Marburg and Ebola Viruses as Aerosol Threats

ELIZABETH K. LEFFEL and DOUGLAS S. REED

ABSTRACT

Ebola and Marburg viruses are the sole members of the genus *Filovirus* in the family Filoviridae. There has been considerable media attention and fear generated by outbreaks of filoviruses because they can cause a severe viral hemorrhagic fever (VHF) syndrome that has a rapid onset and high mortality. Although they are not naturally transmitted by aerosol, they are highly infectious as respirable particles under laboratory conditions. For these and other reasons, filoviruses are classified as category A biological weapons. However, there is very little data from animal studies with aerosolized filoviruses. Animal models of filovirus exposure are not well characterized, and there are discrepancies between these models and what has been observed in human outbreaks. Building on published results from aerosol studies, as well as a review of the history, epidemiology, and disease course of naturally occurring outbreaks, we offer an aerobiologist's perspective on the threat posed by aerosolized filoviruses.

EBOLA AND MARBURG VIRUSES are the sole members of the genus *Filovirus* in the family Filoviridae. Filoviruses are negative-stranded RNA viruses with a lipid envelope that is stable at a neutral pH, as a result of which the virus can survive for long periods in blood, and viral isolation is possible weeks after exposure, even during convalescence. There has been considerable media attention and fear generated by outbreaks of filoviruses, particularly of the Ebola virus strains. In humans filoviruses can cause a severe viral hemorrhagic fever (VHF) syndrome that has a rapid onset and high mortality (23–90%, depending on the virus).

Although transmission during naturally occurring outbreaks is believed to occur from close personal contact with blood or other body fluids, or the failure to practice proper medical hygiene as relates to blood-borne pathogens, in the past 10 years several publications have indicated that filoviruses possess a number of properties that would make them suitable as biological weapons. Studies have shown that filoviruses are relatively stable in aerosols, retain virulence after lyophilization, and can persist for long periods on contaminated surfaces.[1,2] There are allegations that the former Soviet Union weaponized hemorrhagic fever viruses, including the Marburg and Ebola viruses.[3] For these reasons, as well as the high mortality rates, media attention, and fear associated with Ebola virus, filoviruses are classified as category A biological agents by the Centers for Disease Control and Prevention.[4] Category A biological agents are considered to be of greatest concern because of their high mortality rate; low infective dose; ease of dissemination; potential for major public health impact, public panic, or social disruption; and requirement for major public health preparedness measures.[5] This article discusses the history, epidemiology, and disease course of naturally occurring filovirus outbreaks and presents information that is known from animal studies of the potential threat of aerosol exposure to filoviruses.

NATURAL OUTBREAKS OF FILOVIRUSES

The first filovirus to be identified was Marburg virus, made in 1967, after a severe outbreak of VHF that began

Elizabeth K. Leffel, PhD, and Douglas S. Reed, PhD, are with the Center for Aerobiological Sciences, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland.

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14. ABSTRACT
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15. SUBJECT TERMS
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in Marburg, Germany, with subsequent cases appearing in Frankfurt and Belgrade. There were 32 cases and a 23% mortality rate. An analysis of the outbreak concluded that the majority of cases were primary infections as a result of handling tissues from infected African green monkeys, and only nine could be considered secondary cases. Secondary cases were attributed to inadvertent needle sticks and unprotected contact.[6] In one case Marburg virus was transmitted via semen 3 months after the patient had recovered from the disease.[7] Subsequently, sporadic cases occurred from 1975 to 1987, with a total of six cases and three deaths.[8] From 1998 to 2000, a series of cases occurred near Durba in the Democratic Republic of the Congo.[9] All of the cases have been associated with miners working in gold mines near Durba, with 103 cases and a fatality rate of 67%.

There are four known subtypes of the Ebola virus. Near-simultaneous outbreaks of Ebola Zaire and Sudan occurred in 1976, and these two appear to be the most virulent, with the mortality rate approaching 90% for the Zaire strain and 50–60% for the Sudan strain.[4,8] Since the initial outbreak, there have been two additional subtypes identified, Reston and Ivory Coast. Reston and Ivory Coast are virulent for nonhuman primates,[10,11] but the few reported cases in humans have not resulted in any fatalities.[12,13] The Ivory Coast strain, however, does appear to be highly pathogenic in humans.

