Oxford Medicine

Marburg and Ebola viruses

Summary

Marburg and Ebola viruses cause severe and often fatal haemorrhagic disease in humans and non-human primates. They are the two established members of the family Filoviridae and have a distinctive filamentous and irregular morphology with a genome consisting of a very large (about 19 kb) single-stranded RNA of negative polarity. Features of their organization and structure at the molecular level have led to their inclusion in the taxonomic order Mononegavirales, together with the Paramyxoviruses and the Rhabdoviruses.

From its original description in 1967 to 2009, there have been eight reported outbreaks of human Marburg virus infection. The first being three simultaneous outbreaks that occurred in Europe at Marburg, Frankfurt, and Belgrade, following the importation of infected African green monkeys (*Ceropithecus aethiops*) from Uganda. The remaining outbreaks occurred in South Africa 1975; Kenya 1980 and 1987; Democratic Republic of Congo (DRC) (1988–2000); Angola (2004–5) and Uganda (2007 and 2008). These eight episodes involved 449 cases with 369 deaths, an overall fatality rate of 82.3%. All the deaths to date have occurred in the primary cases.

Between 1976 and 2009 outbreaks of human Ebola haemorrhagic fever have been identified in Zaire (1976, 1977, 1995, 2001–2, 2003 and 2007); Sudan (1976, 1979 and 2004); Uganda (2000–1 and 2007–8); Kenya (1980); Côte d'Ivoire (1994 and 1995); and the Gabon (1996 and 1997). All age groups and sexes were affected. In addition, a laboratory-derived infection occurred during the studies of the 1976 Zaire and Sudan epidemic. There is no known endemic incidence of the disease and the mortality rates are based on the limited numbers of epidemics identified. This has involved 2,292-recorded cases with 1,524 deaths, an overall case fatality rate of 66.5%.

A new strain of Ebola virus named Reston was isolated from an epizootic of dying cynomolgus monkeys shipped to the USA (1989, 1990) and Italy (1992) from the Philippines. The virus proved antigenically and genetically distinct from the African Ebola viruses. Human infections documented during the USA epizootic proved asymptomatic. There was no evidence of an epidemiological link with Africa. Therefore, unlike its African counterparts Reston Ebola Virus has proven to date to be non-pathogenic in humans. The high mortality among monkeys makes them an unlikely natural reservoirs. However, six out of 141 slaughterhouse workers studied in the Philippines, who had daily contact with Ebola seropositive pigs, had antibodies against Reston ebolavirus. This marks the first time that Reston ebolavirus has been found in pigs

and the possibility of Ebola transmission to humans from pigs to humans, has occurred (Barrette 2009; World Health Organization (WHO) 2009).

In Africa, the transmission of haemorrhagic fever caused by Ebola and Marburg has been associated with the reuse of unsterile needles and syringes, with the provision of patient care without appropriate barrier precautions preventing exposure to virus-containing blood and other body fluids and preparing bodies for funerals and burial. In addition, the killing and preparing of non-human primates for food was considered the source of SOMP outbreaks. Epidemiological studies in humans indicate that the airborne route does not readily transmit infection from person to person. By contrast, studies of Ebola and Marburg virus infections in non-human primates have suggested possible airborne spread among these species. The risk of person-to-person transmission is highest during the later stages of illness. Studies of individuals who are in contact with an infected patient during the incubation period indicate that infection risk is low.

As the natural history and reservoir of the Filoviruses are unknown, there are no specific precautions for avoiding infection from the natural environment. Non-human primates are not considered the natural reservoir of Filoviruses despite being the source of Marburg virus introduced into Europe and Ebola viruses introduced into the USA and Italy. The precautionary quarantining of non-human primates imported from Africa and Asia for a minimum of six weeks reduces the possibility of introducing a Filovirus infection. Recently, extensive studies undertaken to determine the reservoir of Filoviruses have identified in common species of fruit bat (*Rousettus aegyptiacus*) as a potential candidate (Swanepoel 1996; Towner 2007; Pourrut 2009).

Since there are no licensed vaccines or specific antiviral drugs for the treatment of Filovirus infections, early identification of infected patients or animals is essential. Prevention strategies reducing the risk of transmission in endemic and non-endemic areas rely on the introduction of strict isolation of febrile patients and rigorous use of barrier precautions. Consequently, many institutions and experts consider Filoviruses potential biological weapons (Borro 2002). Filovirus public health and biodefence research require the use of maximum-containment laboratory facilities where Filoviruses are handled under biosafety-level (BSL)-4 containment to protect laboratory workers from infection. There are only a few such facilities that exist worldwide.

History

Filovirus infections were unknown until 1967, when 31 human cases of an acute haemorrhagic fever occurred simultaneously in Marburg and Frankfurt, Federal Republic of Germany, and Belgrade, former Yugoslavia (Martini 1969). Laboratory workers, medical personnel, animal care personnel and their relatives were infected, seven of whom died. The primary cases occurred through contact with kidney tissue, blood, and cell cultures derived from Vervet or African green monkeys (*Ceropithecus aethiops*) imported from Uganda. The virus isolated from patient's blood and tissue was morphologically unique when observed by electron microscopy (see Fig. **31.1a**) and antigenically unrelated to any known mammalian pathogen. This viral agent was named Marburg virus (MBG) after the city of Marburg, where most of the cases and initial work occurred. During 1975 (Zimbabwe), 1980 and 1987 (Kenya) there were sporadic fatal cases identified primarily amongst tourists. The Kenyan cases had included visits to bat infested Kitum caves in Kenya's Mount Elgon National Park (Gear *et al.* 1975; Smith *et al.* 1982; Teepe *et al.* 1983; Johnson *et al.* 1996) (Table 31.1). Apart from the 1987 case, secondary infections proved a risk to health care workers. Further epidemiological investigations in these areas has revealed no information on the origin of these infections occurred.
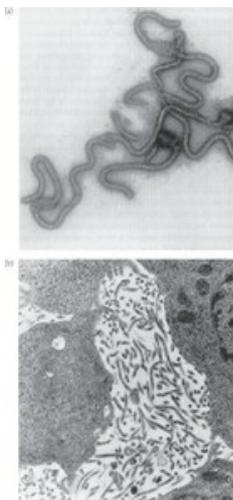

Fig. 31.1

Marburg and Ebola viruses

(a) Electron micrograph, showing filamentous forms of Ebola (Reston) virus (×18 360). (b) Ebola (Sudan) virus thin section, showing virions extruding from cells into extracellular spaces (×14 040).

Courtesy of B. Dowsett

Table 31.1 Human pathogens in the order Mononegavirales			
Family	**Subfamily**	**Genus**	**Human pathogens**
Rhabdoviridae			
		Lyssaviru	Rabies virus
Filoviridae			
		Marburg virus	Marburg virus
		Ebolavirus	Ebola virus
Paramyxoviridae			
	Paramyxovirinae		
		Rubulavirus	Mumps virus, Parainfluenzavirus 2,4
		Respirovirus	Parainfluenza virus 1,3
		Henipavirus	Hendra virus, Nipah virus
		Morbillivirus	Measles virus
		Rubulavirus	Mumps virus, Parainfluenzavirus 2,4
	Pneumovirinae		
		Pneumovirus	Respiratory syncytial virus
		Metaneumovirus	Human metapneumovirus

Between late 1998 and 2000 in Democratic Republic of the Congo the first large outbreak of this disease under natural conditions occurred, which involved 154 cases, of which 128 were fatal, representing a case fatality rate of 83%. The majority of cases occurred in young male workers at a gold mine in Durba, in the northeastern part of the country, which proved to be the epicentre of the outbreak. Cases spread to the neighbouring village of Waste. Family members involved in the close care of patients accounted for some cases, but secondary transmission appeared to be rare.

The largest Marburg outbreak in history occurred between 2004 and 2005, in Angola. It was believed to have started in Uige Province in October 2004 that resulted in 252 cases, with 227 deaths (CFR 88%) countrywide by July 2005. All cases detected in other provinces were linked to the outbreak in Uige (Towers 2006).

From June to August 2007, three confirmed cases occurred amongst mineworkers working in Kamwenge, western Uganda were identified. Of the two miners who cared for the index case who died, one also suffered a fatal illness (WHO 2007).

In July 2008, a Dutch tourist developed Marburg four days after returning to the Netherlands from a three-week holiday in Uganda. The source of the exposure has not been determined, although the woman had visited caves in Maramagambo

Forest western Uganda at the southern edge of Queen Elizabeth National park, where bats were present (WHO 2008; Timen 2009).

There have been 414 primary human Marburg infections documented and an increasing number of secondary cases recorded which have originated from an increasing geographic range in Africa (Table 31.2).

Table 31.2 Summary of human filovirus infections during identified outbreaks						
Year	**Filovirus**	**Source of infection**	**All cases deaths/total**	**Overall mortality rate (%)**	**Source**	**Comments**
1967	Marburgvirus	Germany, Marburg	5/23	22.6	Vervet monkeys	Imported from Uganda
		Germany, Frankfurt	2/6			
		Yugoslavia, Belgrade	0/2			
1975	Marburgvirus	Zimbabwe/South Africa (Johannesburg)	1/3	33.3	Unknown	Index case infected in Zimbabwe. Secondary cases:– travelling companion and nurse
1976	Zaire ebolavirus	Northern Zaire (Yambuka)	280/318	88.1	Unknown	Index case introduced virus into hospital
1976	Sudan ebolavirus	Sudan, Maridi	116/213	53.2	Unknown	Disease amplified by transmission in large active hospital
		Sudan, Nazara	31/67			
		Sudan, Tembura	3/3			
		Sudan, Juba	1/1			Originatedin Nazara cotton factory
1976	Sudan ebolavirus	United Kingdom, Porton Down	0/1	0	Laboratory infection	Needle-stick
1977	(Zaire ebolavirus)	Zaire, Tandala	1/1	100	Unknown	Tandala
1979	Sudan ebolavirus	Southern Sudan, Yambo-Nazar District,	22/34	64.7	Unknown	Nazara, Maridi & local area

1980	Marburgvirus	Kenya, Mount Elgon	1/2	50	Unknown	Visited Kitum cave in national park
1987	Marburgvirus	Kenya, Mount Elgon, Kisumu	1/1	100	Unknown	Expatriate travelling in western Kenya. Visited Kitum cave
1988	Marburgvirus	USSR (Koltsova)	1/1	100	Laboratory infection	Associated with animal studies
1989-1990	Reston ebolavirus	US (Alice, Philadelphia, Reston)	0/4	0	Monkeys	Epizootic-imported monkeys from export facility in Philippines
1990	Reston ebolavirus	Philippines (Luzon)	0/12	0	Monkeys	Export facility
1990	Marburgvirus	USSR (Koltsovo)	0/1	0	Laboratory infection	Associated with animal studies
1992	Reston ebolavirus	Italy, Sienna	0	0	Monkeys	Epizootic-import from export facility in Philippines same as US outbreak in 1989
1994	Côte d'Ivoire ebolavirus	Côte d'Ivoire, Tai forest	0/1	0	Chimpanzees	Contracted in Scientist during post-mortem of chimpanzee —repatriated to Switzerland
1994-1995	Zaire ebolavirus	Gabon (Andok, Mekouka, Minkebe, Mayela-Mbeza, Ovan, Etakangaye)	31/52	60	Unknown	
1995	Zaire ebolavirus	Zaire, Kikwit	245/317	77.3	Unknown	Confined to Bandundo region around Kikwit
1996	Zaire ebolavirus-	Gabon (Mayibout, Makokou)	21/37	57	Chimpanzees	Contact with dead primates hunted for

						hunted for food
1996	Zaire ebolavirus	Russia (Sergiyev Posad-6)	1/1	100	Laboratory infection	Associated with animal studies
1996-1997	Zaire ebolavirus	Gabon (Balimba, Boouee, Lastourville, Libreville, Lolo), South Africa (Johannesburg)	46/62	74.2	Chimpanzee?	Index case hunter Nurse treating imported case from Gabon to Johannesburg
1996	Reston ebolavirus	USA	0	0	Monkeys	Epizootic-Primates imported from Phillipines to quarantine facility in Texas
1996	Reston ebolavirus	Phillipines	0	0	Monkeys	Epizootic-Identified in Export facility. No human infections
1998-2000	Marburgvirus	Democratic Republic of Congo (Durba, Watsa)	128/154	83.1	Unknown	Epicentre of outbreak in young male gold workers in Durba
2000-2001	Sudan ebolavirus	Uganda (Gulu, Masindi & Mbarara Disricts)	224/425	52.7	Unknown	Spread through attending funerals, family case contact & medical centres
2001-2002	Zaire ebolavirus	Gabon (Ekata, Etakangaye, Franceville, Grand Etoumbi, Ilahounene,	53/65	82	Unknown	Community and Nosocomial spread on border region of Gabon and Congo
2001-2002	Zaire ebolavirus	Congo (Abolo, Ambomi, Entsiami, Kelle, Olloba	43/57	75.6	Unknown	
2002	Zaire ebolavirus	Congo (Olloba/Gabon(Etata))	10/11	90.9	Unknown	

2002-2003	Zaire ebolavirus	Republic of Congo (Mbomo & Kelle districts of)	129/143	89	Unknown	
2003-2004	Zaire ebolavirus	Republic of Congo (Mbomo and Mbandza villages in Mbomo district of)	29/35	83	Unknown	
2004	Zaire ebolavirus	Russia (Koltsovo)	1/1	100	Laboratory infection	Guinea pig adapted Zaire ebolavirus
2004-2005	Marburgvirus	Angola, Uige Province	227/252	90.1	Unknown	Community spread through various provinces linked to Uige
2004	Sudan ebolavirus	Sudan (Yambio)	7/17	41-.2	Unknown	Simultaneous outbreak of measles
2005	Zaire ebolavirus	Congo (Etoumbi, Mbomo)	9/11	81.9	Unknown	
2007	Marburgvirus	Uganda (Kamwenge District)	2/3	66.6	Unknown	Origins thought to be in Lead and Gold mines of area
2007	Zaire ebolavirus	Democratic Republic of Congo Kasai Occidental province	187/264	70.8	Unknown	
2007-2008	Bundibugyo ebolavirus	Uganda (Bundibugyo District)	25/149	16.8	Unknown	1st recorded occurrence of new strain
2008	Marburgvirus	Netherlands ex Uganda (Maramagambo Forest)	1/1	100	Cave in Maramagambo bats?	40 year old Dutch women, visited cave in National Park
2008	Ebola-Reston	Phillipines	6 assymptomtic cases	0	Pigs	1st known detection of Ebola-Reston in pigs. 6 assymptomtic cases amongst slaughter house workers.

| 2008-2009 | Zaire ebolavirus | Democratic Republic of Congo | 15/32 | 47 | | Outbreak in Mweka & Luebo of Kasai District |
| 2009 | Zaire ebolavirus | Germany | 0/1 | 0 | Laboratory infection | Animal vaccine studies |

In 1976, a severe and often fatal viral haemorrhagic fever occurred in simultaneous outbreaks in the equatorial provinces of southern Sudan and northern Zaire (Table 31.2). Amongst the several hundred cases of infection identified, fatality rates of about 90 and 60% respectively occurred. Several generations of human-to-human spread contributed to the severity of the outbreak. The two virus strains isolated from patients in Sudan and Zaire where found to be morphologically identical to Marburg but antigenically and biologically distinct (Bowen *et al.* 1977; WHO 1978*a, b*). The virus was named Ebola after a river in Zaire.

Numerous follow-up ecological studies have failed to discover the reservoir. During studies of the Sudanese epidemic in 1976, a non-fatal Ebola infection occurred within the UK in 1977 after a laboratory accident (Emond *et al.* 1977). Just over 6 months after the original outbreaks in Zaire, a 9 year old girl died of acute haemorrhagic fever in Tandala, northern Zaire (Heymann *et al.* 1980). A further small epidemic occurred in the same region of Sudan in 1979 when 22 (65%) of 34 infections were fatal, with transmissibility being associated with person-to-person spread (Baron *et al.* 1983). The geographic area widened when a Swiss zoologist became infected while undertaking an autopsy of a dead chimpanzee in western Côte d'Ivoire in late 1994. This resulted in her contracting a non-fatal severe Ebola illness (LeGuenno *et al.* 1995). Only after her treatment and discharge from a hospital in Switzerland was it discovered that she had suffered from an Ebola infection. A larger-scale outbreak in Kikwit, Bandundu Province, Zaire 1995 demonstrated to a worldwide audience a severe hemorrhagic illness involving some 316 cases, of which 244 died, giving a mortality rate of 77% (WHO 1995*a*). A third of the cases involved healthcare workers. The Kikwit outbreak was similar to the original episode that occurred in 1976, 1000 km to the north. As in previous outbreaks, secondary cases occurred through close personnel contact with infectious body fluids. The uncontrolled spread of infection resulted from a lack of modern medical facilities and supplies that could protect medical personnel from those patients initially affected. Unlike previous Ebola outbreaks, concern centered on the potential for community-wide spread from Kikwit, a large and densely populated area, to the larger cites of Kinshasa and Brazzaville close by. Control of the outbreak coincided with the introduction of protective equipment and barrier nursing techniques (WHO 1995b). The recognition of Ebola has recently extended to a confirmed case in the Côte d'Ivoire of a refugee from neighbouring Liberia in late 1995; other cases were reported to exist in his home village in Liberia (WHO 1995*c*).

During 1996–1997, two small outbreaks occurred in Gabon. These were found in the forest regions of Mayibout and Booué areas and where associated with people hunting and butchery of chimpanzees (Georges 1999). A major outbreak involving 425 cases occurring for the first time in Uganda during 2000–2001. Studies indicated that the three most import risk factors associated with its spread were associated with attendance at funerals of Ebola fever cases, having direct contact with cases and provision of medical care without adequate personal protective measures being used. The geographical spread continued during 2001–3 when Ebola outbreaks with case mortality rates reaching over 80% was recorded in Gabon and for the first time in The Democratic Republic of Congo (DRC) (Okware 2002; WHO 2003; Formenty 2003; WHO 2004). Repeat outbreaks in the DRC in 2007 and in Uganda in 2007–08 produced case fatality rates of 70% and 25% respectively.

Unexpectedly, Ebola virus has also appeared outside Africa when identified amongst cynomologus monkeys (*Macaca fasicularis*) imported into the USA in 1989 (Jahrling *et al.* 1990) and Siena, Italy in 1992 (WHO 1992). Shipments of wild‑caught cynomolgus monkeys originated from the same handling facility in the Philippines, where the presence of the virus was documented. Although a truly Asian origin for these virus strains is not discounted, preliminary serological and sequencing studies, suggest a close similarity with isolates from the 1976 African outbreaks.

The agent

Taxonomy

Ebola and Marburg are members of the family Filoviridae (Kiley *et al.* 1982; Pringle 1991), named for their filamentous appearance under the electron microscope (Fig. **31.1a**). Similarities of genome structure and comparable mechanisms of gene expression suggest that the filoviruses have an evolutionary origin in common with the families Paramyxoviridae (which include measles and mumps) and Rhabdoviridae (which includes rabies) (Sanchez *et al.* 1992). These three virus families are grouped into a taxonomic order (Table 31.1), the Mononegavirales (Bishop and Pringle 1995).

Progressive characterization of the filoviruses revealed substantial differences between the Marburgviruses and Ebolaviruses (Richman 1983) leading to the establishment of two genera, *Marburgvirus* and *Ebolavirus*, within the family *Filoviridae* (Feldmann 1994). The genus *Marburgvirus* contains only one species, *Lake Victoria Marburgvirus*, represented by a single virus, Lake Victoria Marburgvirus (MARV). The genus *Ebolavirus currently* contains five species: *Côte d'Ivoire ebolavirus* (Côte d'Ivoire ebolavirus, CIEBOV), *Reston ebolavirus* (Reston ebolavirus, REBOV), *Sudan ebolavirus* (Sudanebolavirus, SEBOV), '*Uganda ebolavirus*' ('Uganda ebolavirus,' 'UEBOV'), and *Zaire ebolavirus* (Zaire ebolavirus, ZEBOV) (Feldmann 2005; Mason 2008) (Table 31.3). In comparison, the genomes of members of the five ebolaviral species differ genetically by 37–41% at the nucleotide level, and all of them differ from MARV genomes by >65%. Amongst the genus Ebolavirus the three distinct species, Bundibugyo, Sudan and Zaire species have been associated with large outbreaks of Ebola haemorrhagic fever (EHF) in Africa causing death in 25–90% of all clinical cases, while Côte d'Ivoire and Reston have not.

Table 31.3 Filovirus taxonomy				
Order	**Family**	**Genus**	**Species**	**Abbreviation**
Mononegavirales	*Filoviridae*	*Ebolavirus* *Marburg virus*	*Sudan ebolavirus* *Zaire ebolavirus* *Cote d'Ivoire ebolavirus* *Reston ebolavirus* *Bundibugyo ebolavirus* *Lake Victoria marburgvirus*	*SEBOV* *ZEBOV* *CIEBOV* *REBOV* *BUBOV* *MARV*

Molecular biology

Filoviral genomes consists of a single non-segmented negative-stranded RNA about 19 kilobases in length and contain seven genes arranged linearly and that may be separated by intergenic regions. The linear arrangement of genes follows a sequence beginning with a 3′ non-coding untranslated region, the core protein genes, envelope genes, and the polymerase gene attached to the untranslated region at the 5′ end. (Feldmann *et al.* 1993; Sanchez *et al.* 1993). Non-structural proteins are not present. Each gene is flanked by highly conserved transcription and termination sites that differ among the different Filoviruses (Weik 2002).

The genetic sequence of Marburg virus **(**MBG**)** and the available partial sequence of Ebola virus (EBO) indicate that in both cases seven structural proteins are encoded, which are expressed through transcription of monocistronic mRNA species. The first (3') gene of the filoviral genomes, NP a major nucleoprotein (NP; M_r 94–104). The nucleocapsid is composed of RNA, L, NP, VP35, and VP30. Analysis of the NP gene shows a short putative leader sequence at the extreme 3 end followed by the complete nucleoprotein gene. The transcriptional start (3′…UUCUUCUUAUAAUU…) and termination (3′…UAAUUCUUUUU) signals of the MBG NP gene are very similar to those seen with EBO virus. The filoviral *VP35* gene is located immediately downstream of the *NP* gene. The VP35 protein is thought to be a transcriptase–polymerase component that is considered to be the P(NS) protein equivalent of paramyxo-and rhabdoviruses The VP35 and VP35-NP complexes also inhibit both dsRNA-mediated and virus-mediated induction of interferon-responsive promoters and consequently the cellular interferon innate immune response to virus infection. The filoviral matrix or membrane-associated *VP40* gene is located downstream of the *VP35* gene and is the most conserved of all filovirus genes, it encodes the matrix protein VP40 protein (M_r 32 000). The filoviral *GP* gene is a single surface glycoprotein (GP; M_r 170 000). Marburg and ebolavirus encode one and three glycoproteins from their GP genes, respectively The filoviral minor nucleoprotein *VP30* gene (VP30; M_r 32 000), located downstream of the *GP* gene, is another unique component of the filoviral genomes. It encodes a protein, VP30. The N-terminus of VP30 binds directly to single-stranded RNA, and prefers filoviral RNA over unspecific RNA (John 2007) VP30 is also a zinc-binding protein. In the absence of zinc, filovirus-genome transcription is abolished (Modrof 2003)). The *VP24* gene and its expression product is a second matrix or membrane-associated VP24 protein (M_r 24 000). Recent experiments also indicate that VP24 counteracts the interferon-response of virus-infected cells (Reid 2007). The last (5') gene of the filoviral genome is the *L* gene, which encodes the catalytic part (L protein; MARV: 2,331 amino-acid residues, 267 kD; ZEBOV: 2,212 amino-acid residues, 253 kD) of the viral RNA-dependent RNA polymerase holoenzyme, (M_r 267 000); which also contains VP35 (27, 195, 283).

In comparison to other non-structural non-segmented negative-strand (NNS) RNA viruses, filovirus transcriptional signals are very similar to those of members of the paramyxovirus and morbillivirus genera. Nucleotide sequence analysis of the 3' end, including the entire NP and L protein encoding genes of the MBG and EBO genome, has shown similar structure and organization with other NNS RNA viruses. The similarity of the filovirus NP genes and gene products to those of the paramyxoviruses imply a closer biological and phylogenetic relationship to these agents than to rhabdoviruses.

Growth and survival

Filoviruses undergo rapid, lytic replication in the cytoplasm of a wide range of host cells. The mode of entry of Filoviruses into cells occurs by binding to unidentified cell-surface receptors with their spike proteins. Although some ultrastructural studies have suggested that virions are associated with entry by endocytosis. African green monkey kidney cells, such as CV-1 and Vero derivatives, are commonly used to grow filoviruses to high titres. The filoviral nucleocapsid is released into the cytosol subsequent to fusion of the viral and cellular membrane, and complete uncoating of the filovirus genome takes place. The polymerase holoenzyme L/VP35, brought into the cell with the nucleocapsid, transcribes the filovirus genes in a sequence, synethizing the antigenome that is used as the template to synthesize the progeny genes. The filoviral messenger RNA's are abundant in infected cells and are translated into the filoviral structural proteins. The NP, VP30,VP35 and L proteins assemble with the newly synthesized genomes to form RNPs complexes. These recruit the matrix proteins VP40 and VP24 and bud from the cell's plasma membrane, acquiring the GP envelope with its surface projections by budding from the cell membranes (Noda 2006). The entire life cycle occurs in the cytoplasm of the host cell. Nucleocapsids also accumulate in the cytoplasm, forming prominent inclusion bodies.

Laboratory infection of tissue cells shows intracytoplasmic vesiculation and mitochondrial swelling followed by a breakdown of organelles and terminal rarification and condensation. These cytoplasmic changes occur simultaneously with the accumulation of viral nucleocapsid material in intracytoplasmic inclusion bodies and the large numbers of virions extracellularly (Fig. **31.1b**).

The filovirus virion is bacilliform in structure and is composed of a helical nucleocapsid, consisting of a central axis (20–30 nm in diameter), surrounded by a helical capsid (40–50 nm in diameter). A host cell membrane derived lipid outer envelope, with regular 10 nm projections surrounds the nucleocapsid and completes the striking and characteristic appearance under the electron microscope (Fig. **31.1a**). Although there are often structures of varying length, with loops, branches, and other irregularities, evidence exists that infectious particles consist mainly of simple linear forms about 1 μm in length.

Marburg and Ebola virus infectivity are stabilized at ambient temperature (18–20°C) but inactivated within 30 min at 60°C. Ultraviolet and gamma irradiation, lipids, solvents, β-propiolactone, hypochlorite, and phenolic disinfectants all destroy infectivity.

Disease mechanisms

The exact mechanism by which filoviruses cause such a serious illness is being unravelled. There is extensive viral replication in liver, lymphoid organs, and kidneys. Extensive visceral effusions, pulmonary interstitial oedema, and renal tubular dysfunction occurring after endothelial damage leading to hypovolaemic shock are all observations contributing to death. Severe, acute fluid loss accompanied by bleeding into the tissue and gastrointestinal tract is characteristic and leads to dehydration, electrolyte and acid-base imbalance. In primates and clinical cases studied early, lymphopenia is followed by a marked neutropenia (Fisher-Hoch *et al*. 1983). In the later stages of the infection, and in association with the thrombocytopenia, the remaining platelets are unable to aggregate in response to ADP or collagen. It has been suggested that the dysfunction of platelets and endothelia extends to other elements of the endothelial system, such as macrophages (Fisher-Hoch *et al.* 1985). In experimental monkey Ebola infections, virus has been identified in vascular endothelial cells. Humans convalescent from Marburg and Ebola infections have had virus isolated up to 3–4 months after onset in semen, in a patient with uveitis, anterior chamber fluid. There is no evidence of long-term persistence, latency or late degenerative disease in the small number of patients observed or in monkeys recovering from the disease (Fisher-Hoch *et al.* 1992a).

The hosts

Human

Incubation period

Filoviruses are transmitted through direct contact. They enter through small skin lesions or through damaged mucous membranes and initially infect target cells such as macrophages, which transport them through the body. The incubation period for Marburg virus disease is 3–9 days (Martini 1971; Bausch, 2006) and for Ebola virus about 10 days (Bwaka 1999) 5–7 days for needle transmission (Emond 1978) and 6–12 days for person-to-person spread.

Symptoms and signs

The illnesses caused by Marburg and Ebola viruses are virtually indistinguishable. Following exposure and incubation period, both infections have an abrupt onset of illness with initial non-specific symptoms such as fever, severe frontal headache, back pain malaise, and myalgia. Early signs include tachycardia, conjunctivitis, and maculopapular rashes,

which develop after 5–7 days on face, buttocks, trunk or arms and later generalizes over the entire body with desquamation in survivors. Within a week to 10 days, those destined for recovery begin to improve, even though recovery from the severe debilitating effects of the disease often takes weeks. A large number of patients in both Marburg and Ebola outbreaks develop severe bleeding between the fifth and seventh days. Patients with severe infections often experience pharyngitis, severe nausea, and vomiting, progressing to haematemesis and melena. Petechiae, ecchymoses, uncontrolled bleeding from venepuncture sites and post-mortem evidence of visceral haemorrhagic effusions are characteristic of severe illness (Smith *et al.* 1978). Death usually occurs between the 7–10 days (range 1–21 days) after onset of clinical disease and is preceded by severe blood loss and shock. Convalescence is slow and marked by prostration, weight loss, and amnesia for a considerable period after the acute illness (Piot 1978; Formenty 1999). Death occurs usually after 8–16 days of infection due to shock after multi-organ failure, often brought on by secondary bacterial infections.

Clinical laboratory findings include early leucopoenia with left shift of the granulocytes accompanied by, marked thrombocytopenia, and abnormal platelet aggregation. Serum aspartate aminotransferase (AST)/alanine aminotransferase (ALT) enzyme levels are raised and characterized by a high AST/ALT ratio (10–3:1) and γ-glutamyl transpeptidase indicating liver damage. Other findings include raised levels of creatine and urea levels prior to renal failure and hypokalemia because of the diarrhea and vomiting.

As the differential diagnosis in the early acute phase is difficult, other causes need consideration. The most common causes of imported infections showing a severe, acute febrile disease are malaria and typhoid fever. Therefore, delay in differential diagnosis and treatment needs to be minimized. Alternative causes include bacterial diseases such as meningococcal septicaemia, *Yersinia pestis* infection, leptospirosis, anthrax; rickettisal diseases such as typhus and murine typhus; and viral diseases such as sandfly fever, yellow fever, chikungunya fever, Rift Valley fever, hantavirus, and Congo Crimean haemorrhagic fever.

Diagnosis

The diagnosis of Ebola or Marburg should be considered in patients showing acute, febrile illness having visited known epidemic or suspected endemic areas of rural sub-Saharan Africa and Asia, particularly when haemorrhagic signs are present. All tissues, blood, and serum collected in the acute stages of illness contain large amounts of infectious virus. Extreme care should be taken when drawing or handling blood specimens as the virus is stable for long periods at room temperature (Elliott *et al.* 1982). All needles and syringes need discarding to puncture-resistant containers with lids, and incinerated. Specimens of blood should be taken without anticoagulant. Blood or serum should be transferred to a leak proof plastic container and double wrapped in leak proof containers for transportation to a high containment laboratory. Transportation should be under appropriate biocontainment within dry or wet ice according to international transportation or national regulations (Department of Health 2007; WHO 2007) after consultation with any of the global maximum containment reference laboratories.

Inactivation of patients sera with irradiation or heating at 60°C for 30 min render them safe for undertaking immunoassay tests. Although morphologically similar, Marburg and Ebola are immunologically distinct. The immunofluorescence assay (IFA) has been the basic diagnostic test for filovirus infection and the only one that has widespread acceptance for the diagnosis of human Ebola disease (Wulff and Johnson 1979; Rollin *et al.* 1990). A rising antibody in paired serum or a high IgG titre (>64) and presence of IgM antibody, together with clinical symptoms compatible with a haemorrhagic fever, are consistent with a diagnosis. The presence of Marburg antibody is considered a specific result but Ebola virus low-titre, non-specific, false-positive serological reactions do occur. When using IFA the problem of low-titre false positives makes interpretation difficult when undertaking non-human primate and human seroepidemiological surveys. For the IFA, the antigen substrate consists of virus-infected Vero cells dried on to spot slides. Recent advances in molecular biology have expressed the nucleoprotein gene of Ebola virus in a baculovirus expression system. Thus, a large amount of non-infectious protein is a possible source of antigen for many serological tests (IFA, ELISA). There evaluation leading to an improvement in the detection capability within seroepidemiological field studies and in the screening of imported primates.

