

"Filoviruses": a real pandemic threat?

Byron E.E. Martina, Albert D.M.E. Osterhaus*

Keywords: animal reservoir; Ebolavirus; Marburgvirus; pandemic threat

DOI emmm.200900005

Received November 21, 2008 / Accepted January 9, 2009

Introduction

It is generally appreciated that infectious diseases have had a major impact on the population ecology and the course of history. For instance, smallpox, an acute human viral disease caused by variola virus, was one of the most devastating viral diseases known to have affected mankind and human ecology. Consequently, it has had a major influence on the course of history. The prevalence of smallpox around the previous turn of the 19th century was about 50 million people per year and the disease had a case–fatality rate of approximately 30%. Thanks to a world-wide vaccination campaign orchestrated by the World Health Organisation (WHO) using an effective vaccine that had been developed about 200 years earlier, the prevalence of smallpox was reduced to 10 million people by the year 1967. In 1979, WHO announced that the eradication of smallpox from the globe was a fact, the first infectious disease to be eradicated completely. This first success triggered optimism about the feasibility to eradicate all major infectious diseases of the mankind (Snowden, 2008). However, the discovery of the filoviruses, Marburg virus (MARV) and Ebola virus (EBOV) in 1967 and 1976 respectively, refuelled the fear that viruses of this family *Filoviridae* could, similar to smallpox virus, sweep around the globe in a matter of weeks, and kill millions of people. That fear provided the incentive for several countries to establish an infrastructure for studies aiming at the development of intervention strategies for such highly pathogenic

Filoviruses are zoonotic and among the deadliest viruses known to mankind, with mortality rates in outbreaks reaching up to 90%. Despite numerous efforts to identify the host reservoir(s), the transmission cycle of filoviruses between the animal host(s) and humans remains unclear. The last decade has witnessed an increase in filovirus outbreaks with a changing epidemiology. The high mortality rates and lack of effective antiviral drugs or preventive vaccines has propagated the fear that filoviruses may become a real pandemic threat. This article discusses the factors that could influence the possible pandemic potential of filoviruses and elaborates on the prerequisites for the containment of future outbreaks, which would help prevent the evolution of filovirus into more virulent and more transmissible viruses.

(category 4) microorganisms. However, rather than witnessing such a pandemic filovirus outbreak, the world was instead confronted in the early 1980s with the more insidious pandemic viral outbreak of AIDS, caused by the human immunodeficiency virus (HIV). Although AIDS was identified only two and a half decades ago, the HIV pandemic belongs to the most devastating plagues in human history. Nevertheless, the high fatality rate of filovirus infections, the potential of filoviruses as bioterrorist agents and the high media attention for outbreaks caused by viruses of this family, have further increased the fear for outbreaks caused by these viruses. Furthermore, the increasing number of newly emerging and re-emerging virus infections in humans and animals in the past decades, as well as the general perception that the global capacity and infrastructure to adequately respond to such threats are insufficient, had had a negative impact on public confidence in our overall preparedness to combat such challenges. Obviously, the most important question in this regard is whether filoviruses do indeed pose a global threat and whether they may be the cause of future pandemics.

Historical view

The *Filoviridae* family consists of two genera, *Marburgvirus* and *Ebolavirus*, which harbour viruses that are morphologically identical but antigenically distinct. To date, only one subtype of

Erasmus MC, Department of Virology, Rotterdam, The Netherlands.
*Corresponding author: Tel: +31 10 7044066; Fax: +3110 7044760;
E-mail: a.osterhaus@erasmusmc.nl



Figure 1. Filovirus outbreaks reported in Africa. EBOV outbreaks are flagged in red and MARV in blue.

MARV has been described. In 1967, MARV was first identified in patients with a severe febrile syndrome, with signs of haemorrhage and shock, who were admitted to University hospitals in Marburg and Frankfurt (Slenczka & Klenk, 2007). The patients had been working in a pharmaceutical company and the infection was traced back to contacts with African green monkeys (*Cercopethicus aethiops*) imported from Uganda (Bonin, 1969; Kissling et al, 1968; Kunz et al, 1968; Siegert et al, 1967). All the primary cases had been in contact with blood, organs or cell cultures from these animals. All secondary cases involved medical personnel and family members who had been in contact with body fluids of these patients. In 1976, EBOV was discovered as a second member of the family (Pourrut et al, 2005). In contrast to MARV, five subtypes of EBOV have been described; Zaire (ZEBOV), Sudan (SEBOV), Reston (REBOV), Cote d'Ivoire (CEBOV) and Bundibugyo (BEBOV). A summary of all filovirus outbreaks known to date is provided in Table 1 and Figure 1 depicts the locations in Africa from where filovirus outbreaks were reported. Several conclusions may be drawn from the different filovirus outbreaks:

1. The cases of REBOV clearly illustrated that filoviruses could readily be imported into previously unaffected areas by means of international animal transports, nourishing the fear that filoviruses could spread worldwide with devastating consequences.
2. The MARV outbreak in Democratic Republic of Congo (DRC) in 1998 was unique because of the high case–fatality rate,

which had not been observed in previous MARV outbreaks. Furthermore, this outbreak strongly suggested that the reservoir host(s) for MARV would inhabit the caves and mines where the outbreaks started.

3. The Angola outbreak in 2004 represented the first appearance of MARV in western Africa and the largest outbreak reported to date (Ligon, 2005). The epidemiology of this outbreak was different in that a high percentage of children were infected, and the estimated incubation time was shorter with an even higher case–fatality rate of up to 92%.
4. Apparently, filovirus outbreaks are no longer restricted to remote, scarcely populated areas, but may strike in medium-size cities (e.g. Kikwit, 1995) and may be introduced in large cities (e.g. Johannesburg, 1975).

These observations together with the increase in filovirus outbreaks in the last 15 years, point at a seemingly changing epidemiology, and prompt the question whether filoviruses are becoming a threat to the world at large. Is there a need to prepare ourselves for a more extensive spread of filoviruses, which might eventually even lead to a pandemic filovirus outbreak?

Transmission

For a virus to become a real pandemic virus, it must in principle comply with all of the four following criteria:

- i. the population should be immunologically naive towards the virus;
- ii. it should be pathogenic;
- iii. it should have a short generation period;
- iv. it should have a basic reproduction number (R_0) greater than 1 (Box 1).

Glossary

Index case

The first identified case in an outbreak.

Nosocomial infection

An infection acquired while in hospital.

Sentinel animal

An animal intentionally placed in a specific environment to assess the presence of an infectious agent.

Haemostatic system

A system composed of vessel walls, blood platelets and soluble factors responsible for blood coagulation and fibrinolysis.

Parenteral exposure

Exposure of the internal systems of the body *via* any route except the alimentary canal.

Barrier precautions

Any means used to reduce contact with potentially infectious body fluids.

Generation period

The interval between infection and transmission to another person.

Box 1: Basic reproduction ratio

Basic reproduction ratio (R_0) is a key concept in epidemiology and is widely used to study infectious diseases. R_0 is defined as the average number of secondary infections produced by a single infected individual during the entire infectious period. This definition applies to a model where everyone in the population is susceptible. Therefore, determination of R_0 , assuming a SEIR (susceptible–exposed–infectious–recovered) model, is used to estimate the risk of an epidemic or pandemic. When R_0 is <1 , each infected individual produces on average less than one new infection and the disease will die out. When R_0 is >1 , the virus is able to persist in the susceptible population and cause an epidemic. Virus transmissibility is an important parameter that affects R_0 . Transmissibility, which is affected by the route of transmission, is the product of infectiousness and generation time (time between infection and excretion). SARS–corona virus emerged in 2003 as the cause of a fatal respiratory syndrome. The R_0 for SARS was determined to be between 2 and 4 in community-based settings (Lipsitch et al, 2003). The variation of the effective reproduction number reflects the control measures implemented during the SARS outbreak, such as early case detection, prompt contact tracing, strict isolation and quarantine and timely treatment, indicating that the response time and the strength of control measures, have significant effects on the scale of an outbreak and the lasting time of an epidemic. Interestingly, R_0 of the 1918 pandemic influenza virus spread has also been estimated between 3 and 4 for the

community-based setting (Vynnycky et al, 2007). Therefore, the rapid spread of pandemic influenza in 1918 as well as in other pandemics is more likely the result of a short generation time of influenza virus in humans (about 4 days) rather than a high R_0 . Influenza virus is thought to be infectious before the onset of symptoms whereas transmission of SARS coronavirus is thought to occur more than a week after infection and several days after the onset of symptoms (Lipsitch et al, 2003). An important concept in understanding outbreaks and determining the risks of becoming a pandemic is the minimum population size needed to maintain an infection in the population. Highly transmissible, acute virus infections with $R_0 >1$, require a large number of susceptible individuals to persist in the population. For example, it has been estimated that for measles to persist, the threshold population size must exceed 100,000 (Keeling & Grenfell, 1997). On the other hand, vector-borne infections can be maintained in populations with much smaller numbers of susceptible individuals. For newly emerging zoonotic infections with an $R_0 <1$, it is important to understand the disease ecology and identify the animal reservoir. If $R_0 >1$, and the infection is self-sustaining, understanding the ecology of the disease is less important than implementing control measures. Furthermore, it is important to realize that when appropriate control measures are not activated, zoonotic infections with an $R_0 <1$ may adapt to the host population, resulting in an $R_0 >1$.

Table 1. Outbreaks of filovirus infection in humans

Year	Location	Number of human cases	Case–fatality rate (%)	Virus strain	References
1967	Europe: Marburg, Frankfurt, Belgrade	31	26	MARV	(Kissling et al, 1968)
1975	South Africa: Johannesburg	3	33	MARV	(Gear et al, 1975)
1976	Africa: DRC	318	88	ZEBOV	(Johnson et al, 1977)
1976	Africa: Sudan	284	53	SEBOV	(WHO, 1978)
1977	Africa: DRC	1	100	ZEBOV	(Heymann et al, 1980)
1979	Africa: Sudan	34	65	SEBOV	(Baron et al, 1983)
1980	Africa: Kenya	2	50	MARV	(Smith et al, 1982)
1987	Africa: Kenya	1	100	MARV	(Johnson et al, 1996)
1989	USA: Virginia	0	0	REBOV	(Jahrling et al, 1990)
1990	USA: Pennsylvania	0	0	REBOV	(Groseth et al, 2002)
1992	Europe: Siena	0	0	REBOV	(Rec, 1992)
1994	Africa: Gabon	49	65	ZEBOV	(Georges et al, 1999)
1994	Africa: Cote d'Ivoire	0	0	CEBOV	(Formenty et al, 1999)
1995	Africa: DRC	317	77	ZEBOV	(Khan et al, 1999)
1996	Africa: Gabon	37	57	ZEBOV	(Georges et al, 1999)
1996	Africa: Gabon	62	74	ZEBOV	(Georges et al, 1999)
1996	USA: Texas	0	0	REBOV	(Rollin et al, 1999)
1998	Africa: DRC	154	83	MARV	(Bausch et al, 2003)
2000	Africa: Uganda	425	53	SEBOV	(Lamunu et al, 2004; Okware et al, 2002)
2001	Africa: Gabon	124	78	ZEBOV	(Leroy et al, 2002b)
2002	Africa: Gabon	11	91	ZEBOV	(Leroy et al, 2002b)
2003	Africa: DRC	143	90	ZEBOV	(Formenty et al, 2003)
2004	Africa: DRC	35	83	ZEBOV	(Leroy et al, 2004)
2004	Africa: Sudan	17	41	SEBOV	(Towner et al, 2004)
2004	Africa: Angola	252	92	MARV	(Ligon, 2005)
2005	Africa: DRC	11	82	SEBOV	(Rec, 2005; Sanchez & Rollin, 2005)
2007	Africa: Uganda	3	33	MARV	(Towner et al, 2007b)
2007	Africa: Uganda	29	36	BEBOV	(Towner et al, 2008)

DRC, Democratic Republic of Congo.

Box 2: Preparedness plan

As most filovirus outbreaks are the result of nosocomial infections or infections of family members who nurse patients without using protection measures, targeted plans need to be in place to contain filovirus outbreaks. CDC and WHO have published guidelines on how to contain viral haemorrhagic fever in African health care settings (Centers for Disease Control and Prevention & World Health Organization, 1998), which may be adopted according to other settings. Essentially, the actions to be implemented in an outbreak response plan include: (i) direct notification of a suspected case to the authorities, (ii) intensify surveillance to identify cases as early as possible, (iii) active listing,

tracing and follow-up of contact cases, (iv) patient isolation and barrier nursing, (v) inactivation of virus on contaminated materials with, for example, sodium hypochlorite and incineration of clinical waste, (vi) training of health care workers and provision of personal protective equipments, (vii) funerals to be performed by specialized teams that understand the risks involved with body preparation, but are familiar with the local beliefs and customs during burial proceedings, (viii) assignment of a taskforce entrusted with reviewing the outbreak response efforts.

The first two criteria are certainly applicable to filoviruses. The last two criteria are slightly more difficult to apply to filoviruses. Filoviruses are unlikely to be transmitted during the incubation period and transmissibility is generally highest late in the clinical course of infection. Most individuals who have acquired infections in the last few decades were infected by needle-stick injuries or reuse of unsterilized medical devices like needles and syringes. Furthermore, direct contact with blood, body secretions or tissues of infected humans and non-human primates have posed the main risk for virus transmission (Bausch et al, 2007). High numbers of filovirus particles can be found in sweat glands and the human skin (Zaki et al, 1999), suggesting that transmission may occur through direct contact and that nursing patients and preparing bodies for burial without practicing the appropriate barrier precautions, represent an important factor contributing to the spread of the infection. However, it remains unclear how the virus enters the body upon direct contact. It has been shown that administration of filoviruses into the mouth, nose, or conjunctiva of non-human primates resulted in infection (Jaax et al, 1996; Schou & Hansen, 2000). Therefore, it is conceivable that human infections occur through indirect contact of, for example, contaminated fingers with oral mucosa or conjunctiva. The REBOV outbreak and experimental infections carried out with ZEBOV have raised concerns that EBOV may be naturally transmitted by aerosol (Jahrling et al, 1990; Johnson et al, 1995). There is also circumstantial evidence that during the EBOV outbreak in DRC in 1995, some patients became infected through aerosol transmission (Roels et al, 1999). However, although aerosol transmission cannot be completely ruled out, the primary mode of transmission is through direct or indirect contact with an infected body and thus transmission of filoviruses is an inefficient process. Given the relatively limited transmissibility, eventually, the overall R_0 will not be greater than 1. Although initially several patients may become infected by an index case even resulting in a R_0 as high as 2.7 (Legrand et al, 2007), usually R_0 readily drops below 1 when people realize what kind of actions predispose for transmission and relevant measures to prevent further transmission are taken. Obviously, a filovirus may reach any place in the world within days through modern transportation. However, the extent to which the virus will spread after introduction in a new area will largely depend on

the preparedness plan (Box 2) that will be executed upon its introduction and the ability of the health care system to deal with infected patients and prevent further transmission.

Genetic stability and virulence

The question remains, whether there is a risk that filoviruses could mutate and become efficiently transmitted from person-to-person, *e.g.* by aerosol, and therefore may become a real pandemic threat. It has been postulated that if ever ZEBOV would cause an outbreak of significant size in a densely populated urban environment, the evolution towards an airborne variant could occur. Specifically, the argument has been put forward that a large enough epidemic would provide sufficient evolutionary pressure to the virus to give rise to an airborne variant. Since there is some evidence that REBOV infections may be airborne, a variant with an intrinsically high mutation rate could evolve towards an airborne virus. In general, RNA viruses encode error-prone polymerases that lack proofreading mechanisms, allowing these viruses to mutate and evolve under the appropriate selection pressures. It has been hypothesized that MARV strains that were involved in the DRC and Angola outbreaks in 1998 and 2004 respectively, were more virulent than the strains involved in the outbreak in Germany in 1967. Analysing the nucleotide sequences of the glycoprotein gene of both MARV and EBOV from different outbreaks indicates however that isolates from the same outbreak are almost identical in nucleotide sequences, whereas viruses recovered from different outbreaks may vary up to 20%, the majority of the mutations being silent (Towner et al, 2006). The genetic stability observed during the Angolan outbreak of MARV infection indicates that the outbreak was probably caused by a single introduction of the virus by one index case, followed by spread to community members and further amplification by nosocomial transmission (Towner et al, 2006). This sequence of events may explain the relatively limited accumulation of mutations observed during this outbreak. In contrast, the MARV outbreak of 1998 was characterized by circulation of multiple genetic lineages, as well as identical lineages within, but not across clusters of epidemiologically linked cases. This suggested that multiple introductions of MARV in the community had

occurred (Bausch et al, 2006). These sequence data are in agreement with the relatively limited number of secondary infections that were found. Taken together, there is no convincing data to suggest that genetic variation and selection has resulted in more virulent MARV strains during the DRC and Angolan outbreaks. Actually the virulence of the strains was comparable to that of those identified in 1967. More likely factors such as nutrition status, underlying co-infections, and quality of health care were involved in the different case-fatality rates observed among the different outbreaks. Future studies using reverse genetics technology will help us understand the possible biological significance of the few mutations observed in the different strains from different outbreaks. The initial high rate of infection among children in Angola was probably related to needle use and the high mortality recorded in that outbreak may have been related to the parenteral exposure as well, which has been associated with higher mortality rates (Emond et al, 1977). The same degree of genetic stability is observed among the respective EBOVs (Leroy et al, 2002a; Rodriguez et al, 1999). When the geographic distance between the different ZEBOVs is considered, it is estimated that the virus spreads at a constant rate of 50 km per year, and diversity between strains increases with distance (Walsh et al, 2005). Overall, filoviruses evolve slowly, but little is known about the biological consequences of accumulation of mutations. It is interesting to note that REBOV is less virulent than ZEBOV and SEBOV, and several factors may be involved in this difference (Morikawa et al, 2007). For instance, ZEBOV encodes a 17 amino acid peptide in the surface glycoprotein, which is involved in the apoptosis of lymphocytes and induction of immunosuppression (Yaddanapudi et al, 2006). The corresponding peptide in REBOV lacks this immunosuppressive effect. More studies are needed to understand the difference in virulence between viruses like ZEBOV and REBOV, which will help us to understand better the risk of filoviruses to eventually evolve, through mutation or recombination, to become more virulent and perhaps more importantly airborne.

Reservoirs of filoviruses

It is still a mystery as to which animal species constitute the reservoir host or hosts of filoviruses, and how they spread the virus geographically. Despite a lot of efforts to identify the natural reservoir of filoviruses in the last decade, little progress has been made. RNA of ZEBOV was detected in rodents and shrews captured in the Central African Republic, although serology and virus isolation were negative. In several outbreaks of filovirus infections, index cases were associated with visiting a cave or cave-like environments inhabited by bats. Experimental infections of fruit and insectivorous bats showed that filoviruses replicate to high titres in several organs (Swanepoel et al, 1996). Up to three weeks after infection, ZEBOV RNA could still be recovered from faecal samples. Furthermore, infected bats did not develop disease signs, which is an important condition to function as a natural host in which the virus might persist. Recently, evidence was

presented that three species of fruit bats caught near affected villages at the Gabon–Congo border, harboured RNA sequences of ZEBOV (Leroy et al, 2005). The three bat species have a broad geographical range that includes the regions where filovirus outbreaks had occurred. The sequences recovered cluster with strains found during human outbreaks, providing strong indications for the involvement of these bats in either transmission of EBOV to humans or to other types of introduction of the virus into new areas. There is evidence to suggest that the reservoir for MARV can be found in the same or related bat species (Towner et al, 2007a). However, it is not completely clear how humans and non-human primates may become infected by bats. Bat-scratch incidents and certain eating habits involving bat meat are possible risk factors. Clearly, more studies are needed to understand the role of bats in the ecology of filovirus infections of humans and animals. The outbreak in Angola in 2004 was surprising because MARV was not expected in that region, which was about 1,600 km far from the locations where previous MARV outbreaks had occurred (see Figure 1). If MARV would not have been present in Angola initially but was only recently introduced, it is possible that migratory animal species could have carried it over such a long distance, *e.g.* from Zaire to Angola. Birds have been proposed as possible hosts for filoviruses to accomplish such an introduction (Galat & Galat-Luong, 1997). However, there are no experimental studies to backup this hypothesis. It is not clear whether in this particular case bats could have played that role. Interestingly, epidemiological studies have suggested that EBOV has spread as a one-dimensional wave (Walsh et al, 2005). This has prompted some scientists to propose that rivers pose a barrier to the spread of filoviruses rendering both birds and bats unlikely candidate reservoirs. This theory has however been disputed by others (Lahm et al, 2007). Identifying the principle host species of filoviruses will hopefully contribute to new and more successful strategies for preventing and controlling future outbreaks.

Treatment

To date, there is no specific treatment for filovirus haemorrhagic fever and interventions are mainly supportive, aiming to maintain fluid and electrolyte balance, circulatory volume and blood pressure. Several studies suggested that both viral and immune-mediated factors are involved in the pathogenesis of filovirus infections. Development of fatal disease has been associated with high viral RNA copies (Towner et al, 2004). High levels of viral replication presumably lead to necrosis in cells of many organs, including liver and spleen, as can be inferred from microscopic examination of infected tissues (Zaki & Goldsmith, 1999). Therefore, use of effective antiviral drugs could reduce virus-induced pathology in infected individuals, thereby increasing the survival rates. The antiviral drug ribavirin, a nucleoside analogue that inhibits many RNA viruses, has been shown to be ineffective against filoviruses. Encouraging results are being reported with other candidate

drugs (Bray et al, 2000; Huggins et al, 1999) and more efforts should be deployed in developing and testing new antiviral drugs against filoviruses. Alternatively, intervention strategies may be directed against the aberrant host response to filovirus infection. Upon infection with a filovirus, it is believed that the interplay between the immune and haemostatic systems results in increased expression of the tissue factor (TF). The development of disseminated intravascular coagulation might be crucial in the cascade of haemorrhagic complications produced by filovirus infections. Experimental studies conducted in non-human primates indicated that 33% of animals infected with a lethal dose of ZEBOV and treated within 24 h with the TF-specific hookworm-derived inhibitor nematode anti-coagulant peptide c2 (NAPc2), survived the infection (Geisbert et al, 2003). These results should encourage further studies aiming to understand the role of the coagulation system in filovirus pathogenesis that will provide important targets for designing intervention strategies.

Preventive vaccines against filoviruses would also be useful, especially in endemic areas, laboratory settings and for primates whose populations are seriously threatened. Furthermore, the classification of filoviruses as potential bioterrorist agents makes development of a safe and effective vaccine a priority. However, the development of effective filovirus vaccines represents a challenge in the face of a lack of understanding of the correlatedness of protection. The role of antibodies in protection and recovery of the host from a filovirus infection is controversial. On the one hand it has been observed that failure of infected patients to develop filovirus-specific antibody response by the second week of onset of symptoms is associated with poor prognosis. However, in many patients, neutralizing antibodies cannot be detected, and therefore the biological function of filovirus-specific non-neutralizing antibodies remains unclear. Treatment of guinea pigs infected with ZEBOV using hyperimmune equine IgG has been shown to be protective if administered early after infection (Jahrling et al, 1999). Such preparations provided variable results when used on humans and non-human primates (Kuhn, 2008). Also the role of cell-mediated immune response in recovery from infection remains unclear. In mice, T-cells have been shown to play a role in protection against ZEBOV (Warfield et al, 2005). Some evidence exists that responses of CD8+ T-cells are elicited in macaques upon infection as well as by vectored vaccines such as adenovirus-based vaccines (Sullivan et al, 2003). Different vaccine platforms have been investigated and, in general, several vaccines have been shown to be effective in rodent models, but have failed to induce protective immunity in non-human primates (Kuhn, 2008). Surprisingly, most vaccine platforms studied did not induce neutralizing antibody responses or resulted in only very low levels. The immunosuppressive nature of the infectious filovirus has been partially elucidated (Zampieri et al, 2007), however, it remains unclear why inactivated, but adjuvanted vaccines or even vectored vaccines do not induce neutralizing antibody responses. It is of paramount importance to understand why most vaccines failed to induce neutralizing antibody responses and whether neutralizing antibodies are protective. Some of the most

promising vaccination strategies studied involved the prime/boost (DNA vaccine/a subunit vaccine) (Vastag, 2004), and vectored vaccines based on adenovirus or vesicular stomatitis (Geisbert et al, 2008; Sullivan et al, 2006). These vaccines provided protection to non-human primates against lethal infection with EBOV or MARV. Although such vaccination strategies seem effective in protecting animals against homologous challenge, they most likely do not provide a broad-spectrum protection against different filovirus subtypes. Clearly, there is a need to understand the correlates of protection, in order to develop safe and effective vaccines against filoviruses. Viral vectors that can harbour multiple genes, like the complex adenovirus (CAAdVax) or Modified Vaccinia Ankara (MVA), may be suitable candidates for developing pan-filovirus vaccines (Swenson et al, 2008).

Future challenges

One of the daunting challenges in filovirus research is the identification of the animal reservoir for EBOV and MARV. Despite numerous hypotheses on this theme and studies conducted in several vertebrates, little is known about the reservoir and whether or not filoviruses have a single reservoir with a sustained transmission cycle. Furthermore, it is unclear what role vectors play, if any, in the transmission cycle of filovirus infections. In order to address these issues, different studies must be designed for collection of potential reservoir host candidates. In order to facilitate serological studies, methods should be developed that allow large-scale testing of a wide variety of animals in a species-independent way. In addition, serological assays should provide enough cross-reaction between the different EBOV subtypes. The use of sentinel animals should be considered as it may prove to be superior to random testing of wildlife animal species. Experimental studies should attempt to identify animal species that support persistent infection without developing clinical signs. Knowing the reservoir of filoviruses may contribute to making predictions about future outbreaks and estimating the risk that certain events or activities, such as climate change or deforestation, may have on facilitating outbreaks.

Pending issues

- Identification of the animal reservoir(s) for EBOV and MARV.
- Understanding the transmission cycle of filovirus infections.
- Understanding the determinants of virulence of ZEBOV compared to REBOV.
- Understanding the immune response to the virus, viral induction of immune suppression and identifying the correlates of protection for vaccine testing.
- Further development of effective pan-filovirus preventive vaccines.

It is important to understand the determinants of virulence of ZEBOV compared to REBOV infections. Studies using reverse genetics may help to pinpoint the molecular mechanisms by which ZEBOV exhibits high virulence in humans. Studies in both rodents and non-human primates showed that detrimental host responses to filovirus infection may be central to the pathogenesis. Therefore, studies using state-of-the-art technologies such as messenger profiling, should be designed to compare the host responses to REBOV with that against the more virulent ZEBOV. The production of a secretory form of glycoprotein (sGP) by EBOV but not by MARV has also been implicated in the higher virulence of certain EBOV subtypes. However, the emergence of a MARV strain in DRC and Angola with case-fatality rates as high as ZEBOV strains suggests that sGP is not a critical virulence factor. Studies using viruses pseudotyped with the different filovirus glycoproteins will help elucidate the role of several regions in virulence and pathogenesis.

Conclusions

After an era of rapidly increasing control of many infectious diseases in the industrialized world, it was believed that advances in medical technology such as the development and implementation of vaccination strategies and the wide use of antibiotics, would eventually lead to the control and eradication of most of the infectious diseases that have plagued mankind over the centuries. The emergence and re-emergence in the last few decades of a whole range of infectious diseases, most of them caused by viruses coming from the animal world (Kuiken et al, 2003; Osterhaus, 2001), made it painfully clear that infectious diseases will continue to have a significant impact on global health and economies. Major changes in our globalizing society have created a complex mix of interacting predisposing factors that continue to create new niches for such emerging infections (Martina & Osterhaus, 2007). The emergence of HIV, SARS and avian influenza in humans are striking examples of this increasing trend. New viruses with the potential to cause major epidemics and even pandemics in human society will emerge from the animal world. Early warning systems and pandemic preparedness plans should provide us with the tools to combat or perhaps even prevent such events from happening. An unprecedented and successful example of such an internationally coordinated strategy was the containment of the SARS coronavirus, that was on the way to become pandemic in 2003 (Heymann, 2004).

The recent filovirus outbreaks in Africa underscore the need for improved disease surveillance and early warning systems, which become increasingly important for diseases of relatively low occurrence, but with a major public health impact at the international level (Kuiken et al, 2005). The lack of adequate laboratory facilities and integrated disease surveillance systems, especially in regions where filoviruses are endemic, will largely prevent these regions from being monitored for disease trends and initiate the required actions. This is exemplified by the ZEBOV outbreak in Kikwit in 1995, where the disease was first

misdiagnosed as shigella dysentery and was recognized as an EBOV outbreak only after 12 weeks. The international scientific and health communities must assist countries involved in building the appropriate infrastructure that will allow early detection of filovirus outbreaks and spark off an adequate and rapid response. Unfortunately, in areas with social instability, the implementation of such an infrastructure has proven to be an extra challenge. More efforts must also be deployed to educate the general public and medical personnel regarding the mode of transmission of filoviruses and the role of barrier nursing in protecting those in close contact with the patient. For example, the need to isolate patients has not always been fully accepted by the people involved. Furthermore, family members of suspected patients are usually reluctant to bring patients to a hospital, since they associate isolation of patients with death (Ligon, 2005).

In conclusion, it has become apparent over the past 15 years that filovirus infections pose a definite public health risk in those areas where contacts with the still largely unknown reservoir may occur and early warning systems as well as preparedness plans are hard to implement. Obviously, sporadic cases with relatively limited potential for further spread may continue to occur in industrialized countries with more adequate healthcare systems. In spite of the clear public health risks in the endemic areas involved, due to the rapidly decreasing R_0 during outbreaks, which at least in part results from a changing risk perception within the affected communities, outbreaks of filovirus infections have been self-limiting, and have never affected more than 500 people. This clearly limits the potential of these viruses to constitute a real pandemic threat as was recently illustrated in The Netherlands, where an imported case of MARV infection was rapidly contained preventing any secondary cases (Timen et al, 2008).

The authors declare that they have no conflict of interest.

For more information

CDC Filoviruses:

<http://www.cdc.gov/ncidod/dvrd/Spb/mnpages/dispages/filoviruses.htm>

WHO guidelines for preparedness and response to Ebola:

http://www.who.int/csr/resources/publications/ebola/WHO_EMC_DIS_97_7_EN/en/index.html

References

- Baron RC, McCormick JB, Zubeir OA (1983) Ebola virus disease in southern Sudan: hospital dissemination and intrafamilial spread. *Bull World Health Organ* 61: 997-1003
- Bausch DG, Borchert M, Grein T, Roth C, Swanepoel R, Libande ML, Talarmin A, Bertherat E, Muyembe-Tamfum JJ, Tugume B, et al (2003) Risk factors for Marburg hemorrhagic fever, Democratic Republic of the Congo. *Emerg Infect Dis* 9: 1531-1537
- Bausch DG, Nichol ST, Muyembe-Tamfum JJ, Borchert M, Rollin PE, Sleurs H, Campbell P, Tshioko FK, Roth C, Colebunders R, et al & International Scientific and Technical Committee for Marburg Hemorrhagic Fever Control in the Democratic Republic of the Congo (2006) Marburg hemorrhagic fever associated with multiple genetic lineages of virus. *N Engl J Med* 355: 909-919

- Bausch DG, Towner JS, Dowell SF, Kaducu F, Lukwiya M, Sanchez A, Nichol ST, Ksiazek TG, Rollin PE (2007) Assessment of the risk of Ebola virus transmission from bodily fluids and fomites. *J Infect Dis* 196 (Suppl 2): S142-S147
- Bonin O (1969) The Cercopithecus monkey disease in Marburg and Frankfurt (Main), 1967. *Acta Zool Pathol Antverp* 48: 319-331
- Bray M, Driscoll J, Huggins JW (2000) Treatment of lethal Ebola virus infection in mice with a single dose of an S-adenosyl-L-homocysteine hydrolase inhibitor. *Antiviral Res* 45: 135-147
- Centers for Disease Control and Prevention & World Health Organization, C. W. (1998). *Infection Control for Viral Hemorrhagic Fevers in the African Health Care Setting*. Atlanta: CDC.
- Emond RT, Evans B, Bowen ET, Lloyd G (1977) A case of Ebola virus infection. *Br Med J* 2: 541-544
- Formenty P, Hatz C, Le Guenno B, Stoll A, Rogenmoser P, Widmer A (1999) Human infection due to Ebola virus, subtype Cote d'Ivoire: clinical and biologic presentation. *J Infect Dis* 179 (Suppl 1): S48-S53
- Formenty P, Libama F, Epelboin A, Allarangar Y, Leroy E, Moudzeo H, Tarangonia P, Molamou A, Lenzi M, Ait-Ikhlef K, et al (2003) [Outbreak of Ebola hemorrhagic fever in the Republic of the Congo, 2003: a new strategy?] L'epidemie de fièvre hémorragique à virus Ebola en République du Congo, 2003: une nouvelle stratégie? *Med Trop (Mars)* 63: 291-295
- Galat G, Galat-Luong A (1997) [Virus transmission in the tropical environment, the socio-ecology of primates and the balance of ecosystems] Circulation des virus en milieu tropical, socio-ecologie des primates et équilibre des écosystèmes. *Sante* 7: 81-87
- Gear JS, Cassel GA, Gear AJ, Trappler B, Clausen L, Meyers AM, Kew MC, Bothwell TH, Sher R, Miller GB, et al (1975) Outbreak of Marburg virus disease in Johannesburg. *Br Med J* 4: 489-493
- Geisbert TW, Daddario-Dicaprio KM, Geisