A Critical Analysis of the Ebola and Marburg Viruses

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by

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ABSTRACT

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The Ebola and Marburg viruses have been identified as two of the most virulent pathogens to emerge in the past few decades. Every year, more information is collected about these viruses. However, because outbreaks have been so isolated and sporadic, and the viruses are so dangerous and difficult to work with in a laboratory setting, significant gaps and contradictions still exist in the information that has been obtained. The purpose of this thesis is to take a closer look at these viruses in order to fill in some of the gaps and correct some of the inaccurate information.

Several different sources, including journals, texts, and other scholarly works, have been incorporated in order to produce a comprehensive analysis that covers a wide range of issues. The thesis begins by presenting the outbreak history of these two pathogens. The virus structures and classification status are then covered. Later in the work, other topics are discussed including diagnosis methods, transmission, viral pathology, and therapies. The thesis then discusses the progress that has been made in finding the natural reservoirs of the viruses. Finally, a brief analysis is included to conclude the work which discusses future developments and areas of research.
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**Introduction**

“He becomes dizzy and utterly weak, and his spine goes limp and nerveless and he loses all sense of balance. The room is turning around and around. He is going into shock. He leans over, head on his knees, and brings up an incredible quantity of blood from his stomach and spills it onto the floor with a gasping moan. He loses consciousness and pitches forward on the floor. The only sound is a choking in his throat as he continues to vomit while unconscious. Then comes a sound like a bedsheets being torn in half, which is the sound of his bowels opening and venting blood from the anus. The blood is mixed with intestinal lining. He has sloughed his gut. The linings of his intestines have come off and are being expelled along with huge amounts of blood. He has crashed and is bleeding out” (14).

This passage says it all. The Ebola virus, and its cousin the Marburg virus, are two of the most terrifying pathogens the world has ever seen. They come from nowhere, strike quickly, and leave nothing but blood and corpses in their wake. Few recover from infection, and those who do are forever changed from their near-brush with death. Whole families get infected in a week span, and within a month, they’ve all perished. Villages, towns, and camps get completely ravaged. Even hospitals and medical officials aren’t safe. These viruses are merciless.

This paper will explore these dangerous and fascinating pathogens. Only with increased understanding of these viruses will we get any closer to unraveling all the mysteries hidden within the jungles of Africa.
History

The first documented appearance of a member of the filovirus family was in 1967 in Marburg, Germany. Commercial laboratory workers at a biological testing plant suddenly began developing serious health complications from a mysterious illness. It did not take long to determine that the source of the disease was a shipment of green monkeys that had been recently imported from Africa since several of the primates were also sick and displaying similar symptoms as their human counterparts. Following this discovery, health care workers tracked two other shipments of the green monkeys to Frankfurt, Germany and Belgrade, Yugoslavia, where they discovered similar mini-outbreaks taking place (12). Consequently, all of the monkeys from these three shipments were euthanized for containment purposes and the spread of disease was quickly stopped. Overall, 32 human cases were documented (31 were identified at the time and an additional case was added later based on seropositivity). Seven of these infected people died, which is a mortality rate of 23% (7).

The response to this outbreak was very rapid and proficient, particularly considering that this initial appearance occurred nearly forty years ago. Only one generation of secondary transmission, six cases in all, was documented (12). This means that all the cases either involved people that had direct contact with the monkeys or people that had contact with the aforementioned primary sources. The disease was contained before it spread beyond this second level of infected people.

Another important aspect of this initial outbreak was that scientists were able to isolate and identify the Marburg virus very quickly considering the limited technology
available at the time. When this virus was first visualized by electron microscopy, the morphology varied so substantially from all other known viruses that it was placed in a new viral family designated Filoviridae (12). Because of the location of this viral outbreak, the pathogen was the labeled the Marburg virus. Further discussion on the morphology of the Filovirus species will be incorporated in a subsequent section of this paper.

As a direct result of this outbreak, several countries, including the United States, introduced more stringent quarantine and handling procedures on imported primates as well as other animals. However, these procedures have not been uniformly followed in various countries and sites, as the Ebola-Reston outbreak (which will be discussed shortly) demonstrated (7). In addition, a test was introduced a few years later to exclude the Marburg virus from vaccine substrates in order to ensure that the disease was not accidentally spread (12).

Surprisingly, there have only been a few documented reoccurrences of the Marburg virus since its initial appearance in 1967. In 1975, an Australian traveler that was most likely exposed in Rhodesia (now known as Zimbabwe) became ill after arriving in Johannesburg, South Africa. He transmitted the virus, which was identified as Marburg, to his female traveling companion and a nurse, but no further transmission was documented. The male eventually died, while both females recovered (7).

Three subsequent cases were also found in humans in rural African terrains in the 1980s, and like the aforementioned case, only limited transmission was observed (1). One case of Marburg occurred in 1980. An index patient became ill in western Kenya and
infected his physician who was caring for him. Another lone case was identified in South Africa in 1982. A third isolated case occurred in 1987, also in western Kenya, in a 15-year-old male (7). Thus, following the initial outbreak in 1967, seven cases and three (43%) deaths were observed up until the most recent epidemic in 1998 (18).

In late 1998, the largest documented outbreak of Marburg to date occurred in Durba in the Democratic Republic of the Congo. According to reports, several of the infected individuals were illegal gold miners that had been working in abandoned mines (7). Unfortunately, officials from the CDC and WHO were not able to travel to the site of the outbreak for several months because of the remoteness of the area as well as some local fighting that was going on. When health care teams finally arrived in September of 2000, they still found some new cases, which they helped treat and document. The final tally was 99 cases, with a mortality rate of over 80%, which was much higher than the 23-25% rate seen in the previous outbreaks (7).

Following the initial outbreak of the Marburg virus, which caused quite a stir across Europe and beyond, the pathogen has received little attention from the scientific community since because of its sporadic and limited reappearances. Its counterpart in the Filovirus family, however, has maintained a fairly steady presence in the media since the mid-1970s and that is what we will turn to next.

The Ebola virus exploded onto the scene in 1976 via two devastating outbreaks that occurred almost simultaneously. The first epidemic was identified in the western equatorial province of Sudan. It lasted from June through November of 1976, raging through the villages of Nzara, Maridi, and surrounding areas of southern Sudan (1).
When the outbreak finally subsided, 284 cases had been documented, including 117 deaths (1). This represents a mortality rate of 53% for this strain of Ebola, which would subsequently be labeled Ebola-Sudan, after the African region where it was found. According to personal accounts following the outbreak, the disease spread mainly by close personal contact within the hospitals and clinics in the small Sudan villages. As a result, many of the health care workers at these facilities were exposed to and infected with the virus (1).

The second distinct outbreak of 1976 occurred in the Yambuku region of northern Zaire (now known as the Democratic Republic of the Congo or DRC). This epidemic lasted only from September thru October, but tallied 318 cases and 280 deaths (20). In other words, this strain of Ebola, identified as Ebola-Zaire, had a terrifying mortality rate of 88% in this outbreak. Subsequently, researchers found that this Ebola strain was morphologically distinct from the previously mentioned Ebola-Sudan strain. Two additional unique strains have been identified since these initial outbreaks and will be discussed shortly. Following this devastating outbreak in Zaire, researchers arriving on the scene determined that the disease spread primarily via close contact, particularly during traditional burial ceremonies, and by the continuous use of contaminated needles and syringes in the under-equipped hospitals and clinics (1). Another interesting point about this outbreak was that while the Sudan epidemic began a few months earlier than the Zaire epidemic, the first documented case of Ebola came from the Yambuku outbreak (1). Consequently, the name ‘Ebola’ was derived from a river in northwest Zaire that ran fairly near the town of Yambuku (7). Following the isolation of the Ebola virus using
electron microscopy, scientists determined that it had close morphological ties with the Marburg virus and consequently, the two pathogens were placed together in the Filoviridae family.

The two outbreaks of 1976 caught the world by surprise. The speed and destructiveness of this mysterious disease spread a certain amount of alarm and fear around the globe. International teams were sent over to the understaffed African villages, primarily via the World Health Organization (WHO), to help deal with the highly virulent epidemics. However, by the time health care officials arrived, they found that that transmission had, for the most part, ceased (12). Since the African health care teams had never seen anything like Ebola before, misdiagnoses of severe dysentery, malaria, and other illnesses were made for quite some time before anyone realized the potency of the disease with which they were actually dealing with. Consequently, the viral outbreaks had pretty much run their course by the time international aid arrived. Scientists and doctors arriving on the scene, therefore, could not document that much information from the actual outbreaks, but they were able to reconstruct considerable data and information from survivors and from the accounts recorded by health care workers present during the epidemics (12).

One of the most interesting aspects of the information that was collected about these outbreaks dealt with the way the transmission of the virus progressed during the epidemics. Health care officials determined that most medical facilities were forced to close during the course of the outbreaks because the death toll amongst the staff was so high (12). Since Ebola spreads through exchange of bodily fluid, the novice medical
staffs, most of whom did not wear gloves or any other form of protective clothing, were hit very hard. Surprisingly, the closing of hospitals actually inadvertently helped cease the spread of the disease by eliminating major centers for propagation of infection (12). Because the meager medical facilities lacked the proper supplies and educational programs to effectively administer the use of sterilized needles and proper barrier-nursing techniques, they were actually doing more harm than good. With the hospitals closed, most sick persons remained segregated in their homes, which proved to be a more effective means of quarantine (12).

Following the initial outbreaks of Ebola in 1976, very little Ebola activity was documented for the next several years. In late 1976, a laboratory researcher in England was infected with Ebola-Sudan when he was stuck accidentally with a contaminated needle. The scientist recovered and no further transmission occurred (1). In 1977, one case of Ebola-Zaire was noted retrospectively in Tandala, Zaire a few months after the individual had died. Once again, no further transmission was documented in this case (1). In 1979, a recurrent outbreak of Ebola-Sudan occurred in Nzara, Sudan, which was the same site as the 1976 epidemic. In this small outbreak, 34 cases were documented, with a mortality rate of 65% (1). Following this outbreak, Ebola was not positively identified again until 1989. Consequently, since only two small outbreaks and one accidental infection occurred from 1976-1989, the international alarm and intense research work that followed the initial outbreaks quickly diminished and very little was learned about the Ebola disease (12).
In 1989, the Ebola virus made its debut in the United States. Cynomolgus monkeys (*Macaca fascicularis*) that were imported into the U.S. from the Philippines began dying in large numbers in a very rapid and violent manner (20). Tests revealed that the monkeys were infected with a new strain of the Ebola virus which was later named Ebola-Reston. Following the initial identification of the virus, fear gripped the nation and rapid and extreme measures were taken to identify and isolate all possible carriers of the disease. Eventually, the Ebola-Reston strain was identified in monkeys in labs in Reston (VA), Alice (TX), and Pennsylvania (20). Epidemiological studies revealed that all of the infected monkeys could be traced back to one exporter in the Philippines and further investigation found that more infections were occurring in monkeys in captivity near Manila that were intended for exportation (20). Unfortunately, the actual or primary source of the virus was not determined at this time and attempts to research the areas where the monkeys were captured in search of the host also failed because of political instability in the Philippines (12).

The appearance of the Ebola-Reston virus in the United States caused quite a stir because no one knew if this particular viral strain could pass from monkeys to humans. Scientists already found that Ebola-Sudan and Ebola-Zaire could easily and had in the past spread from primates to humans and so they were very concerned about the appearance of this new strain. Fortunately, the Ebola-Reston strain proved to be ineffective in producing illness in humans. Four lab workers at the Reston plant had serological evidence of recent infection because they were found to have antibodies for the virus, but none developed clinical illness (1). Nonetheless, several people were kept
quarantined for a substantial amount of time following the inception of the monkey outbreak and the nation collectively held its breath while the results were still pending. In addition, all of the monkeys at the three labs were euthanized and monkey importation from the Philippines was stopped for quite some time. Despite these measures, Ebola-Reston reappeared in 1990 in monkeys in Virginia and Texas that had once again been imported from the Philippines. As with the first case, no humans were infected, but four lab workers developed antibodies (1). Ebola-Reston also was documented in 1992 in Sienna, Italy, where researchers determined that the virus had been introduced into quarantine facilities by monkeys imported from the same export facility in the Philippines that was involved in the U.S. occurrences (1).

Some interesting information was subsequently discovered about the Ebola-Reston strain. Firstly, this particular strain was found to have a distinctly Asian origin that varied significantly from the African strains previously discovered. Furthermore, this strain was found to have lesser pathogenicity than the other subtypes for both monkeys and humans (12). Perhaps the most startling finding was that, unlike the other known Ebola strains which only could be transmitted via bodily fluids, this strain could spread through air particles. In fact, several quarantined monkeys in the containment facilities were found to have been infected by breathing air that was circulating through vents where sick monkeys were being kept (14). This finding has dangerous implications, which will be discussed later.

A very successful fictional book entitled The Hot Zone by Richard Preston was released shortly after this scare and it is premised on the events that took place in Reston,
Virginia and across the country following the discovery of the Reston strain. This book is largely responsible for introducing the United States to the potential threat that the Ebola virus poses.

Ebola reappeared in Africa with a renewed ferocity in the mid 1990s, with at least five different active sites identified between 1994 and 1996. The first documented outbreak occurred in December of 1994 in the vicinity of Mékouka and other gold-mining camps deep in the rain forests of the African nation of Gabon (1). Overall, 49 cases were documented with a mortality rate of 59%. During the course of the outbreak, the few health care officials on the scene assumed the sickness that was afflicting the miners was yellow fever, but the Ebola-Zaire strain was later positively identified in several blood samples taken from victims of the outbreak (1). Another interesting aspect of this particular Ebola appearance was that several survivors who returned from the rain forest camps reported noticing considerable numbers of “unexplained deaths of great apes, gorillas, and chimps” (20). A research team that entered these rain forests in 1995 in search of these dead primates was unable to find any samples. Nonetheless, this finding is significant because researchers haven’t observed a natural outbreak occurring amongst primates and documenting such an event could yield valuable information in the search for a natural host for the virus.

The next Ebola appearance also occurred in 1994 and was notable in that a new strain of the virus was identified in this episode. Late in the year, a team of Swiss researchers were in the Tai Forest of Ivory Coast (Côte d'Ivoire) conducting an investigation on a report that an outbreak of some sort was taking place amongst the local
monkey population. During the course of the expedition, a female scientist became ill after conducting a necropsy on a wild chimp. The scientist was evacuated to Switzerland where she received treatment and subsequently recovered. No one else contracted the disease (1). Soon after this episode, it was discovered that the mysterious disease that inflicted this woman and was detected in several autopsied chimps in the forest was a new strain of the Ebola virus and it received the label Ebola-Ivory Coast. Also notable of this event was the fact that, because the Swiss researcher was taken to a top rate hospital facility, the clinical information obtained from her hospitalization represents the best-studied clinical case of Ebola (12).

In 1995, the Democratic Republic of the Congo (formerly known as Zaire) was hit once again with a very destructive epidemic of Ebola. Health officials recognized the telltale indications of Ebola very early and called for reinforcements accordingly, but the disease raged through the city of Kikwit and the surrounding area for several weeks nonetheless (1). The outbreak, which was traced back to a patient who worked in a forest adjoining the city, spread through families and hospitals, producing a total of 315 cases and 244 fatal outcomes (mortality rate of 81%) before the flames were extinguished (1). Recall that in the epidemics of 1976, most of the information obtained was based primarily on retrospection. Because this outbreak was more rapidly diagnosed than previous incidents, and health care officials arrived on the scene more quickly, investigators were able to conduct detailed studies while events were still transpiring, which was very important for research purposes.
The other significance of this outbreak was that it served to get the world’s attention focused back on Ebola. The press and tabloid coverage of this outbreak was unprecedented (12). Much of the public interest, particularly in the United States, was attributed to the aforementioned best-seller *The Hot Zone*, which focused on the Ebola-Reston outbreak (21). Because this book introduced the general public to the capabilities of the Ebola virus, people were interested to see how devastating this killer virus really could be. This public interest was also reflected in the international aid effort. The World Health Organization (WHO) headed a collective group composed of several relief organizations which provided support to help control and terminate the epidemic (12). The public also provided large donations for this cause, although officials had some difficulties in properly distributing this money for relief efforts (12). Overall, the Kikwit epidemic was one of the most important and thoroughly studied Ebola outbreaks to date.

Shortly after the Kikwit tragedy, the Ebola-Zaire strain was once again identified in 1996 in two distinct outbreaks in Gabon. The first was in February of 1996 in the Mayibout region (1). On January 24, a hunting party came across a dead chimp in the forest. The party butchered, transported, and consumed the chimp remains and 19 people from the party became sick soon after. The disease then spread to some family members of the hunting party members (1). Overall, 37 cases were documented, 20 males and 17 females with a mean age of 27 years, and the infection had a mortality rate of 68% (20). Fortunately, the rapid identification of the disease and the initiation of proper control measures effectively kept the numbers afflicted low and the outbreak at bay.
The second outbreak in Gabon in 1996 began in the town of Booûé. From July 13 to January 18, the epidemic ripped through Booûé and continued on to the neighboring town of Libreville. Overall, 60 cases were identified with 45 deaths (75% mortality rate) (1). The index case for this outbreak was once again a person who frequented the surrounding forests, in this case, a hunter who was living in a forest camp. From this initial victim, the disease once again spread by close contact with the sick and dead (1). This Ebola-Zaire outbreak also resulted in an appearance of the virus in South Africa. A prominent South African doctor who was treating Ebola-infected patients in Libreville got exposed to the virus in October of 1996. The doctor soon became very ill and officials decided to fly him to Johannesburg to be hospitalized. While the doctor eventually recovered, a nurse caring for the ailing doctor accidentally was exposed to infected blood on October 29 and she subsequently died on November 24 (1,20). Despite only having 2 cases in Johannesburg, officials were so concerned that over 350 possible contacts were identified and put under surveillance for the 21 day incubation period (1).

The Ebola-Ivory Coast and Ebola-Reston strains also made transient appearances in 1996. In January, a man became infected in the Côte d'Ivoire/Liberia region south of the Tai National Park. He recovered and three others were suspected to have been infected, but only the first individual was positively identified to have Ebola (20). In April of the same year, Ebola-Reston was detected in two *Cynomolgus* monkeys being held in a quarantine facility in Texas. Because the monkeys were once again from the Philippines, tests were done at the export facility in Manila and Ebola-Reston was
identified in several monkeys located there. No human infections were discovered in either case (1).

The largest Ebola epidemic to date occurred from August 2000 to January 2001. The infection, identified as the Ebola-Sudan strain, spread through three districts of this impoverished African nation, resulting in 425 cases (20). The bulk of the cases—393 to be exact—were located in Gulu; a few also showed up in the districts of Masindi and Mbarara (27 and 5 respectively) (20). Tragically, 224 people died during this epidemic (53% mortality rate), including 29 health care workers and Doctor Matthew Lukwiya, the beloved Uganda practitioner who first identified the outbreak (20).

The outcome of the Uganda outbreak could have been much worse if it were not for the tremendous international response. More than 20 international non-governmental organizations (NGOs) and government agencies worked together to quell the outbreak (20). All schools were closed in the area for quite some time and the WHO banned funerals in an effort to control the outbreak. In October of 2000, the WHO issued an official statement in which the three most important means of transmission in this particular outbreak were identified. According to the experts, attending funerals of Ebola victims (where it was custom to have ritual contact), intrafamilial contact, and nosocomial (hospital) infection were the three most likely means of transmission (7). The frustrating part about this outbreak was that every time officials thought that they had all the infected individuals isolated and were about to declare the outbreak over, they would discover that someone had slipped through the cracks and infected a whole new lot of
individuals. Keeping track of all possible contact persons is a very challenging task and oversights occur very frequently when dealing with this particularly complicated virus.

Most recently, the Ebola-Zaire strain has appeared on a few different occasions in the Gabon and DRC regions of Africa. From December 2001 to March 2002, 60 cases including 50 deaths were reported in the Ogooue-Invindo province of Gabon (20). At about the same time, an additional 32 cases and 19 deaths were identified in the Cuvette region of the DRC, which is very proximal to the border of the Ogooue-Invindo province (20). Experts believe that these outbreaks are linked because additional Ebola victims were scattered in between the two regions. Overall, 122 documented cases of Ebola-Zaire were identified during this episode and a mortality rate of 79% was calculated (1). In addition, in 2001, a very ill woman displaying symptoms of hemorrhagic fever was flown from Africa to Canada and was admitted to a Canadian hospital. Because it was suspected that she had Ebola, the necessary precautions were taken. Fortunately, it turned out to be an illness of a different sort and this case is only remembered because it would have marked the first time a clinically ill Ebola patient reached the Northern Hemisphere (7).

From February to May of 2003, the Ebola virus reestablished itself in the districts of Mbomo and Kellé in the Cuvette Quest Region of the DRC. Once again, several people were involved in this outbreak, totaling 143 cases and 128 deaths, which is an unbelievable 89% mortality rate (20). While only 13 cases from this recent epidemic were confirmed in the laboratory, the other 130 were epidemiologically linked, which helps confirm the legitimacy of the data. In November of 2003, the Ebola virus flared up
once more in the Mbomo district of the DRC. As of December 24, 2003, the Ministry of Health of the Republic of the Congo had reported 35 cases including 29 deaths in the villages of Mbomo (31 cases, 25 deceased) and Mbandza (4 cases, 4 deceased) in the Mbomo district. However, no deaths had been reported since December 3 and officials were hoping the epidemic had subsided (20).

To summarize, the Marburg virus has been identified in six distinct episodes, with 138 total cases. In the first five appearances, the virus had a mortality rate between 23-35%. However, in the most recent outbreak, the mortality rate soared to over 80%. Meanwhile, the Ebola virus has been identified in over twenty episodes, tallying over 1800 cases. The Ebola-Zaire has proven to be the most prevalent and virulent strain thus far, accounting for over one thousand cases with a mortality rate of 82%. The Ebola-Sudan strain has only struck three times, resulting in about 750 cases with a mortality rate of 54%. However, the Ebola-Sudan episode in Uganda in 2000 remains the largest outbreak to date. Only one person has contracted the Ebola-Ivory Coast strain, and the Ebola-Reston strain has not caused clinical illness in any humans in the five different times the virus has popped up in monkey outbreaks.
Classification

The phylogenetic order of Mononegavirales is composed of four distinct families, *Bornaviridae, Filoviridae, Paramyxoviridae,* and *Rhabdoviridae*. The four viral families are grouped together based on the common feature of being composed of nonsegmented negative-strand RNA viruses (2). The genus *Filovirus*, which is the focus of this paper, is actually so morphologically similar to the *Rhabdovirus* genus that following the first visualizations of the Marburg virus via electron microscopy, many experts proposed that the two families be grouped together. Examples of *Rhabdovirus* diseases include rabies and vesicular stomatitis. Since initial discovery of the Ebola virus, four distinct strains have been discovered and visualized extensively and a much more refined protocol for *Filovirus* morphology has been established. Consequently, in 1982, the family Filoviridae, with only the one genus of *Filovirus*, was created based on “unique morphologic, morphogenetic, physicochemical, and biological features” (2).

The actual disease resulting from these *Filovirus* pathogens is termed Marburg hemorrhagic fever (MHF) and Ebola hemorrhagic fever (EHF). The actual term “hemorrhagic fever viruses” encompasses a select group of viruses that are transmitted to humans by either arthropods or rodents of some kind (22). While the natural host for Ebola and Marburg has not yet been found, most experts feel that the host, once discovered, will fall within one of the aforementioned categories. More on the natural host search will be discussed later. Four different viral families fall within the hemorrhagic fever syndrome classification: *Arenaviridae* (for example, Lassa fever), *Bunyaviridae* (such as Rift Valley fever), *Flaviviridae* (including Yellow fever and
Dengue viruses), and Filoviridae (22). Based on current information, the hemorrhagic fever symptoms resulting from Filoviridae infection are the most aggressive and virulent of all the HF viruses (22).

Presently, the genus of Filovirus is composed of only two species, Marburg and Ebola. While these two distinct viruses share enough qualities to be classified in the same family, they are surprisingly different in many regards. For example, the two species differ in their glycoprotein genes, which are very important genes in establishing viral identities, by at least 55% at the nucleotide level and 67% at the amino acid level (2). In addition, the Marburg strains that were obtained from different samples, such as the 1967 outbreak in Germany and the Zimbabwe episode in 1975, appear to be much more homogenous than Ebola strains that were obtained from different locations and times (1,2). Another difference between the two is that while no subspecies of the Marburg virus have yet to be identified, four distinct subspecies of the Ebola virus have already been discovered.

With recent advances in nucleotide sequence comparison, researchers have been able to define phylogenetic relationships much more precisely and to confirm/disconfirm previously established relationships that were based on peptide and oligonucleotide mapping studies (2). One interesting discovery was that at least two different lineages of the Marburg virus exist. Based on nucleotide sequence analysis of the second and fourth genes in the Marburg virus genome, the Ravn strain, which was obtained from an infected individual in Kenya in 1987, was found to differ significantly from four other
Marburg strains (two of which were obtained from the Marburg outbreak, one from the Zimbabwe episode, and one from an isolated incident in Kenya in 1980) (2).

As for the Ebola virus, four different subspecies have been established: Ivory Coast, Reston, Sudan, and Zaire. Based on the genomes of these four subspecies, they all evolved from a common ancestor and therefore have a monophyletic lineage. Extensive studies have found that the four subspecies vary from one another by 37%-41% at the nucleotide level and by 34%-43% at the amino acid level (2). In addition, several strains of each of these subspecies have been discovered from the numerous documented Ebola outbreaks since 1976. The strains differ in their similarity (2).

The phylogenetic relationship of the filoviruses also provides some interesting information about the evolution of these pathogens. While nucleotide sequence analysis has found that the Marburg and Ebola viruses are only loosely related, an analysis of the amino acid sequences in the structural proteins of these viruses showed a strong relationship between the two (2). Thus, while the nucleotide sequences may began taking divergent evolutionary paths some time in the distant past, the structural proteins have preserved a similar structure and function. This suggests that the two viruses shared a common ancestor and have diverged considerably since. The obvious question that follows is ‘why did they diverge?’ Some experts feel that Marburg and Ebola, as well as the different Ebola subtypes, found different natural hosts and then coevolved with their respective hosts, which consequently resulted in each type becoming genetically different from the others (2). This debate has not yet been settled.
**Structure**

The general structure of filoviruses varies considerably. The actual term filo- is Latin for thread and refers to the unique filamentous shapes that these viruses exhibit (2). Filoviruses are special in that all of the virions, which are complete viral particles with nucleic acid and protein capsids, can take on different shapes (9). This biological feature, termed pleomorphism, is one of the main reasons that filoviruses are so unique. While virions are typically found in the bacilliform shape, they can also be long and either straight or branched, or they can be shorter and either circular, U (termed the Shepherd’s Crook), or 6-shaped (3). Viral analyses have found that Marburg particles are found in the circular form quite often, while the Ebola subspecies tend to exist in long filamentous forms (2).

Viral filaments have a uniform diameter of about 80 nm, but their lengths vary considerably. Marburg virions, which range from 795-828 nm, are typically shorter than Ebola virions, which range from 974-1063 nm (Ebola-Sudan), 990-1086 nm (Ebola-Zaire) and 1026-1083 nm (Ebola-Reston) (2). The actual body of the virus is made up of three distinct parts. The central axis, 19-25 nm in diameter, makes up the core of the structure and is composed of ribosomes, RNA, and histones (also known as the ribonucleoprotein complex) (2). This central material is surrounded by an outer helical layer that is 45-50 nm in diameter and has cross-striations located every 5 nm (2). Another notable feature of filoviruses is the presence of an outer lipid (fatty) envelope, which is derived from the host cell’s plasma membrane. Most filovirus virions also have
spikes on their membrane that are generally 7 nm in diameter and spaced between 5 and 10 nm apart (3).

The genome of filoviruses consists of a single-stranded, negative sense RNA molecule. On average, filovirus genomes are about 19 kilobases long (Marburg is 19.1 kb, Ebola is 18.9 kb), which is much larger than the genomes of the other members of the Mononegavirales order (2). Thus far, entire nucleic acid sequences for a few different strains of Ebola and Marburg have been determined and several different sequences have been found to match up very well, including start and stop transcriptional signals as well as parts of certain genes (2).
Diagnosis

In an outbreak situation involving viruses of such immense potency, rapid and accurate diagnosis is of vital importance. With recent improvements in viral detection technology, testing for the Ebola and Marburg viruses has become more reliable than ever before. Nonetheless, further progress continues to be made.

In the initial outbreaks, diagnosis took quite some time because medical officials were not sure of the cause of the illnesses, and viruses of the Filovirus family had obviously never been seen before. Following isolation of the Marburg and Ebola viruses, in 1967 and 1976 respectively, antibody detection tests performed with the fluorescent antibody technique (also called indirect fluorescent antibody (IFA) tests) were created to clinically diagnose the viruses. The premise of these tests was based on the observation that the viruses induced seroconversions within their host. In other words, following the initial infection, patients were found to produce specific antibodies in response to the presence of the Marburg and Ebola antigens (13). The antibody detection tests, therefore, were designed to isolate these antibodies, which generally begin appearing around day 8-10 following infection (22). As technology continued to advance however, researchers found that these tests often yielded misleading and inaccurate results (13). In addition, the potential benefit of this test has been limited by the fact that the test does not become positive until the patient’s health is improving (22). Many people have been stricken so quickly by these viruses that their body never had the opportunity to produce a sufficient immune response to elicit IFA detection before death claimed them.
A more recently developed technique for diagnostic purposes is referred to as the ELISA test, which stands for enzyme-linked immunosorbent assay. ELISA screening achieved a certain degree of prominence in HIV detection, and is now used in diagnosing several viral infections. Based on the principle of antibody-antibody interaction, this test is extremely sensitive and allows for easy and rapid visualization of results (13). In the case of Ebola and Marburg, ELISA tests are used to detect specific IgM and IgG antibodies. IgM and IgG antibodies are members of the immunoglobulin (Ig) family, which is composed of five classes of structurally related proteins that are distinguished by having different functions in the immune response process (9). In ELISA screening for Ebola, IgM tests become positive very early in convalescence and shortly after, IgG tests usually will also become positive (13). Thus far, it has proved to be very accurate in Ebola and Marburg detection. Nonetheless, the ELISA test is still being evaluated to make sure that it doesn’t have any unforeseen limitations (13).

Presently, several different techniques can be used for diagnosis and confirmation purposes. Viral antigens and antibodies can be detected by ELISA, immunofluorescence, and immunochemistry (IHC). Filovirus infection can also be confirmed by detecting viral RNA using the reverse transcription-polymerase chain reaction (RT-PCR) (22). This technique is particularly useful in detecting lower concentrations of viral RNA, which might slip past ELISA detection (13). Diagnosis can also be accomplished by isolating viral components from infected body fluids and tissues by co-culturing with VeroEG tissue culture cells. When the culture cells are examined using an electron microscope,
characteristic filoviral enclosures loaded with nucleocapsids will be noticeable in the cells if infection has taken place (22).

In epidemic situations, health care officials generally must rely on differential diagnosis techniques to confirm cases because more advanced laboratory processes are not available in the third world African villages where the disease generally strikes. This form of diagnosis involves distinguishing a health condition from various other conditions that share similar signs and symptoms by ruling things out using physical clues and by tracing transmission pathways (9).

In the case of Ebola, diagnosis based on physical findings has always been a challenge. In most outbreaks, the initial patients were misdiagnosed with ailments such as *Shigella* infection (a bacterial intestinal illness), yellow fever, dysentery, and several others (7). Keep in mind that health care facilities in most African villages where outbreaks have occurred have little more than a few beds, some bandages, and an allotment of general antibiotics. Doctors are few and far between, and most who do carry the title are not nearly as educated or experienced as certified physicians here in the United States. In many villages, nursing nuns and volunteers operate the health center (10). In addition, because of the low level of health care in Africa in general, diseases of all sorts (i.e. AIDS, malaria) are much more prevalent than in Western societies. Consequently, diagnosis based on physical symptoms generally has taken a considerable amount of time in the various African outbreaks over the last thirty years.

This delay in diagnosis has played a major role in promoting the various epidemics because the initial victims were not quarantined properly and the necessary
barrier-nursing techniques were not immediately employed. In many documented cases, the initial Ebola patients in an outbreak were even permitted to leave the hospital and return to their friends and family for care, which usually led to further transmission. Also, when these early patients died, family members were allowed to perform traditional funeral ceremonies, which often involved extensive contact with the victim. With the frequency of outbreaks increasing in recent years, as well as better dissemination of Ebola-related information, health care officials are beginning to suspect Ebola and Marburg more readily when a patient reports to the health center with appropriate symptoms. Nonetheless, continued improvement in this area is necessary.

Currently, a new test is being developed which will be able to rapidly detect Ebola components in skin samples (23). Development of such a test will be extremely helpful in outbreak situations by enabling health care workers to make early diagnoses of victims and to keep surveillance of contacts (23).
Transmission

Determining the course of transmission has been a very important issue associated with the Marburg and Ebola viruses. In the early outbreaks, there was quite a bit of confusion and false information concerning this topic. However, much has been learned about how these pathogens spread from host-to-host through on-site observations and experimental work.

The main route of transmission is person-to-person. The virus can spread through direct exposure to bodily fluids, secretions, or excretions (7). Possible routes of infection include mucous-membrane exposure, pharyngeal contamination resulting from swallowing contaminated saliva, infection through small cuts and breaks in the skin, and exposure from accidentally ingesting viral-laden material (13).

Research teams present at recent outbreaks, such as the Kikwit outbreak in 1995, noted that transmission commonly occurred amongst members of the same household through close contact and bodily fluid contact. The caregivers of the household were struck the hardest by secondary infection (12). The same has been true at hospitals, particularly in earlier outbreaks. In African medical facilities, health care workers often did not take the appropriate precautions in treating patients (such as wearing a mask, gown, and gloves) and consequently were infected while caring for sick individuals (1). Researchers have also determined that touching cadavers, a common ceremonial practice in many African communities, has resulted in extensive secondary infections in past outbreaks. Consequently, in recent outbreaks, such as the Uganda epidemic in 2000,
traditional funerals were cancelled and trained burial teams were employed to bury all confirmed and potential Ebola cases to help slow transmission of the virus (11).

In some studies on nonhuman primates, it was discovered that infection can occur through both the mouth and eyes (12). Consequently, the WHO now stresses the importance of health care personnel protecting their face thoroughly when working with infected individuals. This issue becomes especially important when treating late-stage patients because hemorrhaging and viral excretion increases dramatically as the disease progresses, which leads to an increased risk of accidental infection (12). Without a full mask and protective coverings, a splash of blood could easily get into someone’s eyes or mouth as they are trying to help an individual who is bleeding uncontrollably.

Up until recently, it was assumed that Ebola infection always resulted in symptomatic disease. In other words, researchers thought that if the Ebola virus was transmitted to an individual, it was a certainty that the individual would develop clinical illness (7). However, with better technology and surveillance in recent outbreaks, health care workers have had the opportunity to monitor this phenomenon more accurately and efficiently. Surprisingly, they have found that asymptomatic Ebola infection does occur. In the Gabon outbreak, seven individuals were found to have been infected by the Ebola virus without developing noticeable blood antigens or getting sick (7). One big question that stems from this finding deals with the role that these symptomless individuals play in the transmission process. Are they capable of silently spreading the disease to other people in the same fashion that a mono carrier spreads the illness without becoming ill.
him/herself? The jury will remain out on this particular topic until further research is performed.

The prospect of airborne transmission has been explored quite extensively because of the tremendous implications associated with such an ability. Experiments with lower primates have determined that both Ebola and Marburg viruses are stable and infectious in small-particle aerosols (12). In addition, during the 1989-1990 Ebola-Reston outbreak, researchers discovered quite a bit of evidence which seemed to indicate that the virus was spreading amongst the primates via air particles. This evidence included supportive epidemiology data concerning the transmission pattern of the virus amongst and between containment rooms, high levels of viral particles discovered in nasal and oropharyngeal fluid samples, and large collections of viral components within alveoli of the dead monkeys (12).

Besides this Ebola-Reston outbreak, however, transmission through air particles has not been observed in the clinical setting. In recent outbreaks, researchers have specifically sought out EHF cases in which the infected individual(s) had no known direct exposure to the disease, which would possibly indicate airborne transmission. However, up to this point, they haven’t found any substantiated cases of this kind (12). Consequently, excluding the Ebola-Reston subtype which doesn’t lead to clinical infection in humans, the Ebola and Marburg viral diseases do not seem to travel through air particles in the clinical setting.

Much still needs to be learned about how long the virus remains in the body following convalescence. Nonetheless, some interesting data has been discovered in this
area. The Marburg virus has been isolated in some secretions and certain tissues 1-3 months following recovery from illness (12). It was also discovered that transmission of the Marburg virus through semen can occur several weeks after recovery. In one documented case, a male infected a female with the Marburg disease through sexual contact a full three months after he had recovered (7).

The Ebola virus does not seem to remain in the body quite as long as the Marburg virus. In the 1995 Ebola outbreak in Kikwit, researchers kept track of contacts of several survivors for quite some time to see if any late transmission occurred, which might indicate that the virus stuck around in their bodies for some time. No late transmission was documented (12). Researchers have found Ebola RNA in semen as well as vaginal secretions for several months following convalescence. They haven’t been able to isolate an intact virus in these secretions however. In a related study, a testicle from an EHF victim was examined using an electron microscope and found to have significant viral exposure (12). Further, like the Marburg virus, transmission of the Ebola virus via semen has been found to be possible for several weeks following acute illness (7).
Pathology

While the Ebola and Marburg diseases cause similar symptoms and complications, the course of infection is different enough that the two illnesses deserve separate discussions. Therefore, the pathology of Marburg will be covered, followed by an analysis of the acute disease associated with the Ebola virus.

Despite infecting a considerably smaller number of people than the Ebola virus thus far, the clinical manifestations of Marburg hemorrhagic fever are just as lethal. The incubation period ranges from 5 to 10 days following exposure. The clinical syndrome generally is distinguished by a sudden onset of symptoms such as fever, chills, and headache, which are often accompanied by vomiting and non-bloody diarrhea (2). Myalgia, which is defined as a general pain in the muscles, is also often seen in Marburg cases (9). Other common complications include enanthema (which is an eruption on mucus secreting surfaces such as the mouth or vagina), conjunctivitis (inflammation of the outer layer of the eye), and lymphadenopathy (inflammation of the lymph nodes) (2, 9). Patients are generally reported to be extremely drowsy and lethargic as well (7).

The most reliable diagnostic indication is the appearance of a maculopapular rash, generally on the trunk region, around the fifth or sixth day of clinical illness. A maculopapular rash is a particular type of rash that has both macules (flat circumscribed areas of discoloration) and papules (small, superficial raised spots on the skin). The rash associated with Marburg hemorrhagic fever is also noted to result in extensive desquamation, or peeling, as well (2, 9). Thus, this characteristic mark of the Marburg disease is fairly consistent and identifiable.
Around the same time that the rash appears, patients often begin developing several other symptoms such as nausea, vomiting, chest pain, diarrhea, sore throat, and abdominal pain. Generally, the symptoms will continue to increase in severity as the illness progresses. Marburg victims may experience jaundice, pancreatitis, extreme weight loss, delirium, shock, liver failure, and multiorgan dysfunction (7). Further, about a third of patients develop massive hemorrhaging from the gums, nose, puncture sites, and the gastrointestinal tract. Based on documented cases, the onset of this eruption of blood typically occurs during the period of the lowest thrombocyte count, which ranges anywhere from 6 to 12 days after the onset of illness (2).

Laboratory analysis of blood work has revealed additional abnormalities associated with this deadly disease. Blood samples from Marburg victims usually show evidence of leukopenia, or a reduction of white blood cells, and contain high numbers of circulating immunoblasts. In addition, all patients have thrombocytopenia to some degree, or reduced numbers of platelets in the blood, and also exhibit some minor abnormalities on coagulation tests (2).

The Ebola hemorrhagic fever has proved to be just as devastating for victims. The following quotation, taken from a documented account of the Kikwit outbreak, illustrates the severity of the Ebola disease. “The initial symptoms were fever and bloody diarrhea, that was true. But then within a few days there was this massive bleeding from all over, blood pouring out of all the body’s pores and other openings: mouth, nose, everywhere else. Finally, the victims lost consciousness, after which they literally bled to death” (15).
Numerous accounts like this one have been published and all carry the same message: the effects of the Ebola disease are nothing short of terrifying.

The incubation period for Ebola hemorrhagic fever generally ranges between 4 and 10 days, but the official period is between 2 and 21 days (7). An outbreak is not declared over until all possible contacts have been quarantined for the critical 21 day period without any indications of infection. The disease is marked by the sudden onset of a fever, a severe frontal headache, and joint and muscle aches (myalgia) in victims (7). A certain degree of malaise is also often seen in Ebola patients. In addition, health care officials have documented non-bloody diarrhea in 81% of studied cases, vomiting (59%), pain and dryness of the throat (63%), and abdominal pain (13). In many cases, individuals also develop bradycardia (decreased heart rate), conjunctivitis (inflammation of the outer layer of the eye), and pharyngitis (inflammation in the throat behind the pharynx) during the beginning stages of the disease (7, 9). The conjunctivitis can lead to blindness if not treated effectively.

When the 1976 Ebola-Sudan outbreak was in full swing, most patients were hospitalized by the fifth day of infection and were noted to have physical abnormalities such as “deep set eyes, ghost-like expressionless faces, and extreme lethargy” (13). These external indicators remain important in initially diagnosing patients during the chaos of an outbreak.

Around day 5, a maculopapular rash often appears, which resembles the rash associated with rubeola. Based on documented cases, this rash occurs in about half of people afflicted with the disease (13). This figure might be off, however, because the rash
is often very hard to see on dark-skinned individuals. In many situations, the rash is only realized in survivors at a later time, when desquamation, or skin peeling, occurs (13).

An interesting aspect of Ebola pathology is that certain differences have been noted between the different subtypes. For example, extreme chest pain has been documented as a characteristic feature of the Ebola-Sudan strain, appearing in 83% of individuals. Meanwhile, chest pain seems to be very uncommon in Marburg or Ebola-Zaire patients (13). Other, less distinguishable, differences have been noted, but require further documentation for confirmation purposes.

As the Ebola disease progresses through the infection process, health complications become more extensive and severe. Hemorrhagic manifestations occur in 71-78% of patients, with about half of survivors and all of fatal cases developing these massive bleeding episodes (13). Experts believe that the hemorrhaging seen in most cases is the result of the virus adhering to the endothelial lining of blood vessels, which damages them quite extensively (7). The bleeding that results leads to petechia (small, round and flat dark-red spots that are caused by bleeding into the skin or beneath the mucous membrane) and ecchymoses (bluish-black bruise mark on the skin resulting from the release of blood into the tissues from leaking of blood from the vessels) (7, 9). In addition, most victims experience uncontrolled bleeding from orifices, mostly gastrointestinal as opposed to urogenital, and from needle-puncture sites (7, 13). Most that die from Ebola hemorrhagic fever have lost a tremendous amount of blood and are usually in a stupor from shock and tachypnea (rapid breathing) (7).
Based on the information obtained thus far, the Ebola virus (as well as Marburg) seems to be pan tropical. In other words, the virus can invade and affect several different tissues in the body without demonstrating a particular affinity for any one region or organ in particular. Damage is widespread and usually no single organ exhibits the extent of damage that would account for the severe state of shock and extensive bleeding that victims experience (17). This is one of the main reasons why the virus is so difficult to treat and mortality rates are so high. The virus varies and disperses its attack so thoroughly that in most cases, particularly in experimental infections with primates, no clear-cut cause for fatality is ever determined (16).

Despite this variability, some generalities can be made about tissue damage resulting from Ebola infection. Autopsies on Ebola victims found that virtually all individuals experienced characteristic hemorrhaging and widespread necrosis of internal organs. The liver and lymphoid tissue generally exhibited the most necroscopy, or tissue death, but the gonads, spleen, and kidney also experienced extensive damage in victims (22). The autopsy findings also generally included numerous petechial hemorrhages and ecchymoses (defined above) that involved the skin, mucous membranes, and internal organs (22). These marks reflect the widespread bleeding that occurs in most individuals. In addition, the lungs were often found to have experienced hemorrhaging and scattered alveolar damage. The surprising part about this finding is that examiners never found any sign of interstitial pneumonitis or inflammatory response in the lungs despite all the damage (22). Either the disease strikes so quickly that the body cannot produce any defense mechanisms in the lungs, or the virus has a negative effect on the immune system.
as well. This feature is still being explored and much remains to be learned about the virus’s effect on the immune system in general.

In the 1995 Ebola-Zaire outbreak, health care officials arrived on the scene in time to conduct a prospective study on 103 individuals. Based on their analyses, the clinical syndrome associated with Ebola is biphasic and has two distinct stages (13). In the early period following infection, the most notable feature of the patients in the study was extreme asthenia, which is basically general weakness or loss of strength. The second period was marked by hemorrhagic manifestations, hiccups (15% of individuals), and occasional neurological problems. Serious bleeding was observed in 45% of these patients (13). In addition, specific indicators were noted to be common with the patients who ultimately ending up dying. These signs included obtundation (a decrease in sensation or feeling in various areas), anuria (failure of the kidneys to produce urine resulting from a serious drop in blood pressure), shock, tachypnea, and normothermia (9, 13). These various complications proved useful to officials in triage situations as indicators of which patients might make it and which were probably not going to survive.
Therapy

Currently, there is no specific therapy or treatment available for filoviral infection. Left with limited options, physicians and nurses have primarily focused on nonspecific, supportive treatments to help control the various health complications resulting from the disease. Maintaining fluid and electrolyte levels is very important because most individuals become so dehydrated from diarrhea, vomiting, and late-stage hemorrhaging. Health care teams also are careful to maintain oxygenation and blood pressure, which will also fluctuate more and more as the disease runs its course and hemorrhaging becomes more serious (7). Besides monitoring these various things, very little was done in the form of treatment in most of the previous outbreaks. Depending on the quality of the medical center and the skill of the caregivers present at a given outbreak, other measures were occasionally taken to treat specific complicating conditions or infections that resulted from the disease. However, rarely did patients noticeably benefit from these treatments.

A couple of experimental procedures performed during past filoviral episodes have shown some promise. When the laboratory worker accidentally infected himself in England in late 1976, he was treated with convalescent plasma and interferon-α. He recovered from the Ebola-Sudan infection, which indicated that these treatments might have some promise (13). However, skeptics were quick to point out that the lab worker’s condition began to improve at the time of expected recovery and blood work done later was not precise, so many were hesitant to make the claim that these treatments were responsible for the patient’s recovery. Since this time, a substantial amount of
experimentation has been done in cell cultures and in animals (primarily small primates and guinea pigs) to test the possible benefits of convalescent plasma, interferon-α, and ribavirin as well. The results of these studies indicate that these common treatments have no usefulness in treating filoviral infections (13).

Two patients infected with the Marburg virus in 1975 were successfully treated with a drug called Heparin (18). Because of the extensive damage to endothelial tissue that is often found in filoviral infections, doctors in Johannesburg suspected that quite a bit of clotting was occurring in the patients’ circulatory system (officially termed disseminated intravascular coagulation) (13). Therefore, they experimentally began administering Heparin, which is a powerful anticoagulant that is used to decrease the clotting ability of the blood (4). While the drug seemed to be beneficial in these cases, experts warn that Heparin should only being used when proper monitoring systems for coagulation levels are available (13). Consequently, this drug would probably not be very useful in the typical understaffed and undersupplied village health center where most of the past outbreaks have occurred. However, it is an important drug to keep in mind for future filoviral episodes.

Late in the Kikwit outbreak in 1995, health care officials arriving on the scene began giving some of the patients whole blood transfusions using blood from recovered people. Following the transfusion, medical officials noticed significant improvement in many of the patients receiving the blood (13). However, experts are careful to not take this experiment too seriously because there was no control of any kind. Further, no records were kept about such things as day of treatment versus day of infection. In
addition, the condition of subjects when the treatment was administered was also far too variable. In future outbreaks, researchers would like to obtain better and more controlled data to help test the effectiveness of these transfusions. In addition, more studies need to be done comparing the role of whole blood versus frozen convalescent plasma to better understand the beneficial processes that occurred with this treatment (13).

In recent years, with improved technology and better research facilities and teams, some promising treatments have begun to be explored. It was already mentioned that regular convalescent serum, which is a blood serum sample taken from a patient in recovery phase to be used to treat sick individuals, has not demonstrated much benefit in Ebola and Marburg cases. The fact that researchers have not been able to find large amounts of neutralizing antibodies in this convalescent serum supports the aforementioned conclusion (12). However, a hyperimmune horse anti-Ebola serum has been developed that demonstrates neutralizing capacity in vitro as well in some laboratory animals infected with Ebola, such as guinea pigs and baboons (13). Unfortunately, this horse serum has not been as effective in treating rhesus monkeys and mice, which has made researchers question its efficacy in humans (12).

Research teams here in the United States recently managed to produce human monoclonal antibodies that are designed to bind to a specific Ebola virus surface protein. The antibodies were made from some mRNA samples that were extracted from the bone marrow of some survivors of the Kikwit outbreak (12). Using the standard monoclonal antibody production process of fusing antibody-forming cells with tumor cells and then allowing propagation to occur, researchers can produce large quantities of these high-
affinity antibodies, all targeted against the specific Ebola antigen (10). The effectiveness of these antibodies in a clinical setting has not yet been confirmed, but researchers are excited about the possibility of such a standardized, safe, and replenishable treatment option (12).

A vaccine has not yet been developed to prevent filovirus infection. However, some promising work has been done in this department (12). Even if a vaccine is developed, its usefulness is questionable because outbreaks are so erratic and scattered. Further, it would be virtually impossible to inoculate such a large and ever-growing population dispersed primarily amongst rural villages and towns. Nonetheless, experts feel that it is important to continue efforts to develop a vaccine in case it is ever needed in the future. If an outbreak ever occurs on a much larger scale than we have witnessed thus far, a vaccine would certainly become much more valuable. In addition, a lot could be learned from a successful vaccine about the mechanisms of Ebola infection and the body’s immune response (12).

A substantial amount of work has also been devoted to the development of antiviral drugs. Currently, the US Army Medical Research Institute of Infectious Diseases, the National Institute of Allergy and Infectious Diseases, and a few other notable partners are working on a huge project that is aimed at discovering drugs that are effective against various viruses such as respiratory syncytial virus (RSV). Like the filoviruses, RSV is a negative-sense, single-stranded RNA virus that shares several similarities to Marburg and Ebola. Thus far, at least one drug has been identified that has
demonstrated some effectiveness against both RSV and Ebola (12). The drug is still in
the preclinical testing stage, but experts are hopeful that it will live up to the expectations.

For now, health care officials are still reliant on supportive care, despite the
inadequacies of the treatments. The infection and tissue damage is so widespread in fatal
cases that supportive treatment probably does not have much influence on survival. Until
better medications and therapies are developed, the mortality rate is going to remain high
in future outbreaks. However, with the development of new antiviral drugs, effective
passive antibody treatments, and other promising therapies on the horizon, the future for
filoviral treatment looks promising.
Natural Reservoir

Despite all the progress that has been made, many mysteries remain about the filovirus pathogens. Perhaps the biggest mystery concerns the origins of the viruses. While epidemics have often been traced back to an index case or cluster, researchers have yet to determine where the virus originally came from. More often than not, the first individual(s) to contract a filoviral infection in one of the previous outbreaks had some contact with a monkey that was presumably infected with the disease itself. Initially, scientists hypothesized that monkeys represented the natural reservoir for these viruses. However, it did not take long for researchers to eliminate these primates from the list of possibilities because the Marburg and Ebola diseases were far too infectious and pathogenic to monkeys to be maintained for any substantial amount of time. A natural reservoir species must be capable of housing and spreading the virus without experiencing negative effects. In many instances, the filovirus microbes have proven to be more devastating to monkey populations than to human communities. Therefore, it was determined that monkeys can only play the role of an intermediate host in the transmission process.

The search for the natural reservoir continues today. Thus far, very little progress has been made. Because researchers have not identified the host, they know extremely little about how the virus is maintained in nature or how it is transmitted to the index host, whether it be a human or monkey. In addition, some experts speculate that vectors, which are animals (usually insects, such as mosquitoes-malaria) that transmit infectious material from one organism to another, might be involved in the transmission process as
well (7). However, little has been discovered to support this claim as of yet. Until the natural reservoir is determined, many uncertainties will remain.

In past outbreaks, research efforts have often been curtailed. In many instances, the outbreak occurred in such a rural and hard to reach destination that research teams simply did not have the funding or interest to conduct an investigation. In some other cases, civil unrest in or around the area of the outbreak prevented any studies from being conducted. The erratic appearances of these outbreaks also contributed to limiting the amount of studies conducted because officials could not plan a future study for a particular time or place. Also, weather sometimes played a role. The wet season in the jungles of Africa presented a formidable challenge to researchers trying to collect animal and plant samples (12).

In recent years, increasing efforts have been made to discover the primary host. Despite the aforementioned factors that have limited studies, inquiries and investigations of some extent were often made in most epidemics. Even in the first Marburg epidemic in 1967, officials did some light probing to learn about the monkey importer in question and the area where the monkeys were being trapped (18). In the early Ebola outbreaks in Sudan and Zaire, hundreds of rodents, bats, and arthropods were caught and examined (8). However, it was not until much more recently that serious investigations began being consistently and thoroughly conducted.

An extensive study was performed following the Kikwit investigation. Researchers were able to trace the transmission back to an index case. They learned that the man lived in the city of Kikwit, but often rode his bicycle to a forest outside of town,
where he was exposed to a variety of animals and plants (12). Soon after the outbreak
when weather conditions had improved, several international teams visited the area to
conduct a search. In order to collect a variety of samples, investigators decided to throw a
huge net in the tropical forest, which would capture a broad range of vertebrates and
arthropods (12). No evidence of Ebola or Ebola antibodies was found in any of the
numerous species that were sampled. The following year, another team from the National
Institute of Virology in Sandringhan, South Africa, revisited the site to make further
collections, but they did not have any luck either (12). The biggest obstacle noted in these
studies was that the tropical jungle contained such a rich diversity of species that it would
be next to impossible to test them all.

More recently, studies have been conducted in Gabon and the Tai Forest. No
major findings have been reported as of yet (8). For the past ten years or so, the WHO has
been conducting an ongoing study in Tai Forest that is following chimpanzee and
Colobus monkey populations. Periodic epidemics of the strain Ebola-Ivory Coast have
broken out in these populations and researchers hope that with continued monitoring,
they will be able obtain some valuable information about a primary host (8). They have
already determined that reservoir abundance or conditions where the monkeys are in
contact with the reservoir varies seasonally based on the periodicity of past outbreaks (8).
Hopefully, this study will discover more information in the future.

Presently, the three prospects that are being investigated most thoroughly are bats,
insects, and plants. Several different studies and observations suggest that bats may play
a role in transmission, either as the natural host or as vectors. The first indicator was in
the 1976 Sudan epidemic, where it was found that the index case as well as two other primary cases in the Sudan outbreak in 1976 all worked in the Nzara Cotton Factory. Since the three men did not interact with each other outside of their workplace, it was determined that the factory must have been the point of infection (21). At the factory, it was noted that a substantial population of bats of the species *Tadarida (mops) trevori* lived in the roof (21). Unfortunately, the bats were never captured to be tested and it was also noted that several other animals and insects lived in and around the factory, so this finding was not that conclusive (21). Interestingly though, the index case from the 1979 outbreak was also found to work at the Nzara Cotton Factory.

In 1987, a 15 year old boy became ill after visiting a bat cave in western Kenya (7). Once it was serologically confirmed that the boy was infected with the Marburg virus, some researchers conducted an extensive exploration of the cave to examine the possibility of bats being the primary host. Unfortunately, no conclusive evidence was obtained. Further, it was once again noted during this study that several other animal species inhabited the cave, complicating matters further (7).

Robert Swanepoel, at the National Institute of Virology in South Africa, headed up a large study designed to explore the possibility of bats (as well as many others) being the primary host or vector for Ebola (19). Many interesting findings were made. They found that following inoculation, the Ebola virus could replicate in fruit bats as well as several different species of the *Tadarida* genus. Following replication, the virus could also be passed on through the bats’ stool (termed “guano”) (19). The Ebola virions present in this guano could potentially be passed on to human subjects or an unknown
vector. Countering this promising finding, Swanepoel’s team also found that Ebola-Zaire is very pathogenic in the *Tadarida* species tested as well as in many other bat species that are common in Africa (19). This finding suggests that if these bats are involved in the transmission process, they may play more of a transitory role since, like monkeys, they cannot maintain the virus for any long period of time.

Because Ebola virus has proven to be so pathogenic to most animal species naturally and experimentally infected with it, some experts feel that a plant species may be the primary host. Little research has been done on this topic thus far, but some observations seem to support the possibility. For one, the simple fact that no animal host has been found yet despite researchers’ best efforts may indicate that they are looking in the wrong place. Another general observation suggests that since humans and other primates do not have a natural immune response to Ebola infection, the virus may have evolved elsewhere (6). Swanepoel’s study included an analysis of the effects of inoculating 24 different plant species with Ebola to test this hypothesis. Viral replication did not occur in any of the plants and 13 died from the treatment (19). While helpful, these negative results can’t be regarded too highly because the 24 species tested represent such an infinitely small percentage of the total number of floral species indigenous to Africa.

Insects are also being explored as a potential host. Typical of the African environment, arthropods of all sorts have been found around outbreak sites. Up to this point, very few have been examined for the presence of Ebola.
Many challenges are inherent in the general capturing and sampling process aside from the obvious difficulties in finding specimens. Most animal, insect, and plant samples that are obtained were acquired a significant time after the outbreak had run its course. By the time specimens have been collected, the virus may have already worked its way through all the subjects in the designated collection area. In addition, the virus is very likely to not be present in every single member of the primary host species. Because of this fact, when investigators collect and sample only a few members of any given species of plant or animal, their odds of finding the host are increasingly reduced.

Another challenge deals with the different strains of filoviruses. Many experts feel, based on evolved genomic differences primarily, that Marburg and the four Ebola strains could all have different hosts, which would complicate the matter further (2). In fact, the Ebola-Reston strain, which was traced backed to an exporter in the Philippines, may even have an Asian host that is completely distinct from its African counterparts (2).

Researchers have only just begun to unravel this complicated mystery. Only with extensive investigations and surveillance and a little luck will the mystery be solved.
Future Considerations

Judging by the volume of this thesis, quite a bit of information has been learned about both the Ebola and Marburg viruses. Researchers and medical officials alike have spent countless hours both on the frontlines of outbreaks and in the laboratory learning everything they possibly can about these devastating pathogens. Considering how recently these viruses were discovered, the scientific community has certainly made considerable progress and should be commended for it. That being said, a tremendous amount of work still needs to be accomplished in several different areas.

First off, quite a bit remains unknown about the virus itself and various virology issues. The virus has been isolated and the genome has been pieced together. However, little is known about the products and purposes of each of the 12 genes that are present in the filovirus RNA sequence. Further, scientists have yet to determine how the viruses operate in their hosts, particularly humans, to cause infection. In particular, very little is known about the viruses’ interaction with the host immune system. Learning more about these types of things will certainly help in numerous other aspects of research, such as the search for treatments. Therefore, more research in the laboratory is certainly going to be important in the upcoming years.

It was already mentioned that researchers have discovered some promising new treatments and therapies in the last few years. Focus should be placed on the antiviral and antibody treatments because these types of therapies would be most effective in future epidemics. A vaccine would be useful in understanding the pathogenic and immunity-suppressing qualities of the viruses, and might come in handy if a larger outbreak
occurred or the virus mutated to become transferable (airborne transmission). However, at this point, with outbreaks so scattered and small, antiviral and antibody treatments seem to be the most useful. In addition, medical officials should work on developing better and more uniform methods of supportive care for sick patients. A lot can be learned from previous outbreaks, which could be applied to future episodes.

Until better treatments are developed, a considerable amount of money and effort should be devoted to preparation and education in African villages. With proper barrier nursing techniques and clean equipment, much of the nosocomial infections that have occurred in previous outbreaks could be stopped. The international community and organizations such as the WHO and Red Cross need to offer both supplies and instruction to these village health centers so that things such as needles and beds don’t get reused and the staff properly protects themselves with masks, gloves, etc. Not only will this be helpful in controlling Ebola situations, but it will help contain the spread of numerous other diseases as well, such as AIDS. In addition, research teams need to continue developing better diagnostic techniques so that outbreaks can be identified and isolated faster. The skin detection technique has considerable promise and needs to be developed so that it can be applied to the village setting.

The natural reservoir needs to be found as soon as possible. If the source can be determined, officials may be able to devise a way to prevent outbreaks from ever beginning. In addition, if the host were to be determined, researchers could learn so much more information about transmission and viral replication and various other features of the virus that currently remain unclear. While the prospect of finding this primary host
may seem improbable at times because of the infinite number of prospective species of animals, plants and insects, it is vital that investigations are continued or even increased in upcoming years.

One day, we will eradicate these terrible diseases completely. However, plenty of work remains before this end becomes a reality.
References


20. WHO. “Ebola Fact Sheet.”


