, T.W., et al., *Pathogenesis of Ebola hemorrhagic fever in primate models: evidence that hemorrhage is not a direct effect of virus-induced cytolysis of endothelial cells.* Am J Pathol, 2003. **163**(6): p. 2371-82.
23. Johnson, E., et al., *Lethal experimental infections of rhesus monkeys by aerosolized Ebola virus.* Int J Exp Pathol, 1995. **76**(4): p. 227-36.
24. Carrion, R., Jr., et al., *A small nonhuman primate model for filovirus-induced disease.* Virology, 2011. **420**(2): p. 117-24.
25. Ignatiev, G.M., et al., *Immune and pathophysiological processes in baboons experimentally infected with Ebola virus adapted to guinea pigs.* Immunol Lett, 2000. **71**(2): p. 131-40.
26. Bwaka, M.A., et al., *Ebola hemorrhagic fever in Kikwit, Democratic Republic of the Congo: clinical observations in 103 patients.* J Infect Dis, 1999. **179 Suppl 1**: p. S1-7.
27. Feldmann, H., et al., *Effective post-exposure treatment of Ebola infection.* PLoS Pathog, 2007. **3**(1): p. 54-61.
28. Fisher-Hoch, S.P., et al., *Pathophysiology of shock and hemorrhage in a fulminating viral infection (Ebola).* J Infect Dis, 1985. **152**(5): p. 887-94.
29. Geisbert, T.W., et al., *Vesicular stomatitis virus-based vaccines protect nonhuman primates against aerosol challenge with Ebola and Marburg viruses.* Vaccine, 2008. **26**(52): p. 6894-900.
30. Geisbert, T.W., et al., *Postexposure protection of non-human primates against a lethal Ebola virus challenge with RNA interference: a proof-of-concept study.* Lancet, 2010. **375**(9729): p. 1896-905.
31. Hensley, L.E., et al., *Recombinant human activated protein C for the postexposure treatment of Ebola hemorrhagic fever.* J Infect Dis, 2007. **196 Suppl 2**: p. S390-9.
32. Huijbregts, B., De Wachter, P., Sosthene, L, Obiang, N., Akou, M.E., *Ebola and the decline of gorilla Gorilla gorilla and chimpanzee Pan troglodytes populations in Minkebe Forest, north-eastern Gabon.* Oryx, 2003. **37**(4): p. 437-443.
33. Lemonick, M.D., *A deadly mystery. A savage outbreak of Ebola virus is killing gorillas and chimps. Epidemiologists have just figured out why.* Time, 2007. **169**(19): p. 73.
34. Wittmann, T.J., et al., *Isolates of Zaire ebolavirus from wild apes reveal genetic lineage and recombinants.* Proc Natl Acad Sci U S A, 2007. **104**(43): p. 17123-7.
35. Walsh, P.D., et al., *Catastrophic ape decline in western equatorial Africa.* Nature, 2003. **422**(6932): p. 611–614.
36. Walsh, P.D., R. Biek, and L.A. Real, *Wave-like spread of Ebola Zaire.* PLoS Biol, 2005. **3**(11): p. e371.

37. Walsh D., T.B., C. Sanz, D. Morgan, D. Doran-Sheehy, *Potential for Ebola transmission between gorilla and chimpanzee social groups.* The American Naturalist, 2007. **169**(5): p. 684-689.
38. Bermejo, M., et al., *Ebola outbreak killed 5000 gorillas.* Science, 2006. **314**(5805): p. 1564.
39. Vogel, G., *Ecology. Tracking Ebola's deadly march among wild apes.* Science, 2006. **314**(5805): p. 1522-3.
40. Georges, A.J., et al., *Ebola hemorrhagic fever outbreaks in Gabon, 1994-1997: epidemiologic and health control issues.* J Infect Dis, 1999. **179 Suppl 1**: p. S65-75.
41. Georges-Courbot, M.C., et al., *Isolation and phylogenetic characterization of Ebola viruses causing different outbreaks in Gabon.* Emerg Infect Dis, 1997. **3**(1): p. 59-62.
42. Bray, M., *Defense against filoviruses used as biological weapons.* Antiviral Res, 2003. **57**(1-2): p. 53-60.
43. Okware, S.I., et al., *An outbreak of Ebola in Uganda.* Trop Med Int Health, 2002. **7**(12): p. 1068-75.
44. Bukreyev, A., et al., *Successful topical respiratory tract immunization of primates against Ebola virus.* J Virol, 2007. **81**(12): p. 6379-88.
45. Pratt, W.D., et al., *Protection of nonhuman primates against two species of Ebola virus infection with a single complex adenovirus vector.* Clin Vaccine Immunol, 2010. **17**(4): p. 572-81.
46. Weingartl, H.M., et al., *Transmission of Ebola virus from pigs to non-human primates.* Sci Rep, 2012. **2**: p. 811.
47. Roy, C.J., D.S. Reed, and J.A. Hutt, *Aerobiology and inhalation exposure to biological select agents and toxins.* Vet Pathol, 2010. **47**(5): p. 779-89.
48. Leffel, E.K. and D.S. Reed, *Marburg and Ebola viruses as aerosol threats.* Biosecur Bioterror, 2004. **2**(3): p. 186-91.
49. Ryan, C.P., *Zoonoses likely to be used in bioterrorism.* Public Health Rep, 2008. **123**(3): p. 276-81.
50. Zilinskas R., Leitenberg M., with Kuhn J., *The Soviet Biological Weapons Program: A History* 2012, Cambridge, Massachusetts: Harvard University Press.
51. Alibeck K, Handelman S, *Biohazard:The Chilling True Story of the Largest Covert Biological Weapons Program in the World - Told from Inside by the Man Who Ran It*. 1999: Random House.
52. Borio, L., et al., *Hemorrhagic fever viruses as biological weapons: medical and public health management.* JAMA, 2002. **287**(18): p. 2391-405.
53. WHO. *Ebola Hemorrhagic Fever*. Fact sheet N°103 2012 [cited 2012 Feb 11]; Available from: http://www.who.int/mediacentre/factsheets/fs103/en/.
54. Kuhn, J.H., et al., *Evaluation of perceived threat differences posed by filovirus variants.* Biosecur Bioterror, 2011. **9**(4): p. 361-71.
55. Jaax, N., et al., *Transmission of Ebola virus (Zaire strain) to uninfected control monkeys in a biocontainment laboratory.* Lancet, 1995. **346**(8991-8992): p. 1669-71.
56. Khan, A.S., et al., *The reemergence of Ebola hemorrhagic fever, Democratic Republic of the Congo, 1995. Commission de Lutte contre les Epidemies a Kikwit.* J Infect Dis, 1999. **179 Suppl 1**: p. S76-86.

