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Characteristics of Filoviridae: Marburg and Ebola Viruses

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Filoviruses are enveloped, nonsegmented negative-stranded RNA viruses. The two species, Marburg and Ebola virus, are serologically, biochemically, and genetically distinct. Marburg virus was first isolated during an outbreak in Europe in 1967, and Ebola virus emerged in 1976 as the causative agent of two simultaneous outbreaks in southern Sudan and northern Zaire. Although the main route of infection is known to be person-to-person transmission by intimate contact, the natural reservoir for filoviruses still remains a mystery.

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Introduction and History

In 1967 outbreaks of hemorrhagic fever occurred simultaneously in Germany (Marburg and Frankfurt), and Yugoslavia (Belgrade) among laboratory workers having contact with tissues and blood from African green monkeys (*Cercopithecus aethiops*) imported from Uganda. A total of 31 cases in humans with seven fatalities occurred [74, 77]. Subsequently a virus was isolated from blood and tissues of the patients by inoculation of guinea pigs and cell cultures [46, 50, 74, 75] and the virus was named Marburg virus after the city in which it was first characterized (Table 1). The virus also appeared to be highly pathogenic for monkeys, killing all African green monkeys experimentally infected with the virus [35].

After this dramatic episode the virus disappeared from sight until 1975, when three cases of Marburg hemorrhagic fever were reported in Johannesburg, South Africa [18, 33]. The index case patient died 12 days after onset of the disease, while two patients infected secondarily survived. The next Marburg virus outbreak occurred in 1980 when one index patient became ill and finally died in Kenya and an attending physician became infected but survived. In 1987 a single fatal Marburg case was reported in western Kenya (Table 1).

From the first outbreaks several strains of Marburg viruses were isolated; the genomes of two strains have subsequently been sequenced. The Popp strain was obtained in 1967 during the first filovirus outbreak from the blood of infected guinea pigs [17], and the Musoke strain was isolated in 1980 in Kenya and subsequently purified from an infected Vero cell culture [45, 76].

Two cases of laboratory infection with the Popp strain of Marburg virus occurred in Russia in 1988 and 1990 (Table 1). The first took place as the result

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Table 1. Outbreaks of filoviral hemorrhage fevers

Location	Year	Virus/subtype	Human cases (mortality)	Epidemiology
Germany/Yugoslavia	1967	Marburg	32 (23%)	Imported monkeys from Uganda
Zimbabwe	1975	Marburg	3 (33%)	Unknown origin; index case infected in Zimbabwe; secondary cases were infected in South Africa
Southern Sudan	1976	Ebola/Sudan	284 (53%)	Nosocomial transmission and infection of medical staff
Northern Zaire	1976	Ebola/Zaire	318 (88%)	Spread by close contact and by use of contaminated needles and syringes in hospitals
Tandala, Zaire	1977	Ebola/Zaire	1 (100%)	Unknown origin; single case in missionary hospital
Southern Sudan	1979	Ebola/Sudan	34 (65%)	Unknown origin; recurrent outbreak at the same site as the 1976 outbreak
Kenya	1980	Marburg	2 (50%)	Unknown origin; index case infected in western Kenya died, but physician secondarily infected survived
Kenya	1987	Marburg	1 (100%)	Unknown origin; expatriate travelling in western Kenya
Russia	1988	Marburg	1 (100%)	Laboratory infection
Virginia, USA	1989/90	Ebola/Reston	4 (0%)	Introduction of virus with imported monkeys from the Philippines
Russia	1990	Marburg	1 (0%)	Laboratory infection
Italy	1992	Ebola/Reston	0 (0%)	Introduction of virus with imported monkeys from the Philippines
Ivory Coast	1994	Ebola/Ivory Coast	1 (0%)	Contact with chimpanzees; single case
Minouka, Gabon	1994	Ebola/Zaire	44 (57%)	Unexplained deaths in two goldmining camps
Kikwit, Zaire	1995	Ebola/Zaire	315 (77%)	Unknown origin
Texas, USA	1996	Ebola/Reston	0 (0%)	Introduction of virus with imported monkeys from the Philippines
Mayibout and Booué, Gabon	1996	Ebola/Zaire	104 (64%)	2 epidemics (February and July to December)

Modified after Feldmann et al., Archives of Virology, 1996 and Volchkov et al., Virology, 1997

of accident with a contaminated needle, and the researcher died within several days (unpublished data). The second person infected by the serum of a laboratory animal survived after intensive therapy [58].