Filoviruses can be easily isolated from inoculation of fresh or stored (−70°C) specimens of blood or serum collected during the acute phase of illness into Vero monkey kidney tissue cultures cells using laboratory containment level 4 facilities. Vero cells (particularly clone E6) and MA-104 have proved to be the most sensitive and useful cells for the propagation and assay of fresh isolates and laboratory passage filovirus strains. Primary isolation using tissue culture rarely produces a specific cytoplasmic effect, thus evidence of infection is based on the appearance of cytoplasmic inclusion bodies demonstrated 2–5 days after inoculation, by immunofluoresence staining, using antipolyclonal anti-sera or virus subtype or strain-specific monoclonal antibodies. Some filovirus strains such as Ebola Sudan are difficult to grow in primary cultures and success is improved through the intraperitoneal inoculation of young guinea pigs. A monitored febrile response coincides with high levels of virus in the blood, which can be recovered in tissue culture or examined directly by the electron microscope. In each of the Filoviridae outbreaks electron microscopy has proven useful in the identification of Marburg or Ebola in body fluids and tissue, and cell culture supernatants. During the Reston epizootic,

immunoelectron microscopy, when used in conjunction with standard transmission microscopy (TEM) of infected cells, provided consistent results (Geisbert and Jahrling 1990). However, the technique will not distinguish between the Filoviridae strains. Virus may also be detected in a range of specimens, including throat washings, semen and anterior eye fluid. The presence, of Ebola and Marburg in tissues of monkeys, both experimentally and during primate outbreaks, has been demonstrated by electron microscopy (Baskerville *et al.* 1985; Geisbert and Jahrling 1990); and detection of high titres in the blood of infected patients and monkeys indicated the usefulness of an antigen capture ELISA system (Ksiazek *et al.* 1992). Such a system has been of considerable use in the recent Kikwit epidemic. Immunobilized mouse monoclonal antibodies on a solid plastic surface capture Ebola antigen contained in tissue or blood specimens. Rabbit polyclonal anti-filovirus serum detects the antigen. The antigen immunoabsorbent detection assay has proved a rapid and reliable procedure for the early detection of filovirus infections and would prove useful as a method for the routine screening of imported primates.

In recent years, the use of cross-reacting and strain-specific probes for the reverse transcriptase polymerase chain reaction RT-PCR has evolved to the gold standard for Filovirus diagnosis in the field and has taken the place of previously widely used IFAs and ELISAs. Confirmatory diagnosis of RT-PCR-positive samples is usually performed by virus isolation in maximum-containment facilities (Grolla *et al.* 2005; Towner *et al.* 2004).

Currently all outbreaks of EHF and MHF, infections are confirmed by various laboratory diagnostic methods. These include virus isolation, reverse transcription-PCR (RT-PCR), including real-time quantitative RT-PCR, antigen-capture enzyme-linked immunosorbent assay (ELISA), antigen detection by immunostaining, and IgG- and IgM-ELISA using authentic virus antigens (Gibb *et al.* 2001; Ksiazek *et al.* 1992, 1999; Leroy *et al.* 2000; Towner *et al.* 2004; Zaki *et al.*1999; Saijo *et al.* 2006).

Primates

African green monkeys imported from Uganda were the identified source of the Marburg outbreak (Henderson *et al.* 1971), and cynomolgus monkeys the source ebola reston virus. Both species were imported and closely associated with medical research.

The general characteristics of primate filovirus infections amongst experimentally and naturally infected primates suggest that the incubation period varies between 4 and 20 days, during which time the virus replicates to high titre in the liver, spleen, lymph nodes, and lungs. The clinicopathological features noted include high fever, severe weight loss, anorexia, haemorrhages, and a distinctive skin rash in association with splenomegaly, marked elevation of lactate dehydrogenase, alanine aminotransferase and aspartate aminotransferase. The AST levels are constantly 2–10 times higher than that of ALT. Evidence of thrombocytopenia is a pronounced feature but anaemia is not. Both lymphocytopenia and leucocytosis are evident and dependent on the stage of the infection (Fisher-Hoch *et al.* 1983). Severe prostration with diarrhoea and bleeding leads to rapid death in almost all animals. The severity of the disease in primates depends on the filovirus infection and host involved. African green monkeys are far less susceptible to severe or fatal disease due to Ebola virus (Sudan) or Ebola (Reston) than cynomolgus monkeys. However, African green monkeys experimentally infected with Marburg virus after the 1967 outbreak all died irrespective of route of infection (Hass and Mass 1971). Marburg virus infection of rhesus monkeys is less severe or fatal, similar to Ebola (Sudan) infection. Infection by Ebola virus (Zaire) is uniformly fatal in all species so far challenged, regardless of inoculum.

Histopathological findings include severe hepatocellular necrosis, necrosis of the zona glomerulosa of the adrenal cortex, and interstitial pneumonia, all associated with the presence of intracytoplasmic amphophilic inclusion bodies. The necrotic lesions result from direct virus infection of the parenchymal cells. Little inflammatory response occurs at the site of the lesions.

Experimental pathophysiological studies have demonstrated endothelial cell and platelet dysfunction accompanied by oedema, multiple effusions, haemorrhage, and hypovolaemic shock (Fisher-Hoch *et al.* 1985). Recent studies in *Macaca fascicularis* and *Cercopithecus aethiops* suggest that the recently isolated Asian filoviruses are less pathogenic for primates than the African filoviruses (Fisher-Hoch *et al.* 1992b).

Treatment and prognosis

There is no licensed vaccine or effective antiviral drug treatment licensed for use in human. Therapy for Marburg and Ebola virus infection is limited to the provision of supportive measures and general nursing care. In the development of vaccines, recent advances have shown protection in animal models. Amongst the most recent promising vaccines under development are a number of recombinant based systems. The most noticeable those based vesicular stomatitis virus (VSV) that expresses a single filovirus glycoprotein (GP) in place of the VSV glycoprotein. A single dose vaccine has proved capable of protecting non-human primates against *Sudan ebolavirus* (SEBOV), *Zaire ebolavirus* (ZEBOV), *Cote d'Ivoire ebolavirus* (CIEBOV), and *Marburgvirus* (MARV) (Feldmann H *et al.* 2007; Geisbert *et al.* 2009). Recently, a two-

injection filovirus vaccine regime based on an adenovirus vector expressing multiple antigens from five different filoviruses, (ZEBOV NP, ZEBOV GP, SEBOV GP, MARV Ci67 strain GP, MARV Ravn strain GP, MARV Musoke strain NP, MARV Musoke strain GP), proved successful (Pratt *et al.* 2010). All animals in these studies survived the initial filovirus challenge with a different strain or species of filovirus. However, there are a number of significant safety challenges in humans; particularly those with an altered immune status are yet to be overcome.

Intensive supportive care is currently considered most important: prevention of shock, cerebral oedema, renal failure, bacterial superinfection, hypoxia, and hypotension may be life saving. Patient care is complicated by the need for isolation and protection of medical and nursing personnel. The use of plastic patient isolators is a requirement in many countries, including the UK and Europe, despite the return to the strict barrier nursing techniques now favoured in the USA and in the recent African outbreaks in Zaire, Côte d'Ivoire, Gabon, Uganda and Angola.

Epidemiology

Epidemics

Marburg virus disease

A fulminating haemorrhagic fever caused by the Marburg virus was first recognized in August 1967 when it affected laboratory workers in three simultaneous outbreaks in Marburg and Frankfurt, Germany, and Belgrade, former Yugoslavia (Martini 1969). Altogether, there were 31 human infections, of whom 25 had primary infections acquired through contact of blood and tissues from shipments of African green monkeys (*Cercopithecus aethiops*) imported from Uganda, via London. The largest number of primary infections (20) occurred among workers of a Marburg pharmaceutical firm who prepared kidney cells for vaccine production. Among those infected, exposure was attributed to autopsies (13), cleaning of contaminated glassware (5), dissection of kidneys (1), and laboratory accident involving contaminated broken glassware (1). Personnel not in direct contact with contaminated material or who wore protective gloves or masks for work with monkeys was not infected. In Frankfurt, four further primary infections occurred in workers exposed to tissue culture. A veterinary officer carrying out routine post-mortems was the single recorded primary case in Belgrade. Four of the secondary infections occurred in hospital personnel who came into close contact with patients. The fifth was the wife of the veterinary surgeon, who fell ill 10 days after nursing her husband at home. The sixth case involved the wife of a patient who had transmitted the disease through sexual intercourse 83 days after the illness. Seven of the primary cases were fatal, but no fatalities occurred amongst the six secondary cases.

Around 600 animals originating from four shipments reached Europe from Uganda over a 3-week period. Frankfurt received 50–60 animals from two shipments, Belgrade approximately 300 animals from three shipments, and the remainder went to Marburg. All spent between 60 and 87 days in a holding facility in Uganda before being shipped to London, Heathrow where they spent 636 hours in the animal hostel before being forwarded to Germany. Details on the Belgrade enzootic indicated that 46 of 99 animals imported from the first shipment died, and 20 and 30 from the second and third shipments, respectively. This epizootic was characterized by daily deaths of one or more animals throughout the 6-week quarantine period, suggesting ongoing transmission between animals. Epidemiological evidence of the outbreak suggested that transmission between monkeys in quarantine facilities was through direct contact with contaminated equipment. Direct contact with infected blood and tissue was considered the source for all human cases, and there was evidence of aerosol transmission. No epizootics were found in Uganda although uncorroborated information had suggested a large number of monkey deaths in colonies on the island near Lake Kyoga, north of Lake Victoria, to the east of Mount Elgon in Kenya. Ugandan monkeys were captured in this area, transported to Entebbe, where they were held for 3 days prior to shipment.

The first recognized outbreak of Marburg virus disease in Africa, and the first since the 1967 outbreak, occurred in South Africa in February 1975 (Gear *et al.* 1975) (Fig. **31.2b**). The index case involved a young Australian tourist who had hitchhiked through Zimbabwe and died after admission to a Johannesburg hospital. Shortly afterwards two non-fatal secondary cases occurred, one his female travelling companion and a nurse (Table 31.1). Again, there was evidence that the virus persisted in the body; the virus was recovered from fluid aspirated from the anterior chamber of the nurse's right eye 80 days after onset of illness. In January 1980, Marburg re-appeared in Kenya (Smith *et al.* 1982). The index patient was an electrical engineer who acquired the fatal infection in western Kenya. A non-fatal secondary infection occurred 9 days later. This involved a doctor who attended the patient and had attempted mouth-to-mouth resuscitation. In 1987, an isolated case of fatal Marburg disease was again recognized in a 15 year old Danish boy who had been admitted to hospital in Kenya. Nine days prior to the onset of his disease, he also had visited Kitum Cave in Mount Elgon national park. Recent studies of colonies of *R.gypriacus* bats which inhabit these caves have demonstrated molecular and serologically positive for Marburgvirus but there role in transmission is as yet undefined (Kuzmin *et al.* 2010). Presently it is thought that the habitat of the natural host of Marburg virus is within, but not limited to, the Mount Elgon area and possibly overlaps the distribution of Ebola.
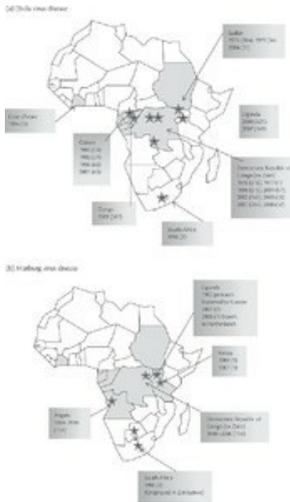
Fig. 31.2
Distribution and dates of filovirus outbreaks in Africa (a) Ebola virus disease and (**b) Marburg virus** disease.