The natural reservoir of filoviruses remains unknown. It is unlikely to be nonhuman primates, because they are especially sensitive to filovirus infection as evidenced by both experimental studies and outbreaks among gorillas and chimpanzees in Africa.[14,15] Some recent outbreaks have been attributed to the consumption or handling of "bush meat."[16] Surveys of wild populations as well as experimental inoculations of animals, arthropods, and even plants have failed to identify a potential reservoir.[17,18] Failure to identify the natural reservoir has prevented any effort at controlling outbreaks of filovirus VHF.

DISEASE COURSE AND DISEASE SIGNS

Ebola and Marburg viruses are communicable primarily through direct contact with infected blood and/or tissues.[4] There is some evidence of infectivity via the respiratory, oral, and conjunctival routes.[4,19] For Ebola viruses, most of the documented cases have been either secondary and/or nosocomial infections.[4,20] Institution of basic isolation procedures is generally sufficient to stop outbreaks. During the 2000 Ebola virus outbreak in Uganda, however, 14 health-care workers were exposed after the institution of isolation procedures.[21] While the possibility of aerosol exposure cannot be ruled out in some cases, it is clear that direct contact is the primary means of transmission.[22] Although a lot of epidemiological evidence of human transmission of disease is not available, what is known suggests that transmission of Ebola virus does not occur before the appearance of symptoms.[4] Experiments in nonhuman primates support this assumption.[6]

Patient complaints at the onset of disease after filovirus infection include sudden onset of fever, headache, myalgia, vomiting, and nonbloody diarrhea.[20,23,24] Full-blown hemorrhagic disease progresses to shock, generalized bleeding, and subsequently death. Pathologic examination of tissues of patients who have succumbed to the disease indicates extensive involvement of neurologic, hematopoietic, and pulmonary tissues.

A number of studies have highlighted the importance of macrophages, monocytes, and dendritic cells as important targets of filovirus infection.[25,26] After the virus infects cells of the mononuclear phagocyte system, the infection is carried in the lymph filtrate to the lymph nodes, spleen, and liver. Although filoviruses do not productively infect lymphocytes, lymphocyte depletion is prominent in both lymph nodes and peripheral blood, and little if any cellular or humoral immune response is detectable.[27,28] Replication occurs in macrophages and dendritic cells, and the virus particles likely enter the vascular system as the macrophages extravasate the endothelium. Resultant release of cytokines, particularly TNF-alpha, may contribute to the endothelial cell damage. Dysregulation of the coagulation pathway results in thrombus formation and triggers the development of disseminated intravascular coagulation (DIC). Liver enzymes become elevated in the latter stages of the disease as viral titers peak. In primates, the animals develop hemorrhagic shock due to the destruction of the endothelium and development of DIC, followed by multiple organ failure and finally death.[26] Similar findings have been reported for human cases.[29]

AEROSOL STUDIES WITH FILOVIRUSES

After the original outbreak of Marburg virus in 1967, there was concern about transmission of Marburg virus, particularly the possibility of aerosol transmission, even though there were few secondary cases. Epidemiological analysis of the outbreak suggested aerosol transmission between shipments of primates had occurred.[30] Haas and colleagues[31] were unable to demonstrate transmission of Marburg virus from infected rhesus macaques to uninfected macaques. However, Jaax and colleagues[32] reported transmission of Ebola virus to uninfected macaques housed in the same room as experimentally infected macaques. The latter study, however, did not exclude the possibility that exposure had occurred from ex-

creted virus that was aerosolized during routine cleaning of the cages rather than "true" primate-to-primate transmission.