In 1976 more than 550 cases of severe hemorrhagic fever with more than 430 fatalities occurred simultaneously in Zaire and Sudan [6, 44]. Subsequently Ebola virus (named after a small river in northwestern Zaire, today the Democratic Republic of Congo) was isolated from patients in both countries and was shown to be morphologically similar to but serologically distinct from Marburg virus [44, 59, 85, 86]. In 1979 Ebola hemorrhagic fever occurred again in the Sudan with 34 cases and 22 fatalities (Table 1) [1].

In 1989 filoviruses were isolated from cynomolgus monkeys *(Macaca fascicularis*) imported into the United States from the Philippines via Amsterdam and New York [12, 13, 41, 52]. During quarantine in a primate facility in Virginia, numerous macaques died, some with symptoms consistent with simian hemorrhagic fever. Subsequent investigations led to a single source in the Philippines that was thought to have furnished all identified infected shipments, including monkeys sent to facilities in Texas and Pennsylvania (Table 1) [36].

This filovirus isolated from the monkeys in Reston, Virginia, was clearly a strain of Ebola virus and was undoubtedly responsible for the deaths, being found in tissues of naturally infected monkeys in high concentrations and because monkeys inoculated with this virus alone died with typical filovirus disease [28]. The use of monoclonal antibodies and genetic sequence analyses suggested differences between the Reston isolate and the Ebola viruses isolated in 1976 from Zaire or Sudan. In addition, Reston virus appeared to be less pathogenic for nonhuman primates and humans.

In 1995 the reemergence of Ebola, subtype Zaire, in Kikwit, Zaire, and in Gabon caused a worldwide sensation, striking as it did after the world had become sensitized to the danger of the disease (Table 1) [15, 16, 34]. Ecological investigations have been conducted after most filovirus outbreaks beginning with the initial Marburg episode in 1967, but the source of filoviruses in nature still remains a mystery.

Classification

Filoviruses are classified in the order Mononegavirales [64] which also contains the nonsegmented negative-strand RNA virus families *Paramyxoviridae*, *Rhabdoviridae,* and *Bornaviridae.* Members of the family Filoviridae include Marburg virus, a unique agent without known subtypes, and Ebola virus, which has four subtypes (Zaire, Sudan, Reston, and Ivory Coast) [26, 48, 64]. In general, filoviruses share their genomic organization with other nonsegmented negative strand viruses: genes encoding the major core proteins (N, P, and their analogs) are located in the 3' terminal part of the of the genome; the gene for the large subunit of polymerase (L) is located in the 5' part, and the rest of the genes, most of them encoding envelope proteins are located in the middle, which is the most variable part of the genome between Mononegavirales [24, 64]. No antigenic cross-reactivity has been demonstrated between Marburg and Ebola viruses. In addition, sodium dodecyl sulfate–polyacrylamide gel electrophoresis profiles clearly distinguish Marburg from Ebola-type virus proteins [25, 45].

Because of their aerosol infectivity, high mortality rate, potential for person-to-person transmission, and the lack of commercially available vaccines and chemotherapy, Marburg and Ebola viruses are classified as biosafety level 4 pathogens (World Health Organization risk group 4).