57. Leroy, E.M., et al., *Human Ebola outbreak resulting from direct exposure to fruit bats in Luebo, Democratic Republic of Congo, 2007.* Vector Borne Zoonotic Dis, 2009. **9**(6): p. 723-8.
58. Jaax, N.K., et al., *Lethal experimental infection of rhesus monkeys with Ebola-Zaire (Mayinga) virus by the oral and conjunctival route of exposure.* Arch Pathol Lab Med, 1996. **120**(2): p. 140-55.
59. Richards, G.A., et al., *Unexpected Ebola virus in a tertiary setting: clinical and epidemiologic aspects.* Crit Care Med, 2000. **28**(1): p. 240-4.
60. Eichner M., Dowell SF., Nina F. , *Incubation Period of Ebola Hemorrhagic Virus Subtype Zaire.* Osong Public Health and Research Perspectives 2011. **2**(1): p. 3-7.
61. Ndambi, R., et al., *Epidemiologic and clinical aspects of the Ebola virus epidemic in Mosango, Democratic Republic of the Congo, 1995.* J Infect Dis, 1999. **179 Suppl 1**: p. S8-10.
62. Geisbert, T.W., et al., *Filovirus Pathogenesis in Nonhuman Primates*, in *Ebola and Marburg Viruses: Molecular and Cellular Biology (Horizon Bioscience)*, H.-D. Klenk and H. Feldmann, Editors. 2004, Taylor & Francis: Norfolk, UK. p. 203-238.
63. Bowen, E.T., et al., *A comparative study of strains of Ebola virus isolated from southern Sudan and northern Zaire in 1976.* J Med Virol, 1980. **6**(2): p. 129-38.
64. Twenhafel, N.A., et al., *Pathology of Experimental Aerosol Zaire Ebolavirus Infection in Rhesus Macaques.* Vet Pathol, 2013. **Ebpub ahead of print**.
65. Fisher-Hoch, S.P., et al., *Haematological and biochemical monitoring of Ebola infection in rhesus monkeys: implications for patient management.* Lancet, 1983. **2**(8358): p. 1055-8.
66. Bonnet, M.J., P. Akamituna, and A. Mazaya, *Unrecognized Ebola hemorrhagic fever at Mosango Hospital during the 1995 epidemic in Kikwit, Democratic Republic of the Congo.* Emerg Infect Dis, 1998. **4**(3): p. 508-10.
67. Geisbert, T., Hensley, LE, *Ebola virus: new insights into disease aetiopathology and possible therapeutic interventions.* Expert Rev Mol Med, 2004. **6**(20): p. 1-24.
68. Sanchez, A., *Filoviridae: Marburg and Ebola Viruses*, in *Fields Virology*, D. Knipe, Editor. 2007, Lippincott Williams and Williams: Philadelphia. p. 1409–1448.
69. Colebunders, R. and M. Borchert, *Ebola haemorrhagic fever--a review.* J Infect, 2000. **40**(1): p. 16-20.
70. Ebihara, H., et al., *Host response dynamics following lethal infection of rhesus macaques with Zaire ebolavirus.* J Infect Dis, 2011. **204 Suppl 3**: p. S991-9.
71. Bowen E.T.W., B.A., Cantell K., Mann G.F., Simpson D.I.H, Zuckerman A.J., *The Effect of Interferon on Experimental Ebola Virus Infection in Rhesus Monkeys*, in *Ebola Virus Haemorrhagic Fever*. 1978, Elsevier/North Holland: Amsterdam. p. 167-171.
72. Reed, D.S., et al., *Aerosol exposure to Zaire ebolavirus in three nonhuman primate species: differences in disease course and clinical pathology.* Microbes Infect, 2011. **13**(11): p. 930-6.
73. Kortepeter, M.G., et al., *Real-time monitoring of cardiovascular function in rhesus macaques infected with Zaire ebolavirus.* J Infect Dis, 2011. **204 Suppl 3**: p. S1000-10.
74. Bray, M., et al., *Haematological, biochemical and coagulation changes in mice, guinea-pigs and monkeys infected with a mouse-adapted variant of Ebola Zaire virus.* J Comp Pathol, 2001. **125**(4): p. 243-53.