Between 1987 and 1998, the only case of Marbug reported were associated with laboratory accidents in the former Soviet Union of which one was fatal **(**Nikiforov *et al.* 1994) outlines the dangers associated with working with these agents in the laboratory.

However, in 1998, the largest natural outbreak of Marburg virus disease to date began in northeastern Democratic Republic of the Congo (DRC). This time, the focus of the outbreak was a town called Durba (population 16 000). A large number of men in this region work for the Kilo Moto Mining Company, which runs a number of illegal gold mines in the area. The Marburg outbreak started in November of 1998, although not reported to any international agencies until late April of 1999, following the death of the chief medical officer in the area from the disease. The outbreak had a case fatality rate of approximately 83 involving 154 cases before the declaration that the outbreak was over in 2000. The disease also spread to neighbouring villages particularly Watsa. Miners had a significantly higher risk of contracting Marburg than the general population of this area, suggesting frequent exposure to the natural reservoir of Marburg virus (Bausch 2006).

The size and extent of this outbreak was eclipsed at the beginning in 2004, when a Marburg outbreak occurred in Angola during October. Due to civil war in the country and a non-existent public health infrastructure, recognition of the outbreak was delayed until almost 6 months later, in March of 2005. The outbreak was declared over in November of 2005, after no additional cases had been reported (Towner 2006).

In 2007 three cases in young males, again associated with miners working in Lead and Gold mines in the Kamwenge District of Uganda was reported (WHO 2007). Finally in 2008, a Dutch tourist developed Marburg four days after returning to the Netherlands from a three week holiday in Uganda. To date, the source of the exposure has not been confirmed, although the woman visited Python Cave in western Uganda where bats were present (WHO 2008, 2009; Timen *et al.* 2009). The Ugandan Ministry of Health closed the cave after this case.

Ebola virus disease (Africa)

Two large simultaneous outbreaks of an acute haemorrhagic fever (subsequently named Ebola haemorrhagic fever) occurred in southern Sudan (WHO 1978*a*) and northern Zaire in 1976 (WHO 1978*b*) (Fig. **31.2b**). The first outbreak was identified in southern Sudan in June, continuing through to November 1976. There were 284 cases, 67 in Nazara, 213 in Maridi, 3 in Tembura, and 1 in Juba. The focus of the epidemic was Nazara, a small town with clusters of houses scattered in dense woodland bordering the African rain-forest zone. The outbreak in Nazara originated in three employees of a cotton factory situated near the town centre. Detailed factory records for the previous 2 years did not show any fatal haemorrhagic disease in Nazara until June 1976. At that time, one or two workers started dying each week. By September, 6 factory workers and 25 of their contacts had developed the same illness, of which 21 died. Before the outbreak died out spontaneously cases were reported in two neighbouring areas. The first was in Tembura, a small town 160 km north of Nazara, where an ill woman went to be nursed by her family. Before she died, three women who cared for her also died of the same haemorrhagic disease. No other cases were discovered. Secondly, the epidemic was dramatically amplified by the larger hospital-associated outbreak (213 cases) in Maridi, following the introduction of a patient from Nazara. Maridi, a town east of Nazara, had an estimated population of 10,000 people. Ninety-three cases (46%) acquired their disease in hospital, and 105 (52%) in the community. Of the 230 staff at Maridi hospital, 72 became infected while at work and 41

died. The highest rate of infection was associated with nursing haemorrhagic patients who, at the height of the epidemic, occupied most wards. After Maridi, further cases (one from Nazara and three from Maridi) were transferred to the regional hospital in Juba. A further three patients were flown to Khartoum (1,200 km north), where two died. A nurse from Juba was the only secondary case identified as a result of these patient transfers.

Overall, there were 151 deaths (mortality rate, 53%) out of 284 cases. The outbreak in Nazara continued until October, infecting 67 people of whom 31 (46%) died, compared to 116 (54%) in Maridi. Studies on 36 families who nursed 38 primary cases indicated that of 232 contacts 30 (14%) developed disease. Similar rates of transmission were observed in subsequent generations, giving an overall secondary attack rate of 12%. Between July and October 1979, 34 cases of Ebola haemorrhagic fever recurred in the Yambio-Nazara District. Five family groups and two individuals became contracted the illness. The index case, a cotton-factory worker, transmitted the virus to several members of his family. The outbreak extended to other families through nosocomal transmission during his hospitalization and the hospitalization of subsequent cases. Mortality in this outbreak was 65% (22 deaths). It was unclear whether the cotton factory was the source of the index case of infection.

Also during September to October 1976, a large outbreak of Ebola haemorrhagic fever took place in the equatorial rain forest of northern Zaire (WHO 1978*b*). The index case had been touring and presented himself to the outpatient clinic at Yamkuka Mission Hospital (YMH) for treatment of acute malaria, and received an injection of chloroquine. He was admitted to hospital 10 days later with gastrointestinal bleeding and died. During the following week, nine other patients who had received treatment at the outpatient clinic of the YMH contracted Ebola haemorrhagic fever. Almost all subsequent cases had received injections or had been in close contact with other patients. The highest incidence was amongst women of 15–29 years, who had attended antenatal and outpatient clinics at this hospital and returned to their villages. After 13 of 17 staff had acquired the disease and 11 patients died, the hospital closed. The major risk factor proved to be the re-use of non-sterilized needles, which were in short supply. Between 1 September and 24 October, there were 318 known cases of Ebola haemorrhagic fever with 280 deaths, a case fatality of 88%. There were over 55 villages in the endemic area (Bumba zone), containing some 550 recorded cases. The overall secondary attack rate was calculated to be 5%, but nearer 20% in close relatives of a case. A further single fatal case was identified in Tandala Hospital in northwestern Zaire in 1977 (Heymann *et al.* 1980). Studies in the Tandala region revealed two possible Ebola cases dating back to 1972, and a 7% Ebola seroprevalence rate in the local population.

In January 1995, a charcoal-maker near Kikwit, a town in Bandudu Province, 550 km east of Kinshasa, was the first fatal case of Ebola (WHO 1995*a*). By early March 12 members of his family had died. Simultaneously, a *Shigella* I dysentery epidemic was taking place and masked the early stages of the Ebola outbreak. Hospitals became sources of infection during February when an Ebola patient entered the Kikwit health centre and later transferred to the general hospital. During April, members of a resuscitating team became ill after handling a patient misdiagnosed as having typhoid. Rapid transmission of unprotected health workers and other patients occurred, many carrying the disease back into the community. Between January and June 315 cases had occurred, of which 244 died (77%). The female: male ratio was 166: 149, which 74%(123) females and 81 per cent (121) males died. Provisional data identified 75 (26%) nurses or students and 61 (21%) housewives who contracted the disease. Two hundred and sixty-six (84%) of the cases resided within the Kikwit North and South Zones de Santé. No cases identified outside the Bandundu region.

The geographic distribution of Ebola virus widened in November 1994 when a non-fatal Ebola haemorrhagic fever case emerged for the first time in West Africa (LeGuenno *et al.* 1995). In the Tai National Park, Côte d'Ivoire, a 34 year old ethnologist infected herself while carrying out a post-mortem on a wild chimpanzee found dead with signs of haemorrhages. Eight days later, she was admitted to hospital in Abidjan for suspected malaria. As there was no improvement in her condition, she was repatriated by Swiss Air Ambulance and admitted to the University Hospital of Basel. As haemorrhagic fever was considered unlikely, she was treated with ciprofloxacin and deoxycyclin for suspected Gram-negative sepsis (typhoid fever), leptospirosis, or rickettsial disease. Retrospective studies isolated the Ebola virus. No clinical illness nor seroconversions were detected among 22 contact persons in the Côte d'Ivoire or 52 hospital and air-ambulance staff based in Switzerland, despite the lack of an early Ebola diagnosis. Late in 1995, a 25 year old refugee from neighbouring Liberia was admitted to the health facility of Gozon, Côte d'Ivoire, confirming the presence of Ebola virus in West Africa (Centers for Disease Control (CDC) 1995*c*). Patient isolation and barrier nursing prevented further spread of infection within and from the health facility. Preliminary investigation of his household contacts in the village of Plibo, Maryland County, Liberia found that two male contacts showed the signs and symptoms of early Ebola infection.

Zaire ebolavirus (ZEBOV) has repeatedly caused large outbreaks in Gabon between 1994 and 2008 (Geordes 1999: WHO 2003; Georges 1998; Pourrut, 2001), in DRC between 2001 and 2005 (Formenty 2003) and in the DRC in 1995 and 2007. In 2007, 103 people (100 adults and three children) were infected by a suspected hemorrhagic fever outbreak in the village of Kampungu, DRC. The outbreak started after the funerals of two village chiefs, and 264 people in four villages fell ill. The Congo's last major Ebola epidemic killed 245 people in 1995 in Kikwit, about 200 miles from the source of the August 2007 outbreak (WHO 2007). In 2004, it resurfaced around Gulu in Uganda (Okware 2002). Again, the disease was amplified in local hospitals and spread into several Ugandan administrative districts. There were at least 425 human cases

and 224 deaths (Bitekyerezo 2002). On 30 November 2007, the Uganda Ministry of Health confirmed an outbreak of severe gastrointestinal disease caused by Ebola in the Bundibugyo District of Uganda. After confirmation of samples tested by the USA National Reference Laboratories and the CDC, the WHO confirmed the presence of a new species of *Ebolavirus* that is now named Bundibugyo. The epidemic ended on 20 February 2008. While it lasted, 149 cases of this new strain were reported, and 37 proved fatal. (Tower, 2008). The fifth ebolavirus, 'Uganda ebolavirus' ('UEBOV') was discovered during this outbreak of 2007. Ebola virus again caused an outbreak that killed 15 and infected 32 people in southern DRC in January 2009. Angola closed down part of their border with DRC to prevent the spread of Ebola after their experience with Marburgvirus in 2 004which killed 227 people.

Finally, the risks of filovirus research were demonstrated in 12 March 2009, when an unidentified 45 year old female scientist from Germany accidentally pricked her finger with a needle used to inject Ebola into laboratory mice. She was given an experimental vaccine never before used on humans. It remains unclear whether or not she was ever infected with the virus or if the experimental vaccine proved beneficial.

