Ebola: A speculative concept that could provide a direct avenue for the detection of an incipient outbreak.

We invite you to read this document written by our CSO, Dr. François Iris. Our analysis, if correct, could provide a direct avenue for the detection of an incipient wave of Ebola outbreak. This document produced in 2008 was not updated.
Do not hesitate to contact us for more information, feedbacks or questions. email: manuel.gea@bmsystems.net

ABSTRACT

Ebola epidemics are currently the cause of considerable concern. However, recurrent episodes (the first in record dating of June 1976) are often separated by intervals of many years without outbreak and very little is currently known of the mechanisms generating these patterns. As a result, an incipient wave of Ebola outbreak remains impossible to detect, the populations primarily concerned becoming aware of the danger only after identification of the first clinical cases.

In an attempt to remedy this situation, we carried out, in 2008, a comprehensive analysis of the data then available. The results highlighted a complex climatology-driven interplay between ecosystems.

Outbreak episodes correspond to periods of maximum fruit production during the rainy season. This is associated with:

1) *dramatic seasonal changes in foraging behaviour in primates, bats, birds, etc*
2) *as well as large increases in insect biomass.*

Our analysis suggests that a ground-based omnivorous insect such as ants, induced to migrate by heavy rainfalls, could act as a reservoir for the maturation and transmission of the infective form of the virus. Ants will be frequently found on fruits and other sugar containing vegetation eaten by bats, apes and other wild life during these periods. Furthermore, in these environments, cadavers of dead animals (whether or not Ebola infected) are also partly disposed of by omnivorous insects such as ants.

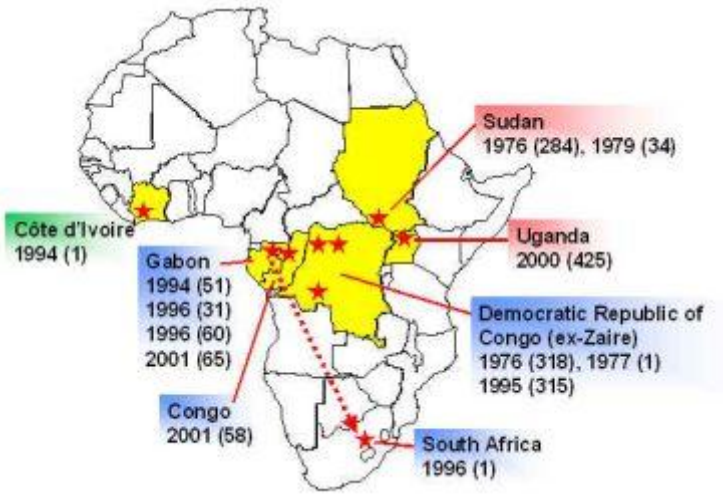
Thus, in years of heavy rainfalls, Ebola-carrying ants might be much more likely to transmit the virus to foraging populations than during years of moderate rainfalls in which ant migrations will be less extensive, thus accounting for the intervals of several years without outbreak. A large proportion of Ebola-infected animals will then succumb to the virus at locations that can be far remove from the infection site. Since the cadavers will then be partly disposed of by ants, this will close the cycle while spreading the infective potential to other ant colonies, thus accounting for a spatiotemporal pattern of outbreaks spreading at the rate of about 50 kilometers/year.

Together with the characteristics of Ebola genetics suggested by our analysis, the above, if correct, could provide a direct avenue for the detection of an incipient wave of Ebola outbreak, thereby allowing timely implementation of protective steps.

FULL TEXT

Epidemiology

Ebola, named after a river in central Africa where it was first seen, is a member of the filovirus family, with the similar Marburg virus being the other member of this class. There are currently five identified ZEBOV strains – Zaire, Sudan, Ivory Coast, Reston and a new Uganda strain – of which all but Reston are fatal to humans. As the nomenclature implies, Ebola is endemic to central Africa. The virus has caused outbreaks in Sudan, Ivory Coast, Gabon, Zaire (now the Democratic Republic of the Congo), Congo, Uganda, and in South Africa. The first recorded outbreak occurred in 1976, and many more have followed. There have also been outbreaks of the Reston strain among imported primates in both the U.S. and the Philippines.



Evolution & spread

Genetic data and information on the timing and location of past ZEBOV outbreaks were combined to determine the merits of two competing hypotheses to explain the emergence and spread of the virus. In the prevailing view, ZEBOV arose from long-persistent local strains after increased contact between humans or great apes and an unidentified reservoir host. But Walsh et al. found support for the alternative hypothesis: that ZEBOV had recently spread to the outbreak regions. This is good news because a virus that spreads at a predictable rate in a predictable direction is far easier to control than one that emerges by chance or at the hands of an unknown trigger.

The virus's spread was modeled based on assumptions of a long-persistent virus versus a recently emerged virus, and the predictions of these competing hypotheses

tested using genetic data, gathered from gene sequences taken from human samples at the different outbreak sites, and information on the spatiotemporal dynamics of the outbreaks. Charting the evolutionary relationships of the viral genotypes identified one major lineage with a most recent common ancestor consistent with the 1976 outbreak. Comparing the path of descent with outbreak localities mirrored the timing of the outbreaks, with new outbreaks directly descending from those preceding.

Analyzing the spatiotemporal pattern of outbreaks revealed a spread at the rate of about 50 kilometers/year -a predictable path unlikely for a persistent virus- with the first epidemic in Yambuku, then spreading south to Kikwit and west to Booué, Gabon. Plotting the geographic distribution of genotypes revealed a clear spatial structure at both local and regional scales: the genotypes from the 2001–2003 Gabon/Congo outbreaks, for example, decreased in genetic similarity as distance increased. Again, this finding is consistent with the recently emerged hypothesis, which predicts a correlation between genotype and geography at different distances. Simulations of viral evolution in a spreading epidemic also showed a consistent spread pattern and a strong correlation between genetic divergence and spatial separation. Though the authors can't say how the virus was transmitted, the simulations showed that a "simple nearest neighbor contact process" could produce the linear, uniform spread rates found here.