75. Ellis, D.S., et al., *Ebola virus: a comparison, at ultrastructural level, of the behaviour of the Sudan and Zaire strains in monkeys.* Br J Exp Pathol, 1978. **59**(6): p. 584-93.
76. Jahrling, P.B., et al., *Ebola hemorrhagic fever: evaluation of passive immunotherapy in nonhuman primates.* J Infect Dis, 2007. **196 Suppl 2**: p. S400-3.
77. Ksiazek, T.G., et al., *Clinical virology of Ebola hemorrhagic fever (EHF): virus, virus antigen, and IgG and IgM antibody findings among EHF patients in Kikwit, Democratic Republic of the Congo, 1995.* J Infect Dis, 1999. **179 Suppl 1**: p. S177-87.
78. Wauquier, N., et al., *Human fatal zaire ebola virus infection is associated with an aberrant innate immunity and with massive lymphocyte apoptosis.* PLoS Negl Trop Dis, 2010. **4**(10:e837).
79. Villinger, F., et al., *Markedly elevated levels of interferon (IFN)-gamma, IFN-alpha, interleukin (IL)-2, IL-10, and tumor necrosis factor-alpha associated with fatal Ebola virus infection.* J Infect Dis, 1999. **179 Suppl 1**: p. S188-91.
80. Baize, S., et al., *Inflammatory responses in Ebola virus-infected patients.* Clin Exp Immunol, 2002. **128**(1): p. 163-8.
81. Geisbert, T.W., et al., *Mechanisms underlying coagulation abnormalities in ebola hemorrhagic fever: overexpression of tissue factor in primate monocytes/macrophages is a key event.* J Infect Dis, 2003. **188**(11): p. 1618-29.
82. Geisbert, T.W., et al., *Treatment of Ebola virus infection with a recombinant inhibitor of factor VIIa/tissue factor: a study in rhesus monkeys.* Lancet, 2003. **362**(9400): p. 1953-8.
83. Rollin, P.E., D.G. Bausch, and A. Sanchez, *Blood chemistry measurements and D-Dimer levels associated with fatal and nonfatal outcomes in humans infected with Sudan Ebola virus.* J Infect Dis, 2007. **196 Suppl 2**: p. S364-71.
84. WHO, *Outbreak(s) of Ebola haemorrhagic fever, Congo and Gabon, October 2001-July 2002.* Weekly Epidemiological Report, 2003. **78**(26): p. 223-225.
85. Kuhn, J., *Filoviruses*. 2008, New York: Springer Verlag/Wien.
86. Baskerville, A., et al., *Ultrastructural pathology of experimental Ebola haemorrhagic fever virus infection.* J Pathol, 1985. **147**(3): p. 199-209.
87. Kortepeter, M.G., D.G. Bausch, and M. Bray, *Basic clinical and laboratory features of filoviral hemorrhagic fever.* J Infect Dis, 2011. **204 Suppl 3**: p. S810-6.
88. Larsen, T., et al., *Pathologic findings associated with delayed death in nonhuman primates experimentally infected with Zaire Ebola virus.* J Infect Dis, 2007. **196 Suppl 2**: p. S323-8.
89. Geisbert, T.W., et al., *Apoptosis induced in vitro and in vivo during infection by Ebola and Marburg viruses.* Lab Invest, 2000. **80**(2): p. 171-86.
90. Zaki, S.R., et al., *A novel immunohistochemical assay for the detection of Ebola virus in skin: implications for diagnosis, spread, and surveillance of Ebola hemorrhagic fever. Commission de Lutte contre les Epidemies a Kikwit.* J Infect Dis, 1999. **179 Suppl 1**: p. S36-47.
91. Kalongi, Y., et al., *Isolated case of Ebola hemorrhagic fever with mucormycosis complications, Kinshasa, Democratic Republic of the Congo.* J Infect Dis, 1999. **179 Suppl 1**: p. S15-7.
92. Pattyn, S., et al., *Isolation of Marburg-like virus from a case of haemorrhagic fever in Zaire.* Lancet, 1977. **1**(8011): p. 573-4.

93. Bray, M., J. Driscoll, and J.W. Huggins, *Treatment of lethal Ebola virus infection in mice with a single dose of an S-adenosyl-L-homocysteine hydrolase inhibitor.* Antiviral Res, 2000. **45**(2): p. 135-47.
94. Connolly, B.M., et al., *Pathogenesis of experimental Ebola virus infection in guinea pigs.* J Infect Dis, 1999. **179 Suppl 1**: p. S203-17.
95. Bray, M., et al., *A mouse model for evaluation of prophylaxis and therapy of Ebola hemorrhagic fever.* J Infect Dis, 1998. **178**(3): p. 651-61.
96. Wahl-Jensen, V., et al., *Use of the Syrian hamster as a new model of ebola virus disease and other viral hemorrhagic fevers.* Viruses, 2012. **4**(12): p. 3754-84.
97. Davis, K.J., et al., *Pathology of experimental Ebola virus infection in African green monkeys. Involvement of fibroblastic reticular cells.* Arch Pathol Lab Med, 1997. **121**(8): p. 805-19.
98. Perry, D.L., L. Bollinger, and G.L. White, *The Baboon (Papio spp.) as a model of human Ebola virus infection.* Viruses, 2012. **4**(10): p. 2400-16.
99. Smith, L.M., et al., *Interferon-beta Therapy Prolongs Survival in Rhesus Macaque Models of Ebola and Marburg Hemorrhagic Fever.* J Infect Dis, 2013.
100. Warren, T.K., et al., *Advanced antisense therapies for postexposure protection against lethal filovirus infections.* Nat Med, 2010. **16**(9): p. 991-4.
101. Geisbert, T.W., et al., *Recombinant adenovirus serotype 26 (Ad26) and Ad35 vaccine vectors bypass immunity to Ad5 and protect nonhuman primates against ebolavirus challenge.* J Virol, 2011. **85**(9): p. 4222-33.
102. Geisbert, T.W., et al., *Single-injection vaccine protects nonhuman primates against infection with marburg virus and three species of ebola virus.* J Virol, 2009. **83**(14): p. 7296-304.
103. Jones, S.M., et al., *Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses.* Nat Med, 2005. **11**(7): p. 786-90.
104. Qiu, X., et al., *Successful treatment of ebola virus-infected cynomolgus macaques with monoclonal antibodies.* Sci Transl Med, 2012. **4**(138): p. 138ra81.
105. Sullivan, N.J., et al., *Immune protection of nonhuman primates against Ebola virus with single low-dose adenovirus vectors encoding modified GPs.* PLoS Med, 2006. **3**(6): p. e177.
106. Sullivan, N.J., et al., *Accelerated vaccination for Ebola virus haemorrhagic fever in non-human primates.* Nature, 2003. **424**(6949): p. 681-4.
107. Sullivan, N.J., et al., *CD8+ cellular immunity mediates rAd5 vaccine protection against Ebola virus infection of nonhuman primates.* Nat Med, 2011. **17**(9): p. 1128-31.
108. Swenson, D.L., et al., *Vaccine to confer to nonhuman primates complete protection against multistrain Ebola and Marburg virus infections.* Clin Vaccine Immunol, 2008. **15**(3): p. 460-7.
109. Marzi, A., et al., *Protective efficacy of neutralizing monoclonal antibodies in a nonhuman primate model of Ebola hemorrhagic fever.* PLoS One, 2012. **7**(4): p. e36192.
110. Olinger, G.G., Jr., et al., *Delayed treatment of Ebola virus infection with plant-derived monoclonal antibodies provides protection in rhesus macaques.* Proc Natl Acad Sci U S A, 2012. **109**(44): p. 18030-5.
111. Neumann, G., et al., *Proteolytic processing of the Ebola virus glycoprotein is not critical for Ebola virus replication in nonhuman primates.* J Virol, 2007. **81**(6): p. 2995-8.

112. Warfield, K.L., et al., *Gene-specific countermeasures against Ebola virus based on antisense phosphorodiamidate morpholino oligomers.* PLoS Pathog, 2006. **2**(1): p. e1.
113. Geisbert, T.W., et al., *Evaluation in nonhuman primates of vaccines against Ebola virus.* Emerg Infect Dis, 2002. **8**(5): p. 503-7.
114. Dye, J.M., et al., *Postexposure antibody prophylaxis protects nonhuman primates from filovirus disease.* Proc Natl Acad Sci U S A, 2012. **109**(13): p. 5034-9.
115. Oswald, W.B., et al., *Neutralizing antibody fails to impact the course of Ebola virus infection in monkeys.* PLoS Pathog, 2007. **3**(1): p. e9.
116. Baize, S., et al., *Defective humoral responses and extensive intravascular apoptosis are associated with fatal outcome in Ebola virus-infected patients.* Nat Med, 1999. **5**(4): p. 423-6.
117. WHO, *Outbreak(s) of Ebola haemorrhagic fever in the Republic of the Congo, January-April 2003.* Weekly Epidemiological Report, 2003. **78**(33): p. 285-9.
118. WHO, *Outbreak(s) of Ebola haemorrhagic fever in the Republic of the Congo, January-April 2003.* Wkly Epidemiol Rec, 2003. **78**(33): p. 285-9.
119. Yevstigneyev, V., *Valentin Yevstigneyev on issues relating to Russian biological weapons.* Yaderny Kontrol (Nuclear Control) Digest (PIR Center), 1999. **4**(11).
120. Leroy, E.M., et al., *Human asymptomatic Ebola infection and strong inflammatory response.* Lancet, 2000. **355**(9222): p. 2210-5.
121. Hensley, L.E., et al., *Proinflammatory response during Ebola virus infection of primate models: possible involvement of the tumor necrosis factor receptor superfamily.* Immunol Lett, 2002. **80**(3): p. 169-79.

Table 1: Lethality and General Disease State Comparison of Rhesus Monkeys and Humans Infected with EBOV

Rhesus Monkey						Human					
Lethality[1]	Viremia[2]	Rash	Route	Study Type[3]	Ref	Lethality	Viremia	Rash	Route	Event	Ref
33% (1/3)	4.00E+07	Yes	IM	NHP model[4]	[74]	88% (280/318)	3.00E+06	Yes	Outbreak	Zaire (Yambuku) 1976	[6]
100% (2/2)	6.00E+06	Yes		Treatment	[30]	75% (3/4)	5.00E+01	Yes		Zaire (Tandala) 1977-78	[12]
100% (2/2)	1.00E+08	Yes		Treatment	[27]	75%% (45/60)	NR	NR		Gabon (Makokou) 1994	[40]
100% (1/1)	1.00E+05[5]	NR		Treatment	[109]	81% (255/315)	3.1E+01	Yes		Zaire (Kikwit) 1995	[26, 56, 77]
100% (3/3)	1.00E+05	Yes		Treatment	[31]	57% (21/37)	NR	Yes		Gabon (Mayibout II) 1996	[87, 116]
100% (3/3)	6.31E+07[5]	Yes		NHP model	[70]	75% (45/60)	NR	NR		Gabon (Booué) 1996	[41, 87]
67% (6/9)	1.00E+07[7]	Yes		NHP model	[73]	79% (97/123)	NR	NR		Gabon/RC 2001	[84]
80% (4/5)	1.00E+07	Yes		Treatment	[110]	90% (129/143)	NR	NR		RC (Kéllé) 2003	[87, 117]
100% (2/2)	3.16E+07	Yes		NHP model	[111]	83% (29/35)	NR	NR		RC (Mbomo) 2003	[87, 118]
100% (4/4)	1.00E+04	NR		Treatment	[112]	75% (9/12)	NR	NR		RC (Etoumbi) 2005	[87]
100% (2/2)	Yes	NR		Vaccine	[113]	70% (186/264)	NR	NR		DRC (Kasai Orientale) 2007	[57]
100% (1/1)	Yes	Yes		Treatment	[114]	47% (15/32)	NR	NR		DRC (Kasai Orientale) 2008	[87]
100% (1/1)	1.00E+06	NR		Treatment	[115]						
100% (1/1)	NR	Yes		NHP model	[58]						
100% (1/1)	3.2E+07	NR		Treatment	[100]						
100% (5/5)	3.00E+06[6]	Yes	IP	NHP model	[19]	0% (0/1)	3.2E+04[6]	Yes	Needle stick	England 1976	[11]
100% (4/4)	NR	Yes		NHP model	[18]	100% (1/1)	NR	Yes		Russia 1994	[119]
100% (2/2)	1.00E+05[5]	NR		Vaccine	[44]	100% (1/1)	NR	NR		Russia 2004	[10]
100% (2/2)	NR	Yes		NHP model	[75]						
50% (1/2)	NR	NR		NHP model	[65]						

Rhesus Monkey						Human					
Lethality[1]	Viremia[2]	Rash	Route	Study Type[3]	Ref	Lethality	Viremia	Rash	Route	Event	Ref
100% (4/4)	NR	Yes	AER	NHP model	[23]	100% (1/1)	NR	Yes	UNK	Zaire 1995	[66]
100% (6/6)	1.00E+07	Yes		NHP model	[72]						
100% (3/3)	2.00E+06	Yes		Vaccine	[45]						
100% (4/4)	6.30E+06[7]	Yes		NHP Model	[64]						
100% (4/4)	NR	Yes	CONJ	NHP Model	[58]	0% (0/11) serosurvery[8]		NA[8]		Gabon 1996	[120]
75% (3/4)	NR	Yes	Oral	NHP Model	[58]						
83% (5/6)	1.00E+07	Yes	NR	NHP model	[28]						
66% (2/3)	NR	NR	UNK	Accidental	[55]						
100% (2/2)	3.16E+05	NR	NR	Treatment	[76]						

[1] percentage (number of deaths/total cases)

[2] maximum PFU/mL observed unless noted otherwise

[3] NHP model development or study designed to evaluate a candidate treatment/vaccine

[4] mouse-adapted EBOV

[5] maximum tissue culture 50% infectious dose in blood ($TCID_{50}$)

[6] guinea pig infective units (GPIU)

[7] qRT-PCR PFU/mL equivalents

[8] asymptomatic individuals, diagnosis based on serology or PCR

Abbreviations: AER = aerosol, CONJ = conjunctival, DRC = Democratic Republic of the Congo, IM = intramuscular, IP = intraperitoneal, NA = not applicable, NR = not reported, RC = Republic of the Congo, Ref = references, UNK = unknown

Table 2: Hematology Comparison of Rhesus Monkeys and Humans Infected with EBOV

Rhesus Monkey						Human					
Lym	**Total Leu**	**Neu**	**Plt**	**Route**	**Ref**	**Lym**	**Total Leu**	**Neu**	**Plt**	**Route**	**Ref**
↓	↑	↑	↓	IM	[74]	NR	↑	NR	↓ (modest)	Outbreak	[6]
↓	NR	NR	↓		[27]	NR	↓	NR	↓		[59]
↓	NR	NR	↓		[31]	NR	NC	NR	NR		[91]
NR	NR	NR	↓		[30]	↓	NR	NR	NR		[78]
↓	↑↓	↑	↓		[70]						
↓	↑↓	↑	↓		[73]						
NR	NR	NR	↓		[110]						
↓	NR	NR	↓		[111]						
NR	NR	NR	↓		[112]						
↓	NR	NR	↓		[114]						
NR	NR	NR	↓		[109]						
↓	NR	NR	↓		[100]						
↓↑	↑↓	↑	↓	IP	[65]						
↓	↑	↑	↓	AER	[23]	↓	↑	↑	↓	Needle stick	[10]
↓↑	↑	NR	↓		[72]	NR	↓	NR	NC		[11]
NR	NR	NR	↓		[45]						
↑↓	↓	↓↑	↓		[64]						
↓	NR	↑	↓	UNK	[28]	↓	↑	NR	NR	UNK	[66]

Abbreviations: AER = aerosol, IM = intramuscular, IP = intraperitoneal, Leu = leukocytes, Lym = lymphocytes, NC = no change from normal, Neu = neutrophils, NR = not reported, Plt = platelets, Ref = references, UNK = unknown

Symbols: ↓ = less than normal, ↑ = higher than normal, ↑ ↓ = higher than normal followed by lower than normal, ↓ ↑ = lower than normal followed by higher than normal

Table 3: Liver and Kidney Panel Comparison of Rhesus Monkeys and Humans Infected with EBOV

Rhesus Monkey							Human						
ALP	**AST**	**GGT**	**CRE**	**S/BUN**	**Route**	**Ref**	**ALP**	**AST**	**GGT**	**CRE**	**S/BUN**	**Route**	**Ref**
↑	↑	↑	↑	↑		[27]							
↑	↑	↑	NC	NC		[31]							
NC	↑	NC	↑	↑		[30]							
NR	↑	NR	↑	↑		[73]							
NR	↑	NC	NC	NC		[110]	↑	↑	↑	NC	NC		[40]
NR	↑	NR	NR	NR	IM	[111]	↑	↑	↑	↑	NC	Outbreak	[59]
↑	NR	NR	NR	NR		[112]	NR	↑	NR	↑	↑		[91]
NR	↑	NR	NR	NR		[114]							
↑	NR	NR	↑	↑		[58]							
↑	↑	↑	↑	↑		[29]							
NR	↑	NR	NR	NR		[100]							
NR	↑	NR	↑	↑	IP	[44]							
NR	↑	NR	↑	↑		[65]							
↑	NR	NR	↑	↑		[23]							
NR	↑	NR	NR	NR	AER	[45]							
NR	↑	↑	↑	↑		[64]	↑	↑	↑	↑	NC	Needle stick	[10]
↑	NR	NR	↑	↑	CONJ	[58]							
↑	NR	NR	↑	↑	ORAL	[58]							
NR	↑	NR	↑	↑	UNK	[28]							