Reston, Virginia

In the late 1980s, an outbreak of Ebola virus occurred in a nonhuman primate holding facility in Reston, Virginia.[33] This outbreak was alarming because initial tests identified the virus as the Zaire subtype, and it appeared to be jumping from animal to animal and room to room in a manner that suggested aerosol transmission. Animal handlers in the facility seroconverted, indicating they had been exposed. Eventually it was determined that this outbreak was not due to the Zaire subtype but instead to a previously unidentified subtype now dubbed Reston. Reston appears to have originated in the Philippines, unlike the other identified subtypes of filoviruses that all originated in Africa. While there was the suggestion that primates were infected with Ebola Reston by aerosol exposure, there is no evidence to indicate that primate-to-primate transmission by aerosol actually occurred. Miranda and colleagues[34] examined an outbreak of Ebola Reston in the Philippines and concluded that the transmission of the virus between cages and buildings in that outbreak was due to poor sanitation and hygiene.

Marburg virus

Only the guinea pig has been successfully adapted as a rodent model of the human disease caused by Marburg virus infection. The time course and pathogenesis in guinea pigs for parenteral exposure to filoviruses are similar to what has been reported for nonhuman primates and humans. A study published in 1995 described the result when guinea pigs were exposed to the Popp strain of Marburg virus by aerosol.[35] Homogenates of guinea pig liver containing 3×10^7 LD_{50} of the Popp strain of Marburg virus were aerosolized with 10% glycerol in a biological aerosol generator. The dose achieved was reported as being in the range of 2–6 aerosol LD_{50}. At this dose, death occurred between 9 and 11 days, and mortality was 100% in the guinea pigs. Some limited disease course and pathogenesis data are included in this study, indicating that guinea pigs exposed to Marburg virus by aerosol also developed coagulation defects, lymphopenia, fever, and other clinical signs that are similar to those that have been reported for humans.

In a subsequent report, Ryabchikova and colleagues[36] provided more information on the pathogenesis of Marburg virus after aerosol exposure in guinea pigs. Macrophages isolated from bronchoalveolar lavage were the first cells to show evidence of viral antigen, approximately 48 hours after aerosol exposure. From there the virus spread into the blood, liver, and peritracheal lymph nodes, findings similar to what had been reported for nonhuman primates. By both viral isolation and histological findings, the most affected organs were the lungs, liver, and spleen. Although the authors did note some defects in blood coagulation and clotting times, aerosol-exposed guinea pigs did not seem to develop the same level of damage to the epithelium and fibrin deposition that are hallmarks of the infection in humans and nonhuman primates.

Although the data from these studies provide valuable information on the disease course and pathogenesis of Marburg virus after aerosol exposure in guinea pigs, it is lacking in some key areas. First, virus counts were reported in terms of guinea pig LD_{50}s, whereas in western countries it is more common to report doses in terms of viral plaque-forming units (pfu). It was unclear how the LD_{50} for guinea pigs relates to pfu counts, as there may be more than one infectious viral particle per pfu of both Ebola and Marburg viruses. In addition, data is reported for only one strain of Marburg virus, the Popp strain, which was isolated in the original outbreak in 1967. At least two other genetically distinct strains, Musoke and Ravn, also have been isolated and studied.[23,24] Finally, the number of animals used in these studies is relatively small.

Bazhutin and colleagues[1] provided the first description of the results of experimental aerosol exposure of nonhuman primates to Marburg virus. African green monkeys were exposed to the Popp strain of Marburg virus using a freeze-dried preparation of the virus aerosolized by a pneumatic sprayer. Six of the 10 monkeys died, with a range in time to death from 13 to 22 days. This paper provides hints of the former Soviet offensive biological warfare program—in particular, the fact that a lyophilized preparation of the virus was aerosolized. There is also some discussion on the preparation of the virus and the fact that the freeze-drying process reduced virulence by nearly 3 logs. The time to death appears extended compared to parenteral exposure of cynomolgus macaques to 1,000 pfu of Marburg virus; however, the doses mentioned in this particular report were extremely low (0.1 to 0.003 guinea pig LD_{50}). Despite the extended time to death, the animals that succumbed developed the coagulation defects and elevated levels of liver enzymes that are hallmarks of VHF. The authors could not determine, however, whether the extended survival time and less than 100% mortality was due to the dose, the preparation of the virus, or the route of exposure.