Though the strength of the individual lines of evidence (timing of origin, spatial spread, and genetic/distance ratio) is not conclusive when considered separately, taken together, they support the hypothesis that a "consistently moving wave of ZEBOV infection" recently spread to outbreak sites in Gabon and Congo. Following its current course, ZEBOV may hit populated areas east of Odzala National Park within 1–2 years and reach most parks containing large populations of western gorillas in 3–6 years. Two Ebola outbreaks have already hit human populations west of Odzala, and over the past two years, the largest gorilla and chimp populations in the world, found in Odzala, have been devastated, the disease is spreading to the last unaffected sector of the park right now. These findings suggest that strategies to protect villagers and some of the last remaining wild apes from future outbreaks would do best to concentrate efforts at the front of the advancing wave.

Reservoirs

The natural reservoir and route of transmission to humans is still unknown for Ebola. Despite extensive surveys of hundreds of species of animals, insects and plants by PCR analysis, there is no definitive answer. Some possible reservoirs include:

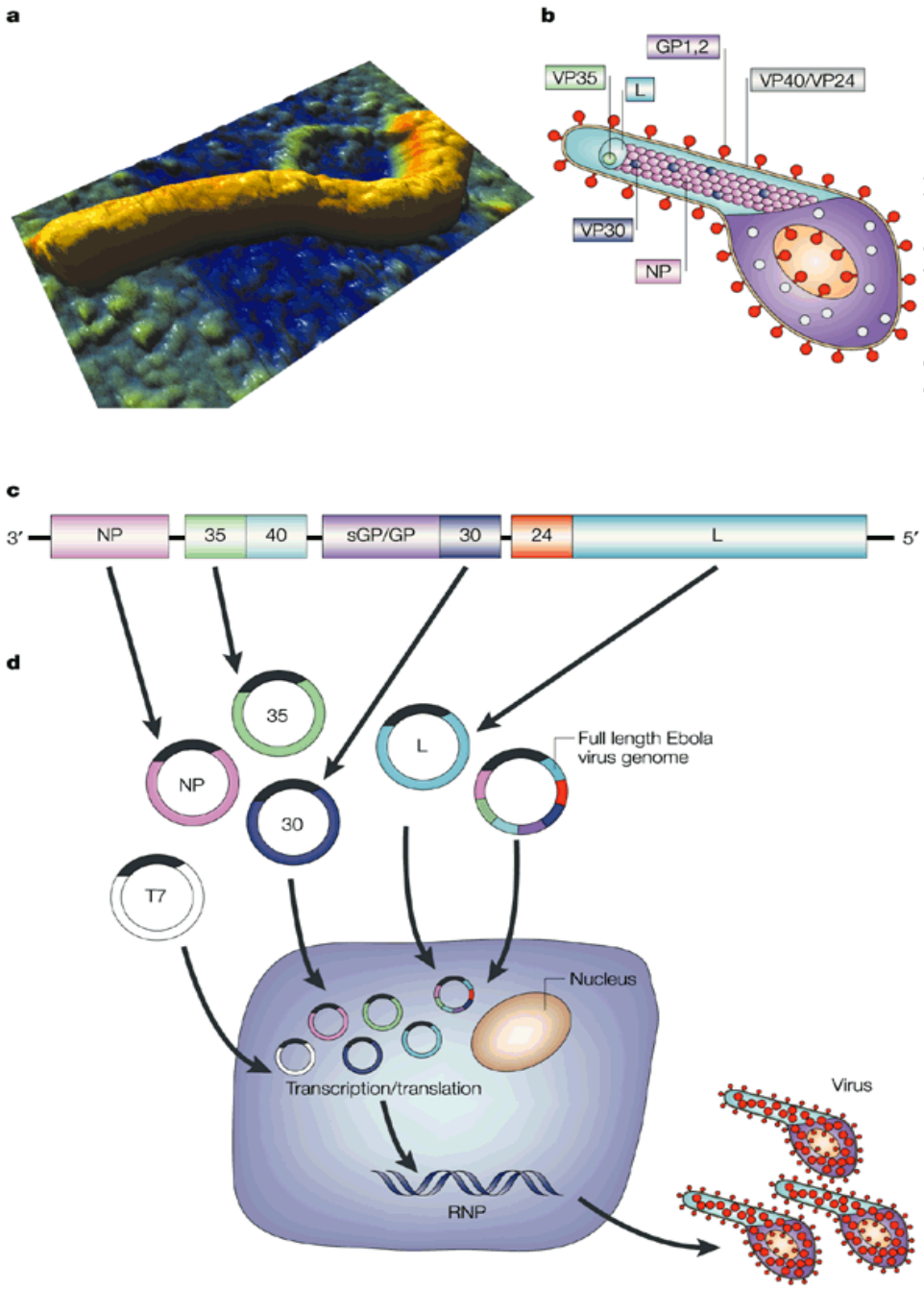
- Bats
- Primates
- Rodents
- Insects

However, when many of these species are infected in a laboratory, the virus proves fatal, thus negating them as potential reservoirs. Indeed, ecological studies of primates in central Africa have shown that Ebola has decimated their populations in recent years. An interesting theory, supported by recent experiments, holds that under certain conditions of immune deficiency, such as that given by HIV, SIV, or other similar diseases, an animal would be able to live with the infection for long periods of time, thus acting as a reservoir for the virus.