Abbreviations: AER = aerosol, ALP = alkaline phosphatase serum levels, AST = aspartate aminotransferase serum levels, S/BUN = serum or blood urea nitrogen; CONJ = conjunctival, CRE = creatinine serum levels, GGT = γ-glutamyltransferase serum concentrations, IM = intramuscular, NC = no change from normal, NR = not reported, Ref = references;
Symbol: ↑ = higher than normal

Table 4: Cytokine Comparison of Rhesus Monkeys and Humans Infected with EBOV

Rhesus Monkey										Human									
IL-1RA	**IL-1β**	**IL-6**	**IL-10**	**MCP-1**	**MIP1α**	**MIP1β**	**TNFα**	**Route**	**Ref**	**IL-1RA**	**IL-1β**	**IL-6**	**IL-10**	**MCP-1**	**MIP1α**	**MIP1β**	**TNFα**	**Route**	**Ref**
NR	NC	↑	↑	↑	↑	↑	↑	IM	[31]	↑	↑	↑	NR	↑	↑	↑	NC	Outbreak	[78]
↑	↑	↑	↑	NC	↑	NR	↑		[70]	↑	↑	↑	↑	NR	↑	↑	↑		[80]
NR	NR	↑	NR	NR	↑	↑	NR	NR	[121]	NR	↓	↑	↑	NR	NR	NR	↑		[79]

[1] asymptomatic individuals, diagnosis based on serology or PCR

Abbreviations: IM = intramuscular, NC = no change from normal, NR = not reported, Ref = references, UNK = unknown

Cytokine abbreviations: IL = interleukin, MCP-1 = monocyte chemoattractant protein-1, MIP1α = macrophage inflammatory protein 1α, MIP1β = macrophage inflammatory protein 1β

Symbols: ↑ = higher than normal

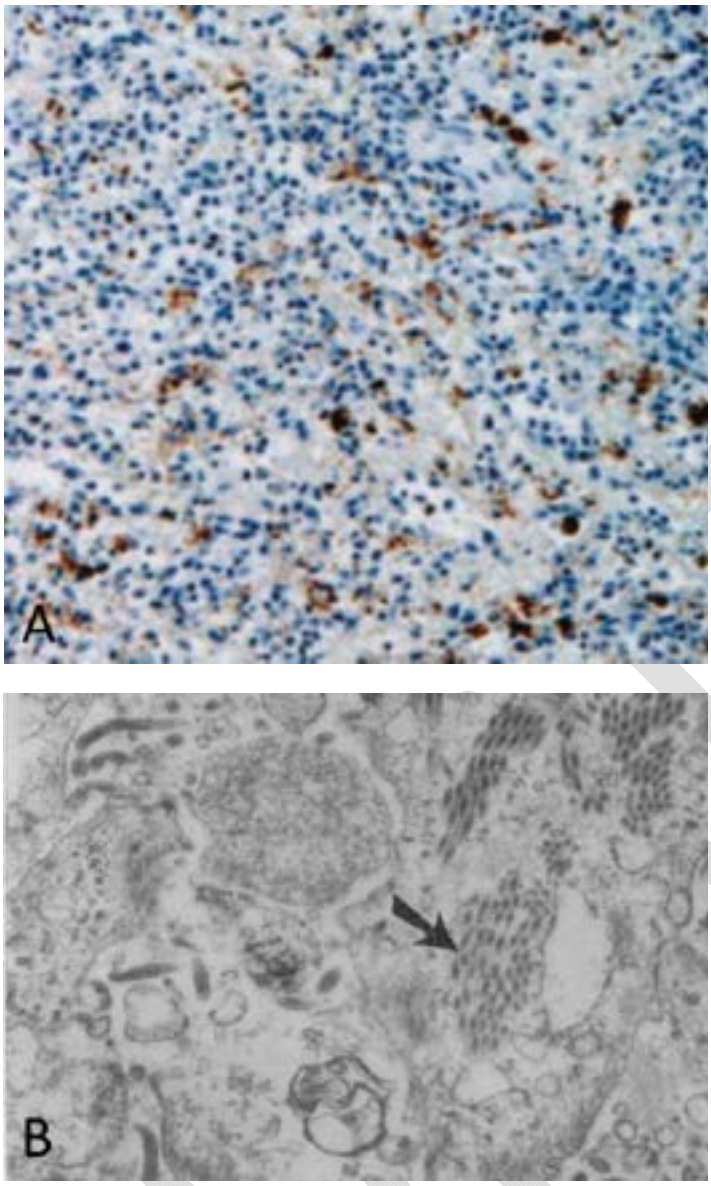

Figure 1: Spleen

A) Scattered foci of immunopositive cells (brown) in the spleen of an EBOV-infected rhesus monkey on day eight PE. Horseradish peroxidase antigen labeling with a hematoxylin counterstain at 20× magnification (adapted from [31]).

B) Large number of EBOV particles with aggregation of ribonucleocapsids (arrow) in the center within the spleen of a rhesus monkey. Lead stain with electron microscopy of necropsy sample from animal euthanized on day six PE at 17,700× magnification (adapted from [75]).

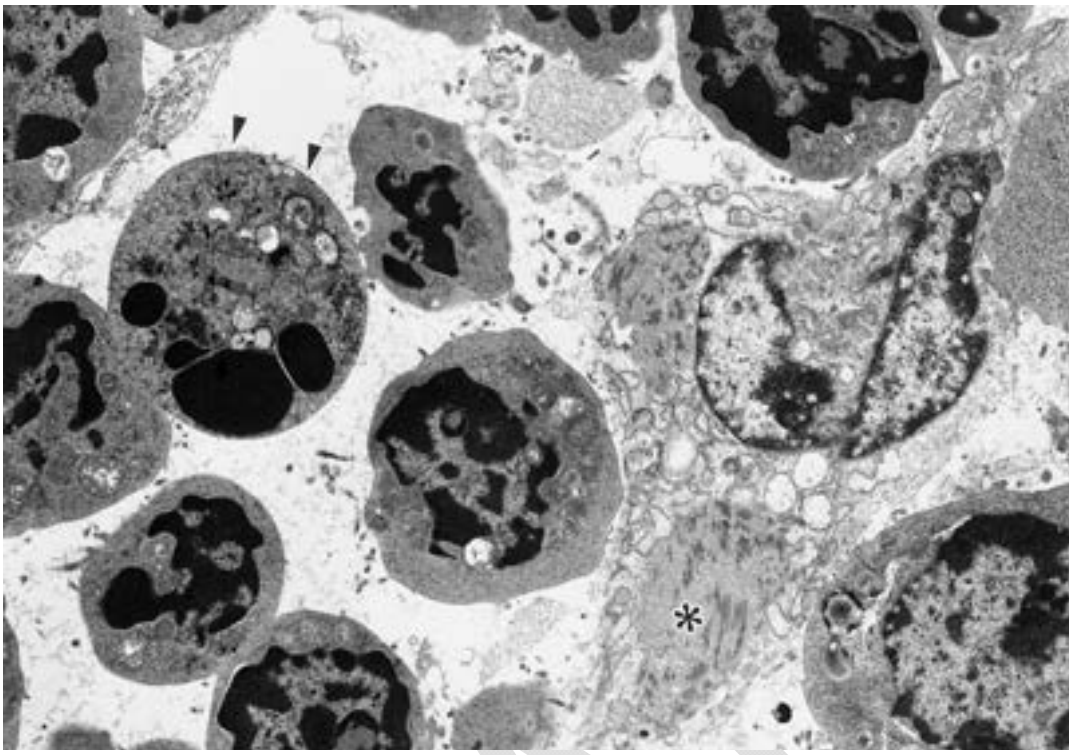

Figure 2: Lymph Node

Transmission electron micrograph from the axillary lymph node of an EBOV-infected rhesus monkey. EBOV inclusion material is marked with a * in the cytoplasm of a degenerative dendritic cell in the T cell area, and an apoptotic lymphocyte is marked with arrows (adapted from [89]).