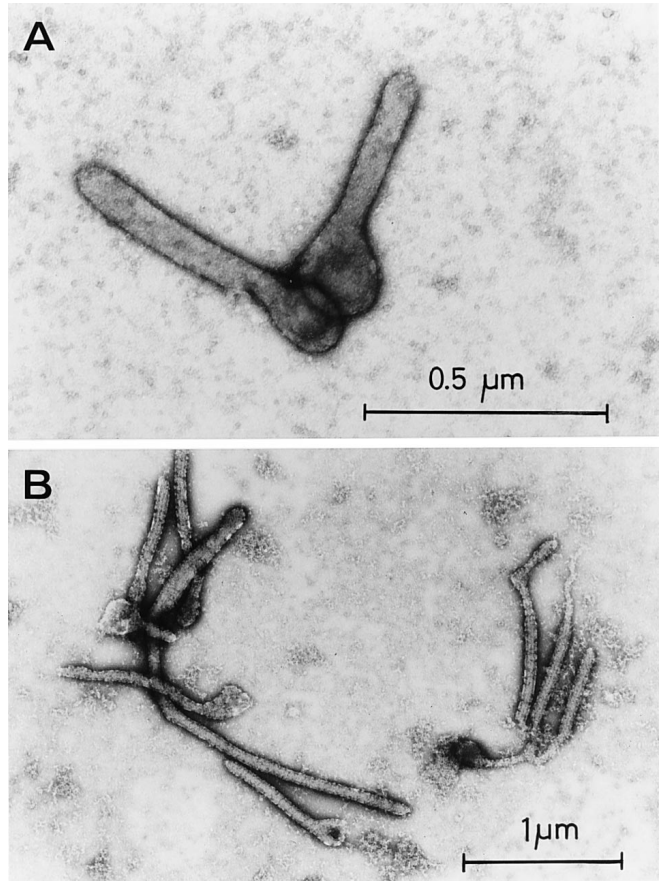

Fig. 1A,B. Electron-microscopic appearance of Marburg virus, stained by negative contrast medium with phosphotungstic acid, pH approx. 7.6. A) ′60,000. B) ′18,000. (Courtesy of H. Gelderblom, Robert-Koch-Institute, Berlin)

Virion Morphology and Structure

Marburg and Ebola viruses are pleomorphic particles which vary greatly in length, but the unit length associated with peak infectivity is 790 nm for Marburg virus (Fig. 1A) and 970 nm for Ebola virus (Fig. 1B) [65]. The virions appear as either long filamentous (and sometimes branched) forms or in shorter U-shaped, 6-shaped (mace-shaped), or circular (ring) configurations (Fig. 2) [57, 62]. Virions have a uniform diameter of 80 nm and a density of 1.14 g/ml. They are composed of a helical nucleocapsid, a closely apposed envelope derived from the host cell plasma membrane, and a surface projection layer composed of trimers of viral glycoprotein (GP) [27]. All filoviruses contain one molecule of noninfectious, linear, negative-sense, single-stranded RNA with a M_r of 4.2×10^6, constituting 1.1% of the virion mass [45, 65].

Marburg and Ebola virus infectivity is stable at room temperature (20° C), but is largely destroyed in 30 min at 60° C [53]. Infectivity is also destroyed

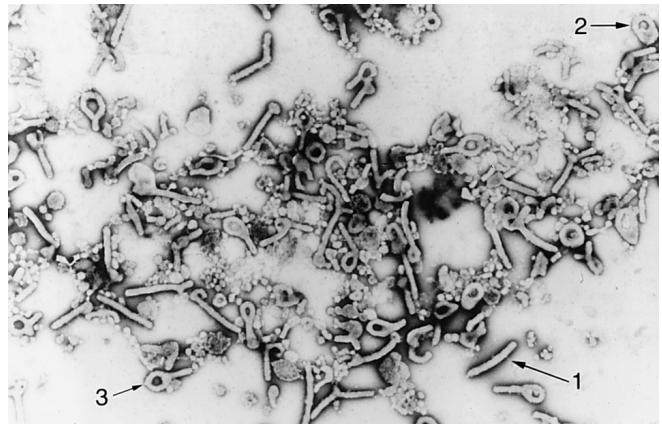

Fig. 2. Marburg virus particles purified from the blood of infected guinea pigs, stained by negative contrast medium. Different forms of the virion are shown: *1*, rod shaped; *2*, ring shaped; *3*, mace shaped. ′10,000. (The virus was purified and concentrated by A.B. et al.; photo by E. Kandrushin, Center for Virology and Biotechnology "Vector," Koltsovo, Russia)

by ultraviolet and γ-irradiation [22], lipid solvents, β-propiolactone, and commercial hypochlorite and phenolic disinfectants.

Genomic Organization, Virion Proteins, and Virus Replication

The nonsegmented negative-strand RNA genomes of filoviruses show the gene arrangement 3′-NP-VP35-VP40-GP-VP30-VP24-L-5'. with a total molecular length of approximately 19 kb (Table 2). They are the largest known genomes for negative-strand RNA viruses (Mononegavirales) [24].