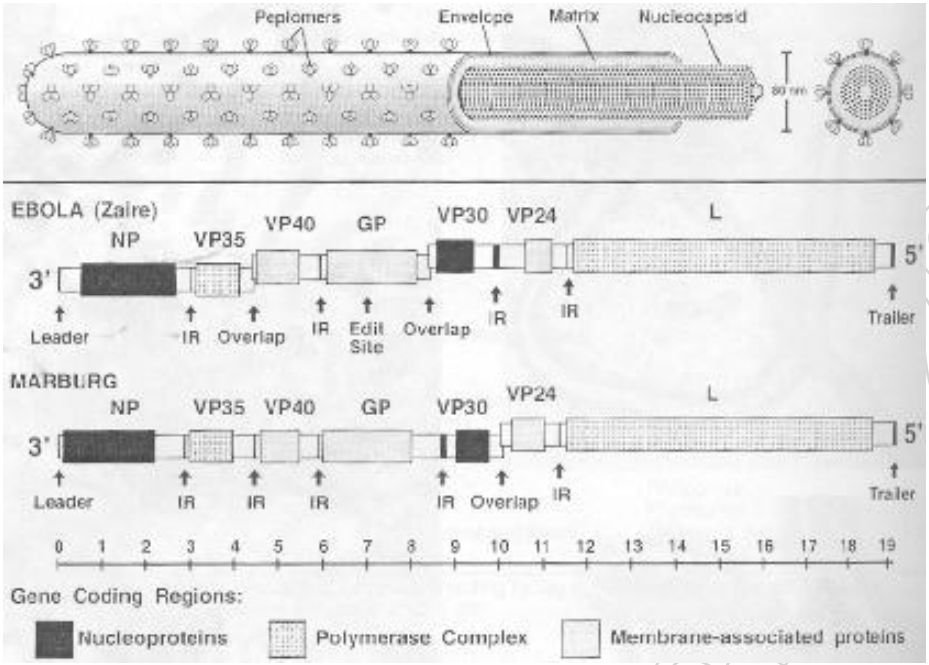
Many experts have put forth a hypothesis that the appearance and increase of outbreaks is directly linked to deforestation and rainfall. As more and more of the dense jungle is cleared for farmland, this raises the possibility that humans will come into contact with previously un-encountered species that have lived in ecological isolation for prolonged periods of time. These unknown species include both the reservoir and the Ebola virus itself.

Genetics

The virions of both Marburg and Ebola viruses are pleomorphic, taking forms ranging from 14,000nm long filamentous particles to small spheres. The virion envelope is derived from the plasma membrane of host cells, and tightly encloses the genome-containing nucleocapsid. The surface of the envelope is studded with 10nm long glycoprotein peplomers that aid in viral entry into host cells.



All Filoviruses have a single-stranded, negative-sense RNA genome, approximately 19,000 base pairs in length. This genome is non-segmented, thus eliminating the possibility of segment recombination between different viral strains or species. The seven genes that exist in both Marburg and Ebola viruses are arranged sequentially along the length of the genome, with non-coding sequences at both the 3' and 5' ends that are thought to play a structural role in initiation of transcription and replication. Sequences indicating transcriptional start and termination sites are conserved between different filovirus species, however there is 37-41% sequence variation in the coding regions between the Ebola and Marburg species.



Key differences between the genetics of the Ebola and Marburg viruses exist in the organization of their glycoprotein (GP) genes. The Marburg virus GP gene encodes one product in one open reading frame, while the Ebola virus GP gene encodes two polypeptides in two open reading frames. The primary product of the Ebola GP gene is a small, secreted glycoprotein that gets released in large quantities from infected cells. The Marburg virus does not produce a similar secreted glycoprotein. SGP is found in large quantities in the serum of infected patients. Although its precise function is unclear, it is suspected to interfere with neutrophil function by binding to their surface Fcβ3 receptors. Through similar interactions with other cell types, SPG may play a role in the inhibition of the inflammatory response.

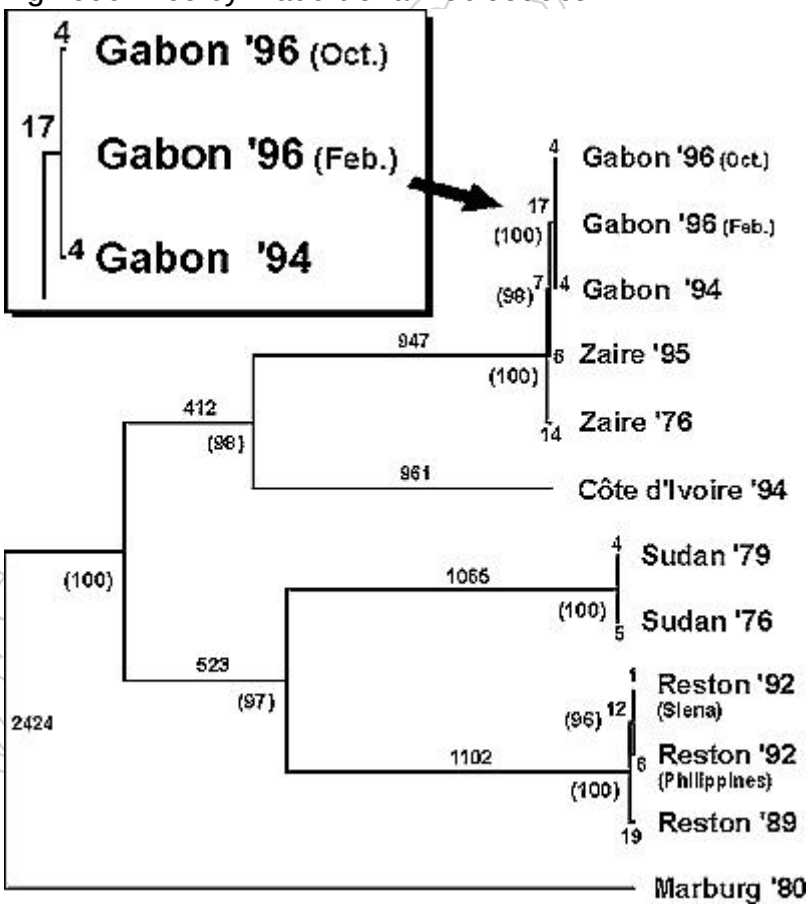
The non-secreted form of the glycoprotein (GP) exists in both Marburg and Ebola viruses and is the major envelope-associated protein of filoviruses. The structure and chemistry of this protein has been extensively studied as it is a prime target for vaccine development. GP has both conserved and variable regions. The variable regions are the most heavily glycosilated with an extended mucin-like structure, and are thus able to tolerate mutations easily. The high glycosylation region spans amino-acids 267 to 310, followed by the mucin-like region, from amino acids 313 to 404.