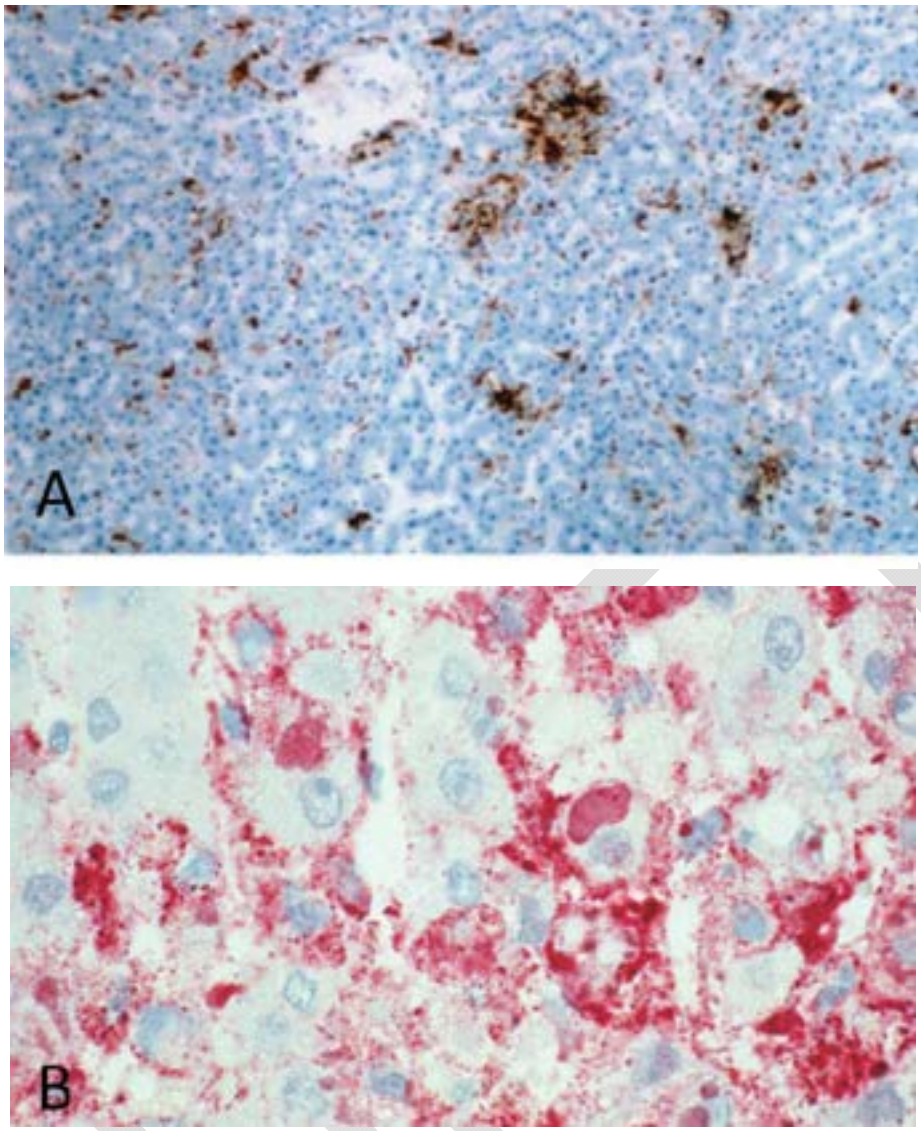

Figure 3: Liver

A) Scattered foci of immunopositive cells (brown) in the liver of an EBOV-infected rhesus monkey on day eight PE. Horseradish peroxidase antigen labeling with a hematoxylin counterstain at 20× magnification (adapted from [31]).

B) 250× magnification showing immunostaining of viral inclusions within hepatocytes as well as abundant antigens in sinusoids and sinusoidal lining cells in an EBOV-infected patient. Immunoalkaline phosphatase staining, naphthol fast-red substrate with light hematoxylin counterstain (adapted from [90]).

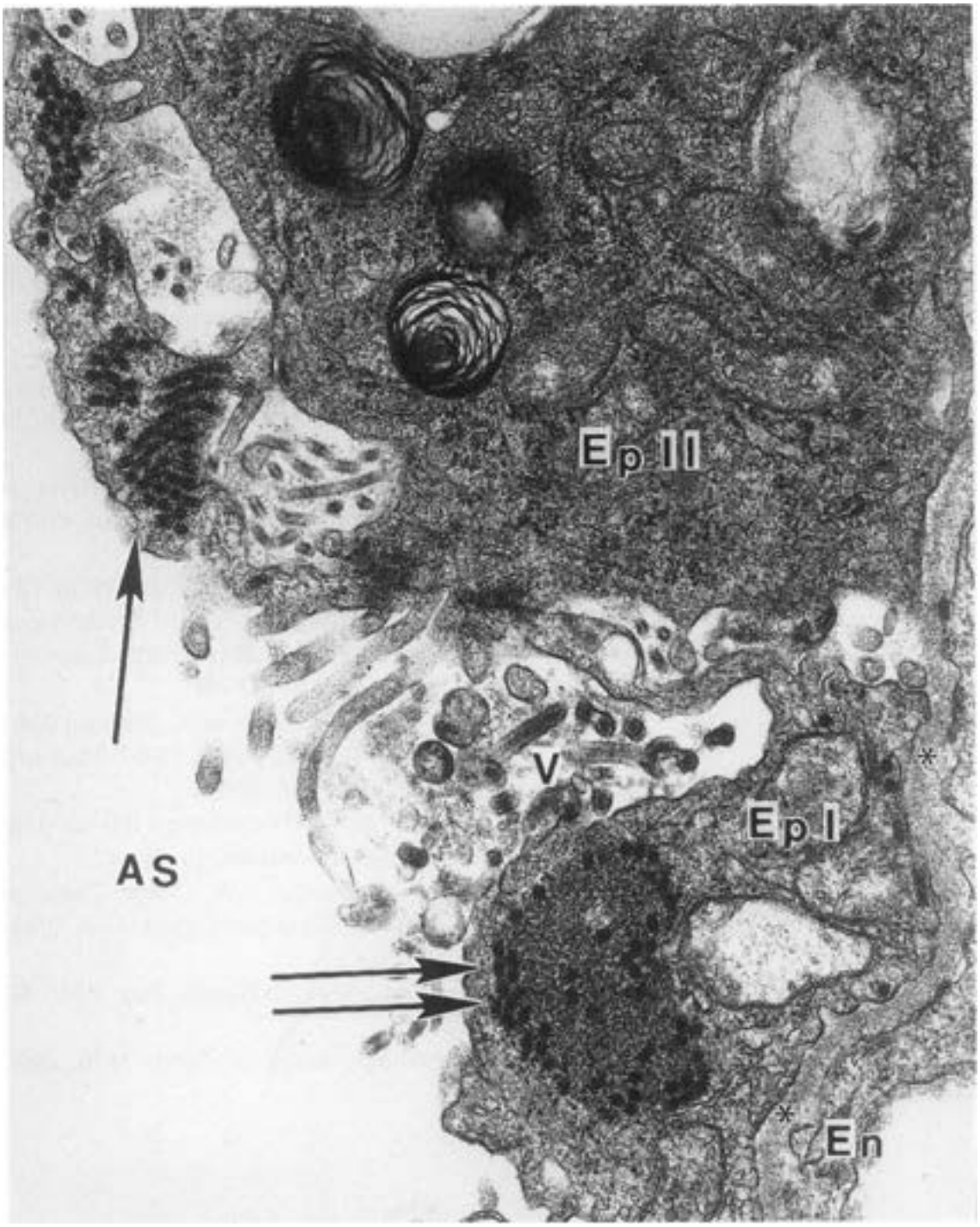

Figure 4 Lung

Extracellular mature virions (V) are evident in the alveolar sac (AS) of a rhesus monkey infected with EBOV. The double arrow shows a large cytoplasmic inclusion containing EBOV ribonucleocapsids within an epithelial cell (Ep). Nucleocapsid filaments, marked by the single arrow, are seen in the cytoplasm of an epithelial cell (on top of a type II pneumocyte). 30,000X magnification (adapted from [23]).