Genes are delineated by conserved transcriptional signals, and the transcription of each gene begins with a start site at the 3' end and terminating with a stop (polyadenylation) site. In addition to common characteristics, there are others that distinguish filovirus genomes from those of rhabdoviruses and paramyxoviruses: (a) transcriptional signals of filoviruses contain a common sequence 3' UAAUU (at the 5' end of start sites and at the 3' end of stop sites) [69], (b) filovirus genes possess the longest 3' and/or 5' end noncoding regions of all negative strand RNA viruses, and (c) the localization of overlapping genes in Ebola and Marburg virus. Gene overlaps were found between VP35 and VP40, GP and VP30, and VP24 and L genes in Ebola virus and VP30 and VP24 in Marburg virus [9, 11, 24, 69]. Overlaps are 18–20 bases in length and are limited to the conserved sequences determined for the transcriptional signals.

The amino acid sequence of each of the seven filovirus polypeptides displays a different degree of identity: VP35 33%, VP40 27%, GP 34%, VP30 33%, VP 24 37% identity, and nucleoprotein (NP) significant identity except for the C-terminal part [8, 9, 11, 69, 71]. A comparison of the complete genomic sequences of the Musoke strain (isolated in 1980) and the Popp strain (isolated in 1967) revealed 94% nucleotide identity [10].

Seven structural proteins are encoded by the genome of which four form the helical nucleocapsid (NP-VP35-VP30-L), two are membrane-associated (VP40-VP24), and one is a transmembrane GP (Table 2).

The GP is the sole structural protein forming the virion surface spikes that mediate virus entry into susceptible host cells through receptor binding [8, 27, 69, 84]. In contrast to Marburg virus, which has a single open reading frame encoding the GP protein, the glycoprotein gene of Ebola virus contains a translational stop codon in the middle, thus preventing synthesis of full-length glycoprotein. The gene product is found in two forms: the transmembrane form, which arises from RNA editing to encode a 120- to 150-kDa glycoprotein that is incorporated into the virion and represents the analog of the Marburg virus glycoprotein, and a secreted form (50–70 kDa), synthesized in large amounts early in infection [72, 81]. Ebola virus GP has been found to contain a furine cleavage site in amino acid positions 497–501; the mature GP consists of two disulfide-linked cleavage products: the amino-terminal 140-kDa fragment, and the carboxy-terminal 26-kDa fragment [83]. Funke et al. [30] demonstrated that the GP of Marburg virus is modified by acylation, and it has been shown that the GP binds to the asial-glycoprotein receptor of hepatocytes [5]. The glycoproteins of filoviruses are highly glycosylated [8, 27, 84]. GP-specific antisera fail to show any cross-reactivity between Marburg isolates and other filoviruses (Table 2) [25].

The VP40 protein is believed to have a matrix protein function based on its large abundance in the vir-

Table 2. Filoviral proteins and their proposed function

Designation	Virus type	Encoding gene	Localization	Proposed function
NP	MBG/EBO	1	Ribonucleocapsid complex	Encapsidation
VP35	MBG/EBO	2	Ribonucleocapsid complex	Phosphoprotein analogue
VP40	MBG/EBO	3	Membrane-association	Matrix protein
GP	MBG/EBO	4	Surface (transmembrane protein)	Receptor binding, fusion
VP30	MBG/EBO	5	Ribonucleocapsid complex	Encapsidation, necessary for transcription and replication
VP24	MBG/EBO	6	Membrane-association	Unknown (minor matrix protein?, uncoating?)
L	MBG/EBO	7	Ribonucleocapsid complex	RNA-dependent RNA polymerase
sGP	EBO	4	Nonstructural, secreted	Unknown

NP nucleoprotein; *VP* virion structural protein; *GP* glycoprotein; *L* Large protein (polymerase), *sGP* small glycoprotein; *MBG* type Marburg filoviruses; *EBO* type Ebola filoviruses
Modified after Feldmann et al., Archives of Virology, 1996

ion, its hydrophobic profile, its removal from purified virion nucleocapsid by nonionic detergents under low salt conditions, and the position of its gene in the genome (Table 2) [11, 21, 69].