As in most ebolavirus-disease outbreaks, it is recognized that burial rituals and caring of and close contact with the sick contributed to the spread of filoviruses (Gear 1975). Most of these outbreaks began with people who had hunted animals in the forest or found dead animals and consumed them. The mechanism of transmission of infection in the Ebola virus outbreaks is mainly by direct contact with infected blood or tissue, by very close and prolonged contact with acutely ill patients, or by inoculation with contaminated syringes and needles. Transmission through the airborne route does not seem to be a factor in the maintenance of any of the epidemics. It is also thought that especially Zaire ebolvirus is causing epizootics among central chimpanzees (*Pan troglodytes troglodytes*) and western lowland gorillas (*Gorilla gorilla gorilla*), which may have contribute dramatically to their population decline (Karesh 2005).

Natural reservoirs

The natural reservoir of the Ebola virus is unknown despite extensive studies, but it seems to reside in the rain forests on the African continent and in the Western Pacific. Between 1976 and 1998, various mammals, birds, reptiles, amphibians, and arthropods from outbreak regions have been studied to determine the natural Fiolovirus reservoir. No *Ebolavirus* was detected apart from some genetic material found in six rodents (*Mus setulosus and Praomys*) and one shrew (*sylvisorex ollula*) collected from the Central African Republic (Peterson 2004).The virus was detected in the carcasses of gorillas, chimpanzees, and duikers during outbreaks in 2001 and 2003, which later became the source of human infections. However, the high mortality from infection in these species makes them unlikely as a natural reservoir.

Plants, arthropods, and birds have also been considered as possible reservoirs; however, bats are now considered the most likely candidate. Bats were known to reside in the cotton factory in which the Ebola index cases for the 1976 and 1979 outbreaks were employed. They have been implicated in Marburg infections in 1975 and 1980. Of 24 plant species and 19 vertebrate species experimentally inoculated with *Ebolavirus*, only bats became infected (Swanepoel 1996). The absence of clinical signs in these bats is characteristic of a reservoir species. In a 2002–2003 survey of 1,030 animals, which included 679 bats from Gabon and the DRC, 13 fruit bats were found to contain *Ebolavirus* RNA (Pourrut 2009). As of 2005, three fruit bat species (*Hypsignathus monstrosus, Epomops frangueti,* and *Myonycteris torquata*) have been identified as carrying the virus while remaining asymptomatic. Studies of Egyptian fruit bats (*Rousettus aegyptiaxcus*) inhabiting the Kitaka Cave, Uganda isolated genetically diverse Marburgvirus RNA from tissue and demonstrated virus specific antibodies (Towers 2009). To date, Gabon is the only country where bat (*Rousettus aegyptiaxcus*) proved to be the reservoirs for both Ebola and Marburg viruses. Thus, various species of bats indicate that they are potential natural host species, or reservoir, of filoviruses.

Reston ebolavirus—unlike its African counterparts—is non-pathogenic in humans. The high mortality among monkeys and its recent emergence in pigs makes them unlikely natural reservoirs.

Epizootics

Ebola virus

Several filoviruses closely related to Ebola were isolated in 1989 and early 1990 from sick or dying cynomolgus macaques in quarantine facilities located in Reston, Virginia, in Texas, and in Pennsylvania (CDC 1990*a*; Jahrling *et al.* 1990). Shipments to a quarantine facility in Siena, Italy in 1992 also contained animals that died with laboratory-confirmed Ebola-like virus infections (WHO 1992). The monkeys involved in each epizootic imported from the Philippines and traced to the same major export facilities. The identification of an Ebola-like virus in each facility led to the termination of all stocks, to reduce the risk of community spread. In the absence of any established link with Africa or African animals, the episode must represent evidence of Asian filoviruses. The animals being co-infected with simian haemorrhagic fever complicated the first reported epizootic in the USA; however, 223 of 1,050 exposed animals died. The natural host and geographic distribution is unknown.

Active transmission was documented at one Philippine export facility (Hayes *et al.* 1992). Antigen capture ELISA using liver homogenates revealed that 85 out of 161 (53.2%) monkeys that died within a 3 month period proved positive for filovirus antigen. The incidence was calculated to be 24.4 per 100 animals. Here captive monkeys were held in gang cages, increasing the opportunity for monkey-to-monkey transmission of virus by close contact with virus-laden blood or body secretions. The source of the infection remains unknown. Laboratory experimentation also shows the presence of high concentrations of viral antigen in pulmonary secretions, raising the possibility that the airborne route (Jaax *et al.* 1995) may spread this ebola-like virus. Parenteral inoculation with virus-contaminated blood is another possible route during the epizootics as all monkeys were tuberculin tested and given antibiotics. The common practice was to inoculate many monkeys with the same syringe and needle.

Serological evidence of filovirus exposure was found in 12 (6%) of 186 people who lived in wildlife collection areas or worked in primate facilities in Manila (Miranda *et al.* 1991). Within the export facility experiencing the epizootic, 22% of employees tested proved positive. No illness was documented in any of the positives. In the Reston facility, five animal handlers were identified as having a high level of daily exposure to sick and dying animals. Four were found by IFA to have had serological evidence of recent infection, three seroconverting during the period of the epizootic. No filovirus illness developed. None of the 16 contacts of monkeys with Ebola-like virus imported into Italy showed any clinical or serological signs of infection.

Since the discovery of the Marburg and Ebola species of filovirus, seemingly random, sporadic fatal outbreaks of disease in humans and non-human primates have given impetus to identification of host tropisms and potential reservoirs. Domestic swine in the Philippines, experiencing unusually severe outbreaks of porcine reproductive and respiratory disease syndrome, have now been discovered to host *Reston ebolavirus* (REBOV). Although REBOV is the only member of *Filoviridae* that has not been associated with disease in humans, its emergence in the human food chain is of concern. REBOV isolates were found to be more divergent from each other than from the original virus isolated in 1989, indicating polyphyletic origins and that REBOV has been circulating since and possibly before, the initial discovery of REBOV in monkeys. The discovery of a number of seropositive abattoir workers in the Philippines having routine contact with the virus and seropositive swine has raised concern about the transmission of Ebola from animal to man (Barrette 2009).

The origin in nature and the natural history of Marburg and Ebola viruses remain unknown. It would seem that the viruses are zoonotic and transmission to humans occurs from ongoing life cycles in animals. Studies attempting to discover the source of the Marburg outbreaks in Europe or Africa, or the recent Ebola outbreak in the USA and Italy, have failed to uncover the reservoir. Whatever their source, person-to-person transmission is a means by which outbreaks and epidemics progress. This involves close contact; secondary cases have rarely exceeded 10%, indicating that transmission is not efficient. Nosocomial infection is a special case; extreme care should be taken when dealing with infected blood, secretions, tissues, and hospital waste.

Prevention and control

Prevention

Without an understanding of the natural history of the viruses, ecological controls capable of preventing the sporadic human cases that have started outbreaks and epidemics in the past are impossible. The exception would be the containment of monkeys, which might have the infections. While there is a strong suspicion that Ebola and Marburg diseases are zoonoses, the search continues for the origin and reservoir host(s) of the virus.

Control strategies

Although both Marburg and Ebola infections are rare events, they represent a dangerous nosocomial hazard. Prompt identification of active cases is essential and is dependent upon an accurate and detailed history (Department of Health and Social Security and Welsh Office 1986; CDC 1995; WHO 1995*b*). It is clear from the filovirus epidemics encountered to date that hospitals have acted as the main amplifier of the disease to the community. Therefore, it is important that physicians working in the areas where haemorrhagic fevers occur should be aware that these diseases exist and that nosocomial spread is a high possibility if not recognized early and patients placed in complete isolation under barrier nursing conditions. In non-endemic areas, it is important to maintain awareness of the current viral epidemiological developments and the threat of importation. Prevention of person-to-person spread of the virus is essential to control. Three weeks prior to illness patients at highest risk have travelled into areas where viral haemorrhagic fever (VHF) has recently occurred; had direct contact with blood, body fluids, secretions, or excretions of a person or animal with VHF; or worked in a laboratory or facility that handles the viruses. The likelihood of acquiring Ebola or Marburg is extremely low if patients do not meet any of the criteria. Contacts of such patients must be placed under surveillance and not allowed to travel. High-risk contact is associated with direct contact with blood or body fluids from acutely ill humans or animals; sexual contact with a convalescent case; or through laboratory accidents. Thus, effective surveillance of high-risk contacts and isolation of further cases ensures rapid control of an outbreak. The cause of fever in patients returning from

Marburg and Ebola endemic areas is more likely to be other infectious disease (malaria or typhoid); therefore, evaluation and treatment of such infections needs urgent attention.

Patient containment

Control of outbreaks in endemic and non-endemic areas has been associated with the introduction of good hospital and laboratory infection control practices, with the isolation of febrile patients, careful handling of laboratory specimens and rigorous use of gloves and disinfectants. The containment of patients in plastic-film patient isolators (Trexlar) is favoured in the UK (Department of Health and Social Security (DHSS) and Welsh Office 1986). These are located within a room having filtered negative air pressure gradients, a separate effluent treatment plant for waste, an in-suite autoclave for solid waste, a shower, and a staff changing room. Many consider that the patient isolator reduces manual dexterity, introduces fatigue, inhibits the effectiveness of intensive care procedures, and hinders communication. Of most concern is that these isolators do not reduce the risk of injury by any sharp instruments and have no provision for resuscitation. This system is not used in endemic areas or recommended in the USA (CDC 1995) since the main risks are associated with direct inoculation of virus in blood or other material and the aerosol hazard considered low risk. Thus, it is recommended to confine patients to isolation in a single room with or without controlled filtered air. The most important consideration is staff training and supervision, the use of gloves and masks, and the mandatory use of a disinfection policy. The recommendations issued concerning the management of AIDS patients are considered adequate for containment of Filoviruses.

The repatriation to Switzerland of an acutely ill Ebola case originating in the Côte d'Ivoire in 1994 and Marburg case from Uganda to the Netherlands in 2008 demonstrates the need for vigilance, since filovirus infection was not considered or diagnosed until the patient had recovered. Had the normal barrier precautions not been undertaken, the potential for spread could have been devastating. Filovirus-infected patients should be isolated and barrier-nursed to prevent secondary infections. Handling, transportation, and testing of clinical material containing a high concentration of viruses should follow international and national guidelines.

Primate guidelines

As non-human primates are known to have introduced Marburg to Europe, and Ebola to the US and Italy, the management of transportation and quarantine facilities should ensure that personnel understand the hazards associated with handling non-human primates. Although the risk of infection is low, guidelines have been issued to minimize such risks in persons exposed to non-human primates during transport and quarantine (CDC 1990*b*; WHO 1990). Those at risk of infection include persons working in temporary or long-term holding facilities and persons who transport animals to these facilities (cargo handlers and inspectors). Monkeys, particularly those imported from Africa and Asia, are potential sources of a range of diseases, and severe illness or deaths in recently imported primates should be reported to health and veterinary authorities and investigated for a variety of infectious agents, including Ebola.

Captive monkeys frequently held in gang cages increase the opportunity for monkey-to-monkey transmission by virus-laden blood or body secretions. Studies of the epizootic in the USA and experimental infection studies have found high concentrations of viral antigen in pulmonary secretions, raising the possibility that filoviruses spread through the aerosol route.

Although the newly recognized Ebola virus from Asia apparently causes a fatal disease in cynomolgus macaques, initial evidence indicates that its ability to produce infection in humans may be less than that of the Ebola and Marburg viruses from Africa. However, because of the known severity of disease caused by other members of the Filoviridae, it would be premature to ignore the possibility of a possible public health threat posed by the Asian filoviruses. Four independent accounts concerning the importation of Filoviridae-infected primates into the USA and Europe from two areas of the world, Asia and Africa, increase the importance of introducing an infrastructure for the recognition, identification, and elimination polices to remove the possibility of spread.

The high degree of transmissibility among monkeys housed in confined conditions indicates the need for early identification of infected animals, both to protect the monkeys and to minimize the risk of human infection. The early detection of filovirus antigenaemia or nucleic acid would allow identification of infected animals before they become ill. Whether their elimination would prevent any outbreak has yet to be proven. At present, the potential threat from filovirus antibody-positive animals remains unclear, although there is no evidence that latent or constant infection has played any role in monkey-associated outbreaks. Improved quarantine and animal handling procedures need to be universally implemented to ensure no future outbreaks associated with wild-caught monkeys. Therefore, contact between monkeys and man should be limited and animal husbandry tightly controlled. Personnel handling animals should wear protective clothing, including rubber gloves and face respirator. All animal waste, cages, and other potentially contaminated items should be treated with appropriate disinfectants.

Finally, the increased concern for wildlife conservation and licensing of exporters/importers will further decrease the risk

of filovirus spread to man.

References

Baron, R., McCormick, J., and Zubeir, O. (1983). Ebola virus disease in southern Sudan: hospital dissemination in intrafamilial spread. *Bull. World Heal. Organ.*, **61:** 997–1003.

Barrette, R. W. et al. (2009). Discovery of Swine as a Host for the Reston *ebolavirus*. *Sci.*, **325:** 204–6.

Baskerville, A., Fisher-Hoch, S. P., Neilds, G. H., and Dowsett, A. B. (1985). Ultrastructure pathology of experimental Ebola haemorrhagic fever virus infection. *J. Path.*, **147:** 199–209.

Bausch, D. G., Nichol, S. T., Muyembe-Tamfum, J. J., *et al.* (2006). Marburg hemorrhagic fever associated with multiple genetic lineages of virus. *N. Engl. J. Med.*, **355:** 909–19.

Bwaka, M. A. et al. (1999). EbolaHemorrhagic Fever in Kikwit, Democratic Republic of the Congo: ClinicalObservations in 103 Patients. *J. Infect.* Dis., **179(suppl. 1):** S1–S7.

Bishop, D. H. L. and Pringle C. R. (1995). Order Mononegavirales. In: F. A. Murphy *et al. (ed.) Virus Taxonomy, Sixth Report of the International Committee on Taxonomy of Viruses,* pp. 265–67. Vienna: Springer-Verlag.

Bitekyerezo, M. C. *et al.* (2002). The outbreak and control of Ebola viral haemorrhagic fever in a Ugandan medical school. *Trop. Doct.,* **32**:10–15.

Borio, L. *et al.* (2002). Hemorrhagic Fever Viruses as Biological Weapons-Medical and Public Health Management. *JAMA,* **287:** 2391–2405.

Bowen, E. T. W., Platt, G. S., Lloyd, G. (1977). Viral haemorrhagic fever in southern Sudan and northern Zaire: preliminary studies on the aetiologic agent. *Lancet*, **1:** 571–73.

Bwaka, D.G. *et al.* (1999). Ebola heamoorhagic fever in Kikwit, Democratic Republic of the Congo: Clinical Observations in 103 patients. *J. Infect.* Dis., **179(suppl.):** S1–S7.

Centers for Disease Control (1990*a*). Ebola virus infection in imported primates⬜Virginia, 1989. *MMWR,* **38:** 831–38.

Centers for Disease Control (1990*b*). Ebola related filovirus infection in non-human primates and interim guidelines for handling non-human primates during transit and quarantine. *MMWR,* **39:** 22–30.

Centers for Disease Control (1995*c*). Management of patients with suspected viral haemorrhagic fever⬜US. *MMWR,* **44:** 475–79.

Department of Health and Social Security and Welsh Office (1986). The control of viral haemorrhagic fevers. London: HMSO.

Department of Health (2007). Transport of Infectious Substances *Best Practice Guidance for Microbiology Laboratories.* http://www.dh.gov.uk/publications.

Elliot, L. H., McCormick, J. B., and Johnson, K. M. (1982). Inactivation of Lassa, Marburg, and Ebola viruses by Gamma irradiation. *J. Clin. Microbiol.*, **16**(4): 704–8.

Emond, R. T. D. (1978). Isolation, monitoring, and treatment of a case of Ebola infection. In: S. R. Pattyn (ed.) *Ebola virus infection,* pp. 27–32. Amersdam: Elsevier/North Holland Biomedical Press.

Emond, R. T. D., Evans, E., Bowen, E., and Lloyd, G. (1977). A case of Ebola virus infection. BMJ, **2:** 541–44.

Feldman, H., Klenk, H. D., and Sanchez, A. (1993). Molecular biology and evolution of filoviruses. *Arch. Virol. Suppl.*, **7:** 87–100.

Feldmann, H., Nichol, S. T., Klenk, H. D., Peters, C. J., and Sanchez, A. (1994). Characterization of Filoviruses Based on Differences in Structure and Antigenicity of the Virion Glycoprotein. *Virol.,* **199:** 469–73.

Feldmann, H. *et al.* (2007). Effective post-exposure treatment of Ebola infection. *PLoS Pathog.*, **3**(1):e2.

Fisher-Hoch, S. P., Platt, G. S., Lloyd, G., Simpson, D. I., Neils, G. H., and Barett, A. J. (1983). Haematological and biochemical monitoring of Ebola infection in rhesus monkeys: implications for patient management. *Lancet*, **2**: 1055–58.

Fisher-Hoch, S. P. *et al.* (1985). Pathophysiology of shock and haemorrhage in a fulminating viral infection (Ebola). *J. Infect.* Dis., **152:** 887–94.

Fisher-Hoch, S. P., Perez-Oronoz, G. I., Jackson, E. L., Hermann, L. M., and Brown, B. G. (1992*a*). Filovirus clearance in non-human primates. *Lancet*, **340:** 451–53.

Fisher-Hoch, S. P. *et al.* (1992*b*). Pathogenic role of geographic origin of primate host and virus strain. *J. Infect. Dis.*, **166**(4): 753–63.

Formenty, P., Hatz, C., Le Guenno, B., Stoll, A., *et al.* (1999). Human Infection Due to Ebola Virus, Subtype Côte d'Ivoire: Clinical and Biologic Presentation. *J. Infect.* Dis., **179**(suppl. 1**):** S48–S53.

Formenty, P. *et al.* (2003). Outbreak of Ebola hemorrhagic fever in the Republic of the Congo, 2003: a new strategy? *Med. Trop. (Marseille),* **63(3)**: 291–95.

Gear, J. S. S. *et al.* (1975). Outbreak of Marburg virus disease in Johannesburg. *BMJ,* **4**: 489–93.

Geisbert, T. W. and Jahrling, P. B. (1990). Use of immunoelectron microscopy to show Ebola virus during the 1989 United States epizootic. *J. Clin. Pathol.*, **43:** 813–16.

Geisbert, T. W. et al. (2009**).** Single-Injection Vaccine Protects Nonhuman Primates against Infection with Marburg Virus and Three Species of Ebola Virus. *J. Virol.,* **14**(83): 7296–7304.

Georges, A. J., et al. (1999). Ebola hemorrhagic fever outbreaks in Gabon, 1994–1997: epidemiologic and health control issues. *J. Infect. Dis.*, **179:** S65–75.

Gibb, T. R., Norwood, D. A., Woollen, N., and Henchal, E. A. (2001). Development and evaluation of a fluorogenic 5′ nuclease assay to detect and differentiate between Ebola virus subtypes Zaire and Sudan. *J. Clin. Microbiol.*, **39:** 4125–30.

Grolla, A., Lucht, A., Dick, D., Strong, J. E., and Feldmann, H. **(**2005). Laboratory diagnosis of Ebola and Marburg hemorrhagic fever. *Bulletin. Soc. Pathol. Exotic.,* **98:** 205–9.

Haas, R. and Mass, G. (1971). Experimental infection of monkeys with the Marburg virus. In: G. A. Martini and R. Siegert (eds.) *Marburg virus* disease, pp. 136–43. Berlin: Springer-Verlag.

Hayes, C. G. et al. (1992). Outbreak of fatal illness among captive macaques in the Philippines caused by an Ebola-related filovirus. *Am. J. Trop. Med. Hyg.*, **46:** 664–71.

Henderson, B. E., Kissling, R. E., Williams, M. C., Kafuko, G. W., and Martin, M. (1971). Epidemiological studies in Uganda relating to 'Marburg agent'. In: G. A. Martini and R. Siegert (eds.) *Marburg virus* disease, pp. 19–23. Berlin: Springer-Verlag.

Heymann, D. L., Weisfeld, J. S., Webb, P. A., Johnson, K. M., Cairns, T., and Berquist, H. (1980). Ebola haemorrhagic fever: Tandala, Zaire, 1977–1978. *J. Infect. Dis.*, **56:** 247–70.

Jaax, M. *et al.* (1996). Transmission of Ebola virus (Zaire strain) to uninfected monkeys in a biocontainment laboratory. *Lancet*, **343**: 1669–71.

Jahrling, P. B. *et al.* (1990). Preliminary report: isolation of Ebola virus from monkeys imported to USA. *Lancet*, **335:** 502–5.

John, S. P., Wang, T., Steffen, S., Longhi, S., Schmaljohn, C. S., and Jonsson, C. B. (2007). The Ebola Virus VP30 is an RNA Binding Protein. *J. Virol.,* **81:** 8967–76.

Johnson, E. D., *et al.* (1996). Characterization of a new Marburg virus isolated from a 1987 fatal case in Kenya. *Arch. Virolol.*, **11:** 101–14.

Karesh, W., and Reed, P. (2005). Ebola and great apes in Central Africa: current status and future needs. *Bull. Soc. Path. Exotic.,* **98:** 237–38.

Kiley, M. P. *et al.* (1982). Filoviridae: a taxonomic home for Marburg and Ebola viruses? *Intervirol.*, **18:** 24–32.

Ksiazek, T. G., Rollin, P. E., Jahrling, P. B., Johnson, E., Dalgard, D. W., and Peters, C. J. (1992). Enzyme immunosorbent assay for Ebola virus antigens in tissues of infected primates. *J. Clin. Microbiol.,* **30:** 947–50.

Ksiazek, T. G. *et al.* (1999). Clinical virology of Ebola hemorrhagic fever (EHF): virus, virus antigen, and IgG and IgM antibody findings among EHF patients in Kikwit, Democratic Republic of the Congo, 1995. *J. Infect.* Dis., **179:** S177–87.

Ksiazek, T. G., West, C. P., Rollin, P. E., Jahrling, P. B., and Peters, C. J. (1999). ELISA for the detection of antibodies to Ebola viruses. *J. Infect.* Dis., **179(Suppl. 1):** S192–98.

Kuzmin, I. V. *et al.* (2010). Marburg Virus in Fruit Bat, Kenya. *Emerg. Infect. Dis.*, **16**(2): 352–54.

Le Guenno, B., Formentry, P., Wyers, M., Gounon, P., Walker, F., and Boesch, C. (1995). Isolation and partial characterisation of a new strain of Ebola virus. *Lancet*, **345:** 1271–74.

Leroy, E. M. *et al.* (2000). Diagnosis of Ebola haemorrhagic fever by RT-PCR in an epidemic setting. *J. Med. Virol.,* **60:** 463–67.

Martini, G. A. (1969). Marburg agent disease in man. *Trans. R. Soc. Trop. Med. Hyg.*, **63:** 295–302.

Martini, G. A. (1971). Marburg virus disease, clinical syndrome. In: G. A. Martini and R. Siegert (eds.) *Marburg virus* disease, pp. 1–9. Berlin: Springer-Verlag.

Miranda, M. E. G., White, M. E., Dayrit, M. M., Hayes, C. G., Ksiazek. T. G., and Burns, J. P. (1991). Seroepidemiology study of filovirus related to Ebola in the Philippines [letter]. *Lancet*, **337:** 425–26.

Miranda, M. E. *et al.* (1999). Epidemiology of Ebola (subtype Reston) virus in the Philippines, 1996. *J. Infect.* Dis., **179**(Suppl 1): S115–19.

Mason, C. (2008). The strains of Ebola. *Can. Med. J.,* **178:** 1266–67.

Modrof, J., Becker, S., and Mühlberger, E. (2003). Ebola Virus Transcription Activator VP30 Is a Zinc-Binding Protein. *J. Virol.,* **77:** 3334–38.

Nikiforov, V. V. (1994). A case of Marburg virus laboratory infection. Zh. *Mikrobiol. Epid. Immun.,* **3:** 104–6, (Russian).

Noda, T. *et al.* (2006). *Assembly and budding of ebolavirus*. Plos Pathogens September; e99. Published online 2006 September 29. doi: 10.1371/journal.ppat.0020099.

Okware, S. I. *et al.* (2002). An outbreak of Ebola in Uganda. *Trop. Med. Int. Heal.,* **7:** 1068–75.

Peters, A., Sanchez, R., Swanepoel, B., and Volchkov, V. E. (2005). Family *Filoviridae.*, *In:* C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger, and L. A. Ball (eds.), Virus Taxonomy - Eighth Report of the International Committee on Taxonomy of Viruses. p. 645–53. San Diego, CA: Elsevier/Academic Press.

Peterson, A. T., Bauer, J. T., Mills, J. N. (2004). Ecology and geographic distribution of filovrus disease. *Emerg. Infect. Dis.,* **10:** 40–47.

Piot, P., *et al.* (1978). Clinical Aspects of EbolaVirus Infection in Yambuku Area, Zaire, 1976. *In:* S. R. Pattyn (ed.), *EbolaVirus Haemorrhagic Fever*, pp. 7–14. Amsterdam: Elsevier/North-Holland Biomedical Press.

Pourrut, X., *et al.* (2005). The natural history of Ebola virus in Africa. *Microbes Infect.,* **7:** 1005–14.

Pourrut, X., *et al.* (2009). Large serological survey showing cocirculation of Ebola and Marburg viruses in Gabonese bat populations, and a high seroprevalence of both viruses in *Rousettus aegyptiacus*. *BioMed. Cen. Infect. Dis.,* **9:** 159–69.

Pratt, W. D. *et al.* (2010). Protection of Nonhuman Primates against Two Species of Ebola Virus Infection with a Single Complex Adenovirus Vector. *Clin. Vacc.Immun.,* **17:** 572–58.

Pringle, C. R. (1991). Order Mononegavirales. *Arch. Virol.,* **117:** 137–40.

Reid, S. P., Valmas, C., Martinez, O., Sanchez, F. M., and Basler, C. F. (2007). Ebola virus VP24 proteins inhibit the interaction of NPI-1 subfamily karyopherin α proteins with activated STAT1. *J. Virol.,* **81:** 13469–77.

Richman, D. D., Cleveland, P. H., McCormick, J. B., and Johnson, K. M. (1983). Antigenic Analysis of Strains of Ebola Virus: Identification of Two Ebola Virus Subtypes. *J. Infect. Dis.,* **147:** 268–71.

Rollin, P. E., Ksiazek, T. G., Jahrling, P. E., Haines, M., and Peters, C. J. (1990). Detection of Ebola-like viruses by immunofluorescence. *Lancet*, **336:** 1591.

Saijo, M.,Niikura, M., Ikegami, T., Kurane, I., Kurata, T., and Morikawa, S. (2006). Laboratory Diagnostic Systems for Ebola and Marburg Hemorrhagic Fevers Developed with Recombinant Proteins. *Clin. Vacc. Immunol.,* **13**(4): 444–51.

Sanchez, A., Kiley, M. P., Klenk, H. D., and Feldman, H. (1992). Sequence analysis of the Marburg virus nucleoprotein

gene: comparison to Ebola virus and other non-segmented negative stand RNA viruses. *J. Gen. Virol.*, **73:** 347–57.

Sanchez, A., Kiley, M. P., Holloway, B. P., and Asuperin, D. D. (1993). Sequence analysis of the Ebola virus genome: organisation, genetic elements, and *comparison with the genome of Marburg virus. Vir. Res.,* **29:** *215–40*.

Smith, D. G., Francis, F., and Simpson, D. I. H. (1978). African haemorrhgic fever in the southern Sudan, 1976: the clinical manifestations. In: S. R. Pattyn (ed.) *Ebola virus haemorrhagic fever,* pp. 1–6. Amsterdam: Elsevier.

Smith, D. H. *et al.* (1982). Marburg-virus disease in Kenya. *Lancet*, **1:** 816–20.

Swanepoel, R., Leman, P.A., Burt, F. J. (1996). Experimental inoculation of plants and animals with Ebola virus. *Emerg. Infect. Dis.,* **2:** 321–25.

Teepe, R. G. *et al.* (1983). A probable case of Ebola virus haemorrhagic fever in Kenya. *E. Afric. Med. J.*, **60:** 718–22.

Timen, A. *et al.* (2009). Response to Imported Case of Marburg Hemorrhagic Fever, the Netherlands. *Emerg. Infect. Dis.,* **15**(8): 1171–75.

Towner, J. S. *et al.* (2004). Rapid Diagnosis of Ebola Hemorrhagic Fever by Reverse Transcription-PCR in an Outbreak Setting and Assessment of Patient Viral Load as a Predictor of Outcome. *J. Virol.,* **78:** 4330–41.

Towner, J. S. *et al.* (2007). Marburg Virus Infection Detected in a Common African Bat. PLoS One, **2:** article e764 [Epub Aug. 22, 2007] http://www.plosone.org.

Towner, J. S. *et al.* (2008). Newly discovered Ebola virus associated with hemorrhagic fever outbreak in Uganda. *PLos Pathogens,* **4**(11): e1000212. doi:10.1371/journal. ppat. 1000212.

Towner, J. S. *et al.* (2006). Marburgvirus genomics and association with a large hemorrhagic fever outbreak in Angola. *J. Virol.,* **80:** 6497–516.

Weik, M., Modrof, J., Klenk, H. D., Becker, S., and Mühlberger, E. (2002). Ebola Virus VP30-Mediated Transcription Is Regulated by RNA Secondary Structure Formation. *J. Virol.,* **76:** 8532–39.

World Health Organization International Commission to Sudan. (1978*a*). Ebola haemorrhagic fever in Sudan; 1976. *Bull. World Heal. Organ.*, **56:** 247–70.

World Health Organization/International Commission to Zaire (1978*b*). Ebola haemorrhagic fever in Zaire; 1976. *Bull. World Heal. Organ.*, **56:** 271–93.

World Health Organization (1990). Interim guidelines for handling nonhuman primates during transit and quaratine. *Wkly. Epidemiol. Rec.*, **65:** 45–52.

World Health Organization (1992). Viral haemorrhagic fever in imported monkeys. *Wkly. Epidemiol. Rec.*, **67:** 142–43.

World Health Organization (1995*a*). Ebola haemorrhagic fever, Zaire. *Weekly Epidemiol. Rec.*, **70:** 241–42.

World Health Organization (1995*b*). Viral haemorrhagic fevers management of suspected cases. *Wkly. Epidemiol. Rec.*, **35:** 249–52.

World Health Organization (1995*c*). Ebola haemorrhagic fever confirmed case in Côte d'Ivoire and suspected cases in Liberia. *Wkly. Epidemiol. Rec.*, **70:** 359.

World Health Organization (1996). Ebola haemorrhagic fever. *Wkly Epidemiol. Rec.*, **71:** 320.

World Health Organization (2003). Outbreak(s) of Ebola haemorrhagic fever, Congo and Gabon, October 2001 – July 2002 *Wkly. Epidemiol. Rep.,* **78**(26**)**: 223–25.

World Health Organization (2004). Ebola haemorrhagic fever in the Republic of the Congo—Update 6. *Wkly. Epidemiol. Rec.*, 6 January.

World Health Organization (2005). Outbreak of Ebola haemorrhagic fever in Yambio, south Sudan, April – June 2004. *Wkly Epidemiol. Rec.,* **80:** 370–75.

World Health Organization (2007). Ebola virus haemorrhagic fever, Democratic Republic of the Congo—Update. *Wkly. Epidemiol. Rec.,* **82:** 345–46.

World Health Orgaization (2007). *Guidance on regulations for the transport of infectious Substances*

www.who.int/csr/resources/publications/biosafety/WHO_CDS_EPR_2007_2cc.pdf.

World Health Organization (2007). Marburg haemorrhagic fever, Uganda. *Wkly Epidemiol. Rec.,* **82:** 297–8.

World Health Organization (2008). Case of Marburg Haemorrhagic Fever Imported into the Netherlands from Uganda. 10 July.

World Health Organization (2009). Ebola Reston in pigs and humans, Philippines. *Wkly Epidemiol. Rec.,* **84**: 49–50.

Wulff, H. and Johnson, K. M. (1979). Immunoglobulin M and G responses measured by immunofluorescence in patients with Lassa or Marburg virus infections. *Bull. World Heal. Organ.*, **57:** 631–35.

Zaki, S. R. *et al.* (1999). A novel immunohistochemical assay for the detection of Ebola virus in skin: implications for diagnosis, spread, and surveillance of Ebola hemorrhagic fever. Commission de Lutte contre les Epidemies a Kikwit. *J. Infect. Dis.,* **179(Suppl.1):** S36–47.

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