A precursor form of GP, known as GP0, is differentially cleaved post-translationally by furin, creating two forms of GP, GP1 and GP2. GP1 and GP2 dimerize, and then the heterodimers form trimers that make up the virion surface spikes, or peplomers, which are critical for viral entry into host cells. In addition to the glycosylation and mucin-like domains described above, the GP1 fragment contains an apparently fragmented head-domain extending from residues 70 to 95, 105 to157, 168 to 175 and 214 to 226. The GP2 form (amino acids 502 to 672) contains two heptad repeat regions (HR1 and HR2), connected by a 25-residue linker containing a CX6CC motif to the internal fusion loop. The crystal structures of post-fusion GP2 fragments have revealed that the two heptad repeat regions form antiparallel a-helices and that a

CX6CC motif forms an intrasubunit disulphide bond between Cys 601 and Cys 608. HR1 extends from residues 554 to 598 and HR2 from residues 599 to 632.
GP has been implicated to have direct immunosuppressive and cytopathic effects, such as the inhibition of T-cells proliferation in response to mitogen. Other envelope proteins include VP40, which is suspected to facilitate budding by promoting interactions between newly formed nucleocapsids and the plasma membrane, and VP24, which does not have a defined function.

In addition to the envelope and secreted glycoproteins, the other major category of proteins of the filoviruses is the ribonucleocapsid complex. The major structural protein of this complex is nucleoprotein (NP). NP ranges in size from about 96-104kD in different filovirus species and plays an important function in binding genomic RNA. The other proteins of the ribonucleocapsid are polymerase, VP30, and VP35, all of which are involved in transcription and replication.

Viruses from the same outbreak show identical GP gene sequences, but unique virus sequences were found in each of the three different outbreaks in Gabon. Although the viruses causing the Gabonese outbreaks clearly belong to the Zaire subtype, they were distinct from viruses that had caused disease in Zaire. The GP from the Gabon spring 1996 viruses differs from the sequence of the Gabon fall 1994 viruses by 4 nucleotides. The GP sequence from the Gabon fall 1996 viruses differs from that of the Gabon spring 1996 virus by 4 additional nucleotides

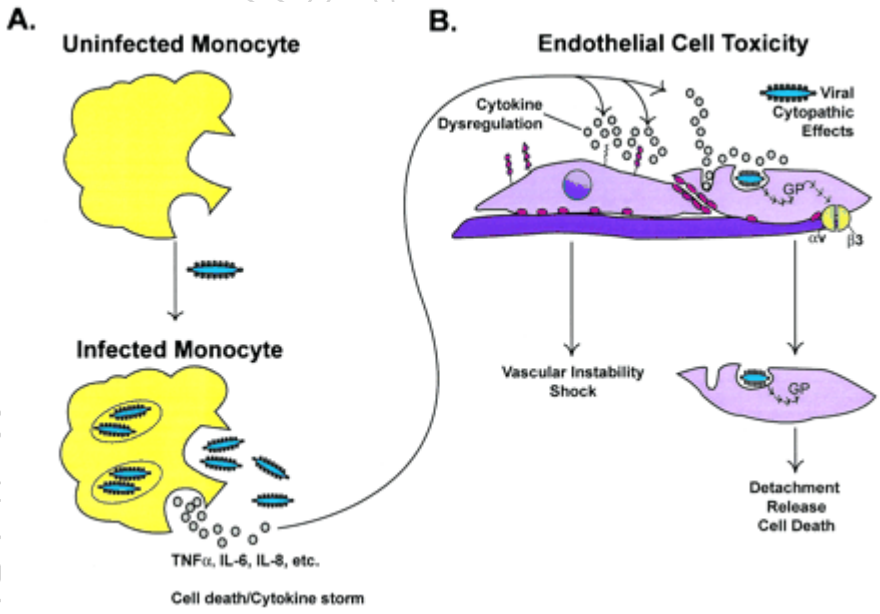


Phylogenetic tree showing the relationship between the Ebola viruses that caused outbreaks of disease in Gabon and previously described filoviruses. The

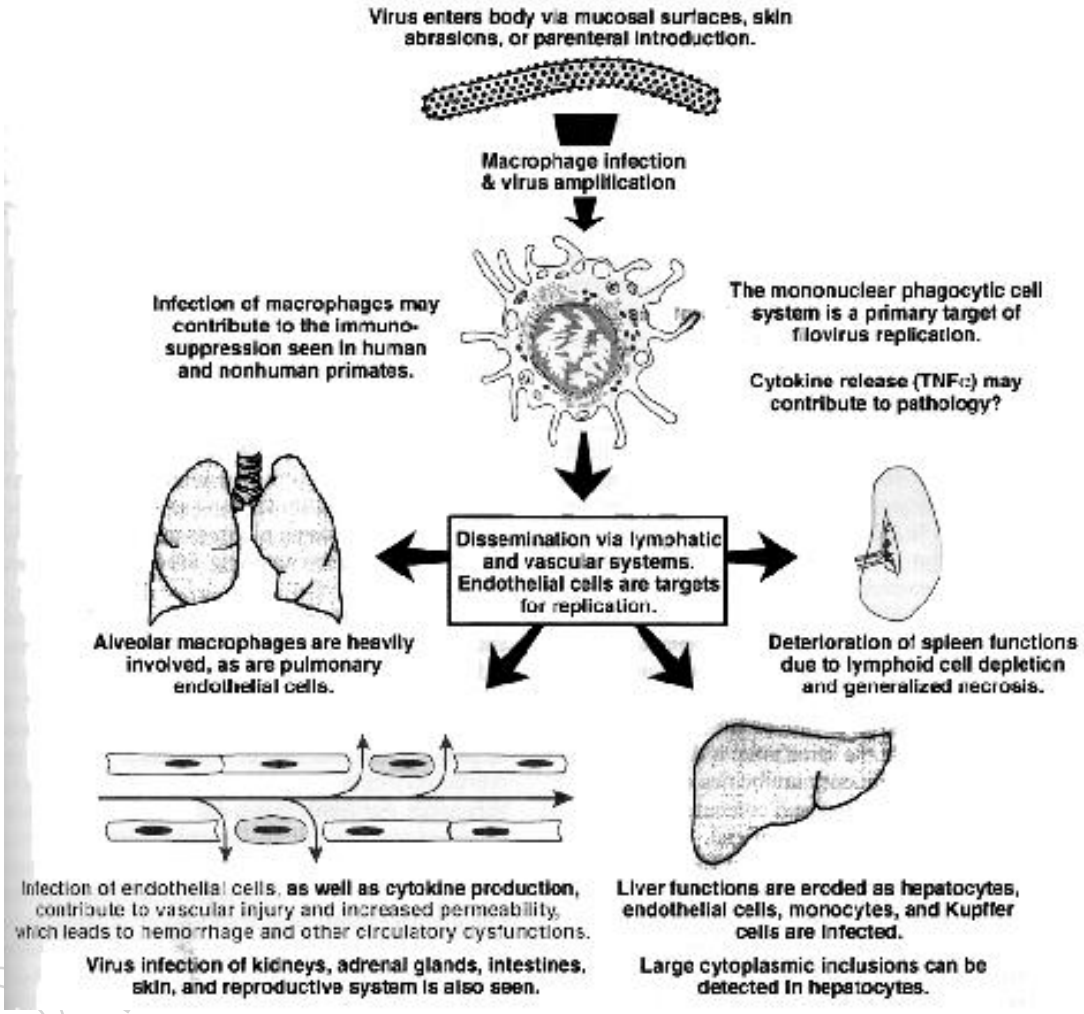
entire coding region for the glycoprotein gene of the viruses shown was used in maximum parsimony analysis, and a single most parsimonious tree was obtained. Numbers in parentheses indicate bootstrap confidence values for branch points and were generated from 500 replicates (heuristic search). Branch length values are also shown.

Pathology

Initial filovirus infection is followed by an incubation period lasting 4-10 days, before symptoms develop. Initial symptoms include fever, chills, myalgia, and malaise. As infection progresses, symptoms escalate to nausea, vomiting, abdominal pain, chest pain, coughing, shortness of breath, edema, diarrhoea, headache, and coma. The bleeding that characterizes hemorrhagic fevers is particularly severe in filovirus infection. Mucosal hemorrhage, oozing from venipuncture sites and a macropapular rash are common by day 5 of infection and often useful diagnostic indicators. Worsening symptoms or signs of recovery are usually observed around day 7, coincident with the antibody response, with the majority of patients succumbing to death induced by metabolic disturbances and shock.
The pathogenesis of infection is similar for all observed species of filoviruses. Dendritic cells, monocytes and macrophages are implicated as sites of early infection, impairing the immune response from the beginning. These infected cells not only become ineffective in combating the virus, but may also serve as vehicles to transport virus to other locations throughout the body, such as the lymph nodes, liver, lungs, and spleen. It has also been shown that infection of cells with Ebola virus inhibits the activation and expression of immunomodulatory and antiviral genes, such as MHC Class I, IRF1, and PKR, allowing infected cells to escape immune surveillance.



While infection of macrophages and dendritic cells disrupts their normal immunologic functions, it may also cause aberrant production of extremely high levels of cytokines such as TNF-α, IL-2, IL-10, and IFN-γ. These cytokines may contribute directly to the pathology of the disease. Monocytes have also been implicated as a possible source of the overproduction of tissue factor (TF), a cell-surface glycoprotein that plays a key role in the initiation of coagulation protease cascades. These cascades result in the accumulation and deposition of thrombin and fibrin causing microthrombi to form in capillaries and microvasculature, block blood supply to vital organs. This disseminated intravascular coagulation provides another mechanism for the widespread organ failure associated with filovirus infection.
Characteristic pathology is seen in all filovirus infections. Extensive necrosis of the liver, spleen, kidneys, and gonads commonly noted, with the liver showing both the most severe necrotic damage and the highest viral titers. Infection also results in damage to endothelial cells, and particularly capillary endothelium. This increases capillary permeability and is responsible for the uncontrolled bleeding associated with infection. The extent to which the spleen and lymph nodes are damaged may be a direct contributor to the outcome of the disease, with survivors escaping irreparable damage to these organs.



Immune responses

The typical immune response to Ebola infection is characterized by an impaired innate immune response, abnormal and deleterious cytokine secretion profiles of innate and adaptive immune cells, and significant apoptosis of lymphocytes. It is important to note that the nature of the immune response to Ebola is intimately associated with the disease pathology. By suppressing certain parts of the immune system and causing an inappropriate activation of others, Ebola produces rapid, severe and typically fatal damage to the host while avoiding its own destruction by the immune system.

Dendritic cells infected with Ebola exhibit general suppression of cytokine secretion; in particular, secretion of IFNα, an important immunomodulatory and antiviral cytokine, is eliminated. Suppression of IFNα secretion is believed to significantly contribute to the defects in activating adaptive immunity that are observed in later stages of Ebola infection/disease. Dendritic cells (DCs) infected with Ebola also exhibit defects in their ability to stimulate T cells, and impaired response to other interferon-inducing stimuli. The molecule responsible for the inactivation of IFNα production is the viral protein weighing 35kDa, known as VP35. VP35 is believed to interfere with gene transcription of important immune factors. Because inactivated Ebola can also lead to impairment of IFNα production, it is believed that Ebola structurally expresses VP35 before it enters a cell and replicates.
Monocytes and macrophages infected with Ebola also exhibit defects in IFNα secretion, and changes in the secretion of other cytokines. In particular, infected monocytes/macrophages were observed to secrete increased levels of IL-1β, IL-6, Il-8, and TNF-α, as well as RANTES, MIP-1α, and monocyte chemotactic protein-1. Since monocytes and macrophages are motile cells, they can also serve to transport Ebola virus to other parts of the body, such as the lymph nodes, lungs, spleen, and liver.
Infected dendritic cells, monocytes, and macrophages (antigen-presenting cells = APCs) have also been observed to up-regulate expression and secretion of tissue factor (TF), which is thought to be responsible for the development of disseminated intravascular coagulation (DIC) through over-activation of the clotting system and formation of microthrombi on the microvascular endothelia. DIC is thought to be an important factor in the necroses and organ failures observed in the later stages of Ebola disease.
It is important to consider both the typical dysfunctions of the innate responses to Ebola, as well as the types of innate responses that would be most advantageous to control of Ebola infection when examining the adaptive responses to the virus, because of the profound influence (which can be beneficial or detrimental) that innate immunity has on adaptive immunity, and the implications thereof for treatment, control, and ultimately vaccination against the virus.

Adaptive immunity

It is widely held that robust innate and adaptive responses are both necessary for

control and clearance of Ebola infection. The profound impact Ebola infection has on innate immunity has been described above, and those factors in conjunction with specific impairment of the branches of adaptive immunity are important determinants in the pathology of Ebola infection, particularly in the intermediate and end stages of the disease. Early presence of specific anti-Ebola antibodies, and proper functioning of helper and cytotoxic T cells in coordinating cytokine release and antibody switching and clearing Ebola-infected cells are the factors most strongly correlated with increased likelihood of survival from Ebola infection.

Ebola has not been observed to infect T or B lymphocytes. However, Ebola infection leads to significant apoptosis of T lymphocytes, evidence for which is provided by the increased levels of Fas, FasL, perforin, IFNγ, and sometimes CD28 in infected individuals, and the disappearance of CD3, CD8, and T-cell receptor mRNA in later stages of infection. It is important to note that in fatal cases, the levels of these factors continued to rise until death, suggesting relentless cytotoxic killing of T cells. This killing is possibly mediated by infected monocytes and macrophages, both of which are capable of producing both membrane and soluble Fas ligand. One study found that IL-2 and IL-4 mRNA were barely or never detected in the peripheral blood mononuclear cells of either human survivors or fatalities, presumably due to the impact of Ebola infection on T-cell activation, proliferation, and survival.

Because of the significant effect Ebola has on T cells, it is expected that B cell functioning (specifically, production of antibody) would be impaired. This has been observed to be the case, as the typically observed humoral response to Ebola is not very robust, and is characterized by low anti-Ebola IgG and no anti-Ebola IgM in most fatal cases. This is potentially due to impaired B-cell activation as a result of reduced T cell and dendritic cell levels. Survivors mostly exhibit rapid and robust responses to Ebola characterized by the production of IgG and IgM targeted at the viral nucleoprotein, the 40kDa viral protein, and the 35kDa viral protein.

There is some evidence that Ebola may benefit from antibody-dependent enhancement of infection through binding of complement component C1q to Ebola-specific IgG and IgM/virus complexes. This C1q/Ab/virus complex is then bound by C1q receptor on susceptible cells. The potential of facilitating ADE may be influenced by antibody titer, in that high levels of antibody may be sufficient to neutralize the virus, whereas a sub-neutralizing antibody titer may contribute to the possibility of C1q being able to bind an antibody-virus complex, facilitating its uptake into vulnerable cells. It is uncertain how significant a role ADE may play in vivo, but it is an important consideration, particularly with regards to vaccine development.

Successful immune response

The impact that Ebola has on the adaptive immune system, and the adaptive immune responses that increase the likelihood of survival, are still being elaborated. What is known is that most survivors have a consistent profile of early and robust Ebola-

specific IgG and IgM responses, as well as expression of inflammatory cytokines and evidence of a cytotoxic response to infected cells, both of which are moderated by the control and elimination of viral antigen. These adaptive responses, in turn, may be heavily dependent on the rapid and intact functioning of the innate immune system, and it is likely that the production of type I interferon is necessary in order to appropriately activate elements of innate and adaptive immunity.
It is essential to note that the elements of a successful immune response to Ebola are similar to the elements of an anti-Ebola immune response that initiate and aggravate pathology. Pro-inflammatory cytokines must be released at the appropriate stage of infection and not too early; and the antibody response must be fast and robust, or else it might end up facilitating virus entry into cells. The elements of both successful and failed immune responses to Ebola need to be further elaborated in order to fully understand the range of effects the virus has on the immune system, and how those effects influence the immune response as well as pathology. At this point, only the most general information is available on what constitutes a successful immune response; since the likelihood of survival when infected with Ebola is still below 50% even by the most conservative standards, and the pattern of Ebola incidence has not yet been fully elaborated, it is difficult to characterize what in fact determines survival or death.

Outbreaks

1976 (Ebola-Sudan) Sudan, 27th June. 284 cases, 53% deaths.
Occurred in Nzara, Maridi and the surrounding area. The outbreak in Nzara appears to have originated in the workers of a cotton factory. Disease was spread mainly through close personal contact within hospitals. Many medical care personnel were infected. This outbreak was the first recognition of the disease.

1976 (Ebola-Zaire) Zaire [Democratic Republic of the Congo (DRC)] 1st Sept. 318 cases, 88% deaths.
Occurred in Yambuku and surrounding area. Originated from a teacher returning from northern Zaire (boundary with southern Soudan). Disease was spread by close personal contact and by use of contaminated needles and syringes in hospitals/clinics.

1976 (Ebola-Sudan) England. 1 case, survived.
Laboratory infection by accidental wound with contaminated needle.

1977 (Ebola-Zaire) Zaire, June. 1 case, died.
Noted retrospectively in the village of Tandala.

1979 (Ebola-Sudan) Sudan, 2d Aug. 34 cases, 65% deaths.
Occurred in Nzara. Recurrent outbreak at the same site as the 1976 Sudan epidemic.

1989 (Ebola-Reston) USA. No cases.

Ebola-Reston virus was introduced into quarantine facilities in Virginia, Texas, and Pennsylvania by monkeys imported from the Philippines. Four humans developed antibodies to Ebola-Reston virus but did not become ill.

1990 (Ebola-Reston) USA. No cases.
Ebola-Reston virus was introduced once again into quarantine facilities in Virginia, and Texas by monkeys imported from the Philippines. Four humans developed antibodies but did not become ill.

1992 (Ebola-Reston) Italy. No cases.
Ebola-Reston virus was introduced into quarantine facilities in Sienna by monkeys imported from the same export facility in the Philippines that was involved in the episodes in the United States. No humans were infected.

1994-1995 (Ebola-Zaire), Gabon, Dec. 49 cases, 59% deaths.
Outbreak occurred in the Andock, Mékouka, and Minkébé area, far northeastern Gabon, in gold-panner encampments near the Nouna River and spread to the Minkouka area, far from the encampments.

1994 (Ebola-Ivory Coast). Ivory Coast. 1 case, survived.
Scientist became ill after conducting an autopsy on a wild chimpanzee in the Tai Forest. The patient was treated in Switzerland.

1995 (Ebola-Zaire), Republic of the Congo (formerly Zaire) Jan-June. 315 cases, 77% deaths. Occurred in Kikwit and surrounding area. Traced to index case-patient who worked in forest adjoining the city. Epidemic spread through families and hospitals. Four separate hospitals were implicated in the outbreak. The first hospital and the center of the outbreak was Kikwit General Hospital (the hospital that treated a laboratory technician). The second was Kikwit II Hospital. The third was the hospital in Mosango, where one of the medical personnel who cared for the laboratory technician was transferred. The fourth hospital was in Yassa Bonga, approximately 250 km north-west of Kikwit.

1995 (Ebola-Zaire), Liberia, 15th Nov. 1 case, survived.
Contracted Ebola in Liberia. Came from the town of Plibo, Liberia, but primarily lived in the bush. Surveillance around this town did not reveal any additional cases.

1996 (Ebola-Zaire), Gabon, 24th Jan. 31 cases, 68% deaths.
Occurred in Mayibout area on the Ivindo River. A chimpanzee found dead in the forest was eaten by people hunting for food. Nineteen people who were involved in the butchery of the animal became ill; other cases occurred in family members.

1996 (Ebola-Zaire), Gabon. 23th jul- 13th Nov. 60 cases, 75% deaths.
Occurred in Booué area with transport of patients to Libreville. Index case-patient was a hunter who lived in a forest camp. Disease was spread by close contact with

infected persons. A dead chimpanzee found in the forest at the time was determined to be infected.

1996 (Ebola-Zaire), South Africa. 27th Oct. 2 cases, 50% deaths.
A medical professional travelled from Gabon to Johannesburg, South Africa, after having treated Ebola virus-infected patients and thus having been exposed to the virus. He was hospitalized, and a nurse who took care of him became infected and died.

1996 (Ebola-Reston), Philippines. No cases.
Ebola-Reston virus was identified in a monkey export facility in the Philippines. No human infections were identified.

2000-2001 (Ebola-Sudan), Uganda, 12th Oct. 425 cases, 53% deaths.
Occurred in Gulu, Masindi, and Mbarara districts of Uganda. The three most important risks associated with Ebola virus infection were attending funerals of Ebola hemorrhagic fever case-patients, having contact with case-patients in one's family, and providing medical care to Ebola case-patients without using adequate personal protective measures.

2001-2002 (Ebola-Zaire), Gabon and The Republic of the Congo, 11th Dec. 122 cases, 79% deaths.
Outbreak occurred over the border of Gabon and the Republic of the Congo. Suspected to have begun with hunters handling a dead boar that was infected with the virus.

2002-2003 (Ebola-Zaire), Gabon and The Republic of the Congo, 31st Dec. 143 cases, 89% deaths.
Outbreak occurred in the districts of Mbomo and Kellé in the Republic of the Congo's Cuvette Ouest Region and in the neighbouring villages of Gabon. Suspected to have originated in the consumption of infected gorilla meat.

2003-2004 (Ebola-Zaire), Gabon, 17th Nov. 35 cases, 83% deaths.
Outbreak occurred again in the districts of Mbomo and Mbandza.

2004 (Ebola-Sudan), Sudan, 24th May. 17 cases, 41% deaths.
Outbreak occurred in the district of Yambio, Western Equatoria, south Sudan.

2005 (Ebola-Zaire), The Republic of the Congo, 18th May. 12 cases, 75% deaths
Outbreak occurred once more in the districts of Mbomo and Kellé in the Republic of the Congo's Cuvette Ouest Region.

2007 (Ebola-Zaire), The Republic of the Congo, 11th Sept. 264 cases, 71% deaths.
Outbreak occurred in the province of Kasai Occidental, associated with typhoid and *Shigella dysenteriae* type 1.

2007-2008 (Ebola New Species), Uganda, 28th Nov. 149 cases, 25% deaths.
Outbreak occurred in Bundibugyo District, western Uganda. First reported occurrence of a new strain.

Environmental peculiarities

Rain season:

Soudan (repeatedly affected by ebola outbreaks): April to October. But, nine months rain season in the extreme south for only one week at most in the far north (July-August in Kartoum). Nzara & Maridi are in the extreme south.

Cameroon (never yet affected by ebola outbreaks): Rains start in March in the south, reach the center by mid-April and the far north in May. The general pattern is similar to that observed in Zaire (double rain season).

Central African Republic (never yet affected by ebola outbreaks): Rains from Early April to mid-June. The general pattern is similar to that observed in Zaire (double rain season).

Ex-Zaire (repeatedly affected by ebola outbreaks): Rain falls throughout the year but very heavy in the west from March to end of May and again from August to November and in the south from November to early April.

Congo (repeatedly affected by ebola outbreaks): Starts in mid-January and ends in mid-May, except in the south where it ends in late April. The general pattern is similar to that observed in Zaire (double rain season).

Equatorial Guinea (never yet affected by ebola outbreaks): Abundant from mid-April to end of May. The general pattern is similar to that observed in Zaire (double rain season).

Gabon (repeatedly affected by ebola outbreaks): Abundant from mid-March to mid-April and continuing through May. The general pattern is similar to that observed in Zaire (double rain season).

Uganda (repeatedly affected by ebola outbreaks): Less obvious wet and dry season. Bwindi National Park (gorilla & chimpanzee populations) is a rain forest at considerable elevation and can be wet and cool at any time of year. Heavy rains tend to occur in November, April and May. Intermittent rains start in March, heavy rain clustering in April & May and short bur frequent rains occurring in November.

Fire season: From the first week of May to end of June in southern Democratic Republic of Congo and northern Angola. Each year, during the dry season, the region experiences widespread annual agricultural burning (pasture renewal and land clearing).

Theoretical patterns

A. Reservoir and spread.

The outbreaks occur systematically at the end of wet seasons in area presenting gallery tropical forest or continuous tropical forest and strike wild animals (small antelopes [duiker] wild pigs and primarily apes [gorillas & chimpanzees]) before the apparition of human outbreaks (15752448). This suggests that the Ebola virus emerges from its cryptic reservoir in a specific geo-temporal and enviro-climatic context. The reservoir is still really unknown although fruit bats have been found to carry asymptomatic Ebola virus infection during outbreaks in humans and great apes between 2001 and 2003 in Gabon and the Republic of the Congo (16319873). But here, only small vertebrates were tested. The outbreak periods correspond to period of maximum fruit production during the rainy season. This is associated with 1) dramatic seasonal changes in fruiting and foraging behaviour in primates, bats, squirrels, and birds, as well as 2) large increases in insect biomass (3.9 times greater during the rainy season from mid-October to mid-December in Gabon). Thus, a ground-based omnivorous insect such as ants, induced to migrate following heavy rainfalls (flooded burrows), presents the likeliest probability to act as a seasonal reservoir for the maturation and transmission of the infective form of the virus, all the more so since ants will be frequently found on fruits and other sugar containing vegetation eaten by bats, apes and other wild life during these periods. Furthermore, in these environments, cadavers of dead animals (whether or not ebola infected) are also at least in part disposed of by omnivorous insects such as ants.
Thus, in years of heavy rainfalls, ebola-carrying ants would be more likely to transmit the virus to foraging animals (primates, bats, etc.) than during years of moderate rainfalls in which ants migration could be less extensive, thus accounting for the geo-temporal and enviro-climatic observations and the intervals of several years without outbreak. A large proportion of ebola-infected animals will succumb to the virus at locations that can be far remove from the infection site. Since the cadavers will then be partly disposed of by ants, this will close the cycle while spreading the infective potential to other ant colonies, thus accounting for a spatiotemporal pattern of outbreaks spreading at the rate of about 50 kilometers/year.

B. Viral mechanism of successful infection.

The virus appears to initially infect antigen presenting cells (APC). This suggests a variant of the strategy employed by AIDS.
In order to dampen direct immune responses from memory cells, AIDS primarily infects T4 lymphocytes, thereby curtailing the main route of adaptive immune

responses. Thus, AIDS does not have to escape non-self detection. It must simply be very efficient at binding to either the CR2 receptor on B lymphocyes (which then interacts with T4 cells, leading to effective infection of the latter) or the CCR5 and CXCR4 co-receptors on T4 cells.
In the case of Ebola, the situation appears similar in that it must efficiently curtail the development of adaptive immune responses. To do so, it targets APCs, which, subsequently to infection cannot efficiently present antigens to effector cells anymore. However, the progeny of this initial infection must escape detection by existing activated effector cells.

This suggests a mechanism.
The viral trimeric, GP1-GP2 protein complex that studs its external phospholipids envelope must be detectable as potentially non-self by APCs but not as definitely non-self by activated B cells and T lymphocytes. Here, three GP1 viral attachment subunits assemble to form a bowl-shaped structure, cradled by the corresponding three GP2 subunits, while a glycan cap projects from each GP1 subunit a mucin-like domain that hides access to the receptor-binding site in the bowl.
This mucin-like dome would be the structure that enables escape from immuno-detection by antigen-presenting memory B cells and activated T cells. As a result, the aspragines/threonines (n-linked glycosylation sites) and possibly serines (O-linked glycosylation sites, if any) involved in the constitution of this cap should be highly conserved, together with non-aromatic amino acids in the bowl-like receptor-binding domain.
However, the GP1 subdomains forming the bowl-like receptor-binding structure should be fully exposed for effective infection.
A likely strategy could rely upon cleavage of the domain containing the mucin-like structure, thereby exposing the binding site. This could be achieved using short-lived proteases released by APCs following detection of non-self structures. To this effect, the virus could rely upon partly glycosylated GP1 trimeric structures. Indeed, the size and structure of the viral particle suggest that heterogeneity in the glycosylation patterns of its exposed GP1 trimers may be expected. Such a scheme would naturally open the door to the induction of a full-scale immune response. This could explain why outbreaks only occur following contact with heavily infected individuals or animals. Here, a mass-effect would be considerably more effective than sporadic low-level exposures.
This leads to the problem of why some individuals survive an epidemic while most infected individuals die of it.

C. Surviving an epidemic.

The mechanism whereby the C1 complement system may lead to antibody-dependent enhancement of infectivity has been exposed above.
It is likely that two different mechanisms could be concurrently at play in survival.
One would rely upon previous low-level exposure and the ensuing constitution of small antigen-presenting memory B cells populations but much larger memory

responsive T cells population. This would lead to cell-mediated immune responses rather than humoral responses, hence bypassing the negative effects associated with antibodies & complement responses, while the other would rely upon particular HLA haplotypes that would heighten the responsiveness of memory T cells.
Here, heterogeneity in the glycosylation patterns of viral GP1 trimers could be one factor but the GP2 protein thereby exposed is also likely to play a significant role in the generation of haptens targeting NK to virus infected cells. In fact, the data available suggests that variations in the GP2 protein are likely to be one of the main factors discriminating various ebola strains.
Should this be the case, then it could be expected that

1. The clustering pattern of N-linked glycosylation sites within the GP1 segment (codons encoding asparagines/threonines within the variable region) should be conserved between the various ebola strains (residues 227 to 270 and 368 to 310).
2. The clustering of non-aromatic amino acids in the head domain of GP1 (particularly between residues 105 and 157) should also be highly conserved between the various ebola strains, and more particularly the Cys spacing pattern (108; 121; 135; 147).
3. The GP2 coding sequence should show high variability between strains at the interface between the cleavage site and the internal fusion loop (approx residues 502 to 520) and again at the interface between internal fusion loop and HR1 heptad repeat regions (approx residues 540 to 570). But the disulfide bond sites at Cys 511 and 556 should be highly conserved

Conclusions.

Given the theoretical nature of the elements described above together with their current lack of biological corroboration, the concepts exposed here can only be regarded as highly speculative. However, these theoretical elements not only fit the field observations, they could also explain several unresolved mysteries attached to Ebola epidemics.
Thus, if correct, the above might provide a direct avenue for the detection of an incipient wave of Ebola outbreak (from ground-based omnivorous insects, such as ants, migrating following heavy rainfalls), thereby allowing the timely implementation of protective/preventive steps.

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2008.