The VP24 protein has a highly hydrophobic amino acid composition concentrated within five hydrophobic domains. The function of the VP24 is unclear, although it is possibly another membrane-associated viral protein acting as a minor matrix protein or taking part in the uncoating of the virion during infection. It may also be an analogue of the small hydrophobic protein of paramyxoviruses (Table 2) [9, 69].

The NP is the primary structural protein associated with filovirus nucleocapsids [70, 71]. The NPs of filoviruses are phosphorylated [4, 21] and appear in two forms differing in M_r by about 2 K (94 and 92 K, respectively) [4]. The NPs of filoviruses can be divided into a hydrophobic N-terminal half, which contains all the cysteine residues, and a hydrophilic C-terminal half, which contains most of the proline residues and is extremely acidic. The VP30 is a second nucleoprotein (Table 2) [9, 21, 45, 69].

The RNA-dependent RNA polymerase or L protein of filoviruses is the largest (M_r 267 K) and least abundant viral protein both in the virion and in infected cells [45, 68]. The predicted amino acid sequence of the Marburg virus L protein contains three regions in its N-terminal half and a putative adenosine triphosphate binding motif in the C-terminal region that are conserved in the polymerases of paramyxoviruses and rhabdoviruses (Table 2) [54].

The position of the VP35 gene in the genome corresponds to that of the phosphoprotein genes of rhabdoviruses and paramyxoviruses. It is very likely that this protein has a role in replication similar to that of the P protein of related viruses [11, 69].

The mode of entry of Marburg and Ebola viruses into cells remains unknown, although uncoating is presumed to occur in a manner similar to that of other negative-strand RNA viruses. Virion assembly involves budding from the plasms membranes of preformed nucleocapsids which can also accumulate in the cytoplasm, forming prominent inclusion bodies [57].

Pathology and Course of Infection in Experimental Animals

Monkeys, guinea pigs, suckling mice, and hamsters have been experimentally infected with Marburg and Ebola viruses [2, 7, 46, 51, 56, 66, 67]. Marburg virus and the Zaire subtype of Ebola virus are highly virulent, with infection usually ending in death. The Sudan subtype of Ebola virus differs in that it often causes a self-limiting infection. The Reston subtype of Ebola is even less pathogenic for monkeys and guinea-pigs than other Ebola subtypes [6, 7, 28, 51].

In rhesus and African green monkeys inoculated with Marburg virus or the Zaire subtype of Ebola virus the incubation period is usually 4–16 days, during which time the virus replicates to a high titer in liver, spleen, lymph nodes, and lungs. With the onset of clinical disease severe necrosis of these target organs, which is most evident in the liver, and interstitial hemorrhage, which is most evident in the gastrointestinal tract, occur [29, 40, 56]. In the liver and the other target tissues, necrotic lesions are caused directly by virus infection of the parenchymal cells with typically very little inflammation in the lesion sites. The pathophysiological basis for the hemorrhagic shock syndrome is not known, but prostaglandin-mediated dysfunction of endothelial cells and platelets during infection has been suggested as a potential mechanism [29]. Another possible mechanism for the development of hemorrhagic fever is the combination of viral replication in endothelial cells [73] and the enhancing effect of virus-induced cytokine release on permeability [23].

Pathology and Course of Infection in Humans

Of all the viral hemorrhagic fevers, filovirus infections have the highest case fatality rates (30–90%), and their hepatic involvement and hemorrhagic manifestations are usually striking. Gross pathological changes in fatal Marburg and Ebola hemorrhagic fever cases include hemorrhagic diatheses into skin, mucous membranes, visceral organs, and the lumen of the stomach and intestines. Swelling of spleen, lymph nodes, kidneys, and especially brain can be observed. Microscopic changes include focal necroses of liver, lymphatic organs, kidneys, testes, and ovaries [19, 50, 55]. Hepatocytes often contain large eosinophilic intracytoplasmatic inclusion bodies, which are coincident with massive accumulation of viral nucleocapsids visible by electron microscopy.

The origins of the pathophysiological changes that make Marburg and Ebola virus infections of humans so devastating are still not understood. Necrosis of

parenchymal cells of organs such as liver is correlated with the presence of large numbers of virions or virus antigens in the same cells in target tissues. Endothelial cells have also been shown to be targets for viral replication, and at times in situ fibrin deposition suggestive of disseminated intravascular coagulation has been noted. During crucial early stages of infection, damage of liver cells is not the only basis for clinical manifestations. The high AST-ALT ratio and normal bilirubin level suggest an involvement of extrahepatic targets of infection [29, 33].
Extensive visceral effusions, pulmonary interstitial edema, and renal dysfunction follow the increased endothelial permeability and are important components of the shock syndrome seen in filovirus infections. Filoviruses can infect cultured endothelial cells as well as macrophages with the resulting cytopathic effect.
In addition to severe thrombocytopenia, remaining platelets are unable to aggregate normally and there is additionally an early, profound lymphopenia followed by a dramatic neutrophilia with a left shift.

Clinical Features and Patient Management

After an incubation period of usually 4–10 days there is an abrupt appearance of illness with initially nonspecific symptoms, including fever, severe headache, malaise, myalgia, bradycardia, and conjunctivitis [32]. Deterioration over the following 2–3 days is heralded by pharyngitis, severe nausea, and vomiting, progressing to hematemeses, melena, prostration, and obtundation. Bleeding is manifested as petechiae, eccymoses, uncontrolled bleeding from venepuncture sites, and postmortem evidence of visceral hemorrhagic effusions. There is often a macropapular rash appearing around day 5 which is a valuable differential diagnostic feature [32]. Death in shock usually occurs 6–9 days after onset of clinical disease. Abortion is a common consequence of infection, and infants born to mothers dying of infection are fatally infected. Clinical laboratory findings include an early lymphopenia, subsequent neutrophilia, and marked thrombocytopenia accompanied by abnormal platelet aggregation. Hepatic serum enzyme levels are elevated.
No specific drug for treatment of Marburg and Ebola hemorrhagic fever is as yet available. Ribavirin, an antiviral drug used to treat several other hemorrhagic fevers, had no in vitro effect on Marburg and Ebola viruses and is unlikely to be of any clinical value. Human convalescent plasma containing virus binding antibodies has been used in the past, but efficacy in improving the clinical status has not been demonstrated. The most important therapy is intensive supportive care which include prevention of shock, cerebral edema, renal failure, platelet and clotting factor depletion, bacterial superinfection, hypoxia, and hypotension. Care is complicated by the need for isolation and protection of medical and nursing personnel.

Immunology and Protection

The mechanism of recovery from filovirus infection has not been established in either humans or experimental animals. Development of a vigorous cell-mediated immunity appears to be the most likely mechanism of disease recovery, although no definitive proof has been presented. In fatal filovirus infections the host dies with high viremia, there is generally no evidence of an immune response, and the reason for the failure to respond is unknown. The filovirus major glycoprotein is highly glycosylated [8, 25, 27, 84], and this may modulate its interaction with the immune system. Furthermore, filovirus GPs contain a 26 amino acid motif similar to that found in retroviral p15E, a sequence known to have immunosuppressive effects in several experimental systems [8, 82, 84]. The secreted glycoprotein of Ebola virus has recently been found to interact with neutrophils through CD16b, the neutrophil-specific form of the Fcg receptor III, whereas the transmembrane glycoprotein was found to interact with endothelial cells but not neutrophils [88].
Attempts to develop inactivated vaccines by formalin or heat treatment of cell culture grown virus have been at best marginally successful [38, 39, 49], and even then careful balance of the challenge virus dose and virulence is needed to achieve protection. Recently, however, protection against Ebola virus infection could be achieved by immunizing guinea pigs with plasmids encoding the viral nucleoprotein and the secreted or transmembrane forms of glycoprotein [79, 87]. Protection against a lethal dose of Marburg virus has been achieved by a similar approach using immunization with the GP cDNA [79].

Diagnosis

Filovirus disease should be included in the differential diagnosis of acute, febrile illness in anyone who has recently traveled in rural sub-Saharan Africa,

particularly when hemorrhagic manifestations are present. A specific characteristic is the macropapular rash which is not seen in other viral hemorrhagic fevers, with the exception of Dengue fever and sometimes Lassa fever. Infectious diseases which must be considered for differential diagnosis are malaria, typhoid fever, shigellosis, other bacterial diseases, such as meningococcal septicemia, plague, leptospirosis, anthrax, and relapsing fever, rickettsial diseases, and viral diseases such as yellow fever, Chikungunya fever, Rift Valley fever, hemorrhagic fever with renal syndrome, Crimean Congo hemorrhagic fever, Lassa fever, and fulminant viral hepatitis [31].

Acute sera or postmortem tissue specimen from patients as well as materials collected during ecological investigations may contain filoviruses and should be handled using the maximum feasible precautions such as respirators, gloves, gowns, and BSL-4 laboratory safeguards until subjected to a process that inactivates virus. Inactivation is successfully achieved using γ-irradiation, formalin fixation, or RNA extraction. Acetone fixation and heating, however, may leave small amounts of infectious virus.

Reverse transcriptase polymerase chain reaction is one of the most powerful tools of diagnosis of filovirus infection [80]. Antibodies to filovirus can be detected by immunofluorescence assays using acetone-fixed virus-infected cells inactivated by γ-radiation [42, 43]. Although this test is usable under field conditions, it has resulted in many false positive diagnoses. An enzyme-linked immunosorbent assay using a mild detergent extract of infected Vero cells adsorbed to plastic plates has been shown to be more reliable [47]. In addition, the western blot method has been standardized and evaluated for the diagnosis of filovirus infections [20].

Vero cells are most commonly used for the isolation and propagation of fresh and laboratory passaged strains of the viruses. MA-104 cells and SW13 cells have also been successful in primary filovirus isolation [51]. In some circumstances primary isolation in guinea pigs (for Marburg virus) or suckling mice (for Ebola virus) may be required.

Epidemiology

The origin in nature and the natural history of Marburg and Ebola viruses remain a total mystery. It seems that the viruses are zoonotic, transmitted to humans from ongoing life cycles in animals. However, all attempts to backtrack from human index cases in Africa or from monkey epidemics in Africa and the Philippines have failed to uncover the reservoir. There are speculations about a potential reservoir in rodents or bats. Viral replication in arthropods, however, has been excluded [78].

Whatever the source, person-to-person transmission by intimate contact is the main route of infection in human filoviral hemorrhagic fever outbreaks [1]. Although aerosol transmission has not been implicated in human outbreaks to date, it cannot be discounted. Marburg virus, at least, is transmissible to nonhuman primates in the laboratory by aerosols [3, 63]. Furthermore, the outbreak of disease caused by the Reston subtype of Ebola virus among quarantined monkeys in 1989 and 1990 was transmitted by droplets and perhaps small-particle aerosols [60, 61]. Finally, nosocomial transmission via contaminated syringes and needles was a major problem in the Ebola outbreak of 1976 and to some extent even that in 1995.

Conclusion

Wild monkeys are an important source for the introduction of filoviruses, as clearly demonstrated in 1967 for Marburg [50], in 1989–1990 and 1992 for Ebola Reston [41], and in 1994 for Ebola Ivory Coast [48]. Quarantine of imported nonhuman primates and professional handling of animals are essential to prevent introduction of these agents into humans (for guidelines see [14]).

Although the transmission of filoviruses occurred through monkeys, they are not considered to be the natural reservoir of the virus. Rather, viruses are thought to be transmitted to humans from other animals whose identities remain unknown. Despite the failure to detect filoviruses in the natural environment the possibility still exists that an aerosol transmission cycle in bats or other mammals plays some role in the natural history of filoviruses.

Feldmann et al. [26] discuss several factors allowing the emergence/reemergence of hemorrhagic fever caused by filoviruses: (a) international commerce and jet travel; (b) interhuman spread during normal social transactions or sexual intercourse; (c) limited knowledge concerning the genetics and pathogenesis of the agents; (d) limited experience in diagnosis and case management; (e) the import of nonhuman primates; (f) an unknown reservoir; and finally (g) the potential of evolution due to the high error rate of the virus-encoded polymerase and the lack of repair mechanisms [37]. The chronology of epidemics in human and nonhuman primates shows that filovi-

ruses are classical prototypes of emerging and/or reemerging pathogens which was made all too clear by the recent outbreaks of Ebola subtype Zaire in Kikwit and Gabon.

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