In 1995, Lub and colleagues[37] reported results from studies with rhesus macaques exposed to Marburg virus by aerosol. After first being found in lungs on day 3, the virus quickly spread to the liver and peritracheal lymph nodes on day 4 and then beyond. Fever was not seen until day 6 or 7, when it rapidly increased to 40.5°C. Be-

tween days 6 and 7, the authors noted an increase in blood coagulation time and a decrease in thrombocytes. Animals that were not euthanized died on day 10 or 11 postexposure.

Ebola virus

In 1995, Johnson and colleagues[38] reported lethal experimental infection of rhesus monkeys by aerosol exposure to Ebola Zaire. Two monkeys were exposed to a dose of ~400 pfu and another two to a dose of ~50,000 pfu. All four animals died or were euthanized after becoming moribund between days 7 and 9 postexposure. This is within the same time frame that rhesus monkeys die from parenteral exposure to Ebola Zaire.[39] On necropsy, all four animals were found to have a mild, moderate pneumonia with ample viral antigen found by immunohistochemistry in the bronchial epithelium and alveolar macrophages. There was also abundant evidence of infection and necrosis in the lymph nodes that drain the lungs. What is not clear from these studies is how these findings might differ from necropsies of monkeys that succumbed to Ebola Zaire by parenteral exposure. In a more recent report Geisbert and colleagues[26] found little evidence of pneumonia or viral antigen in the lungs of cynomolgus monkeys infected by subcutaneous injection of Ebola Zaire. Findings in terminal samples from rhesus macaques were more varied, from little if any evidence of necrosis to widespread damage in the lungs.[39,40] Findings similar to those reported for cynomolgus macaques in other species of nonhuman primates indicate that the lack of viral antigen in the lungs after parenteral exposure is not a unique finding in cynomolgus macaques.[41]

DISCUSSION

This perspective presents what is known about filoviruses as it pertains to their potential use as a biological weapon dispersed by aerosol. The high mortality rates, coupled with the knowledge that these viruses possess properties considered desirable in biological weapons, explains the considerable concern about their potential use. However, this concern must be couched with an understanding of the paucity of data concerning that potential. Without data there can be little understanding of the level of threat that filoviruses present. For example, it is not clear from the available data whether filoviruses would cause large-scale infections and deaths if disseminated by aerosol over a city without extensive preparation or modification ("weaponization").

It is clear in the animal models studied that filoviruses can infect by the aerosol route and that extraordinarily low doses are lethal for both guinea pigs and nonhuman primates. The epidemiological data from natural outbreaks would suggest, however, that the aerosol infectious dose for humans is considerably higher, that the survivability of filoviruses as a respirable particle is very short outside of controlled laboratory conditions, or that infected patients do not expire infectious virus particles. Better-developed animal models and studies of the aerobiological properties of filoviruses need to be conducted to better understand these apparent differences, which are critical to evaluating the threat posed by filoviruses.

More work needs to be done to develop both the guinea pig and nonhuman primate models to determine whether there are differences in the disease course and pathogenesis after aerosol exposure as compared to parenteral exposure. A comparison of the disease after aerosol exposure in multiple species of nonhuman primates would be advisable considering the differences in disease course, pathogenesis, and time to death that have been observed after parenteral exposure.[41,42] Our own studies currently in progress have suggested differences in the virulence of aerosolized Marburg virus that are dependent on the strain of Marburg virus and strain of guinea pig employed. Vaccines that protect against injection of filoviruses must be reexamined for efficacy against aerosol exposure. In our view, additional work is needed, particularly in the development of animal models, before the nature of the biological weapon threat posed by filoviruses can be truly understood and addressed.

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Address reprint requests to:
Douglas S. Reed, PhD
Center for Aerobiological Sciences
U.S. Army Medical Research Institute
of Infectious Diseases
1425 Porter Street
Fort Detrick
Frederick, MD 21702-5011

E-mail: doug.reed@det.amedd.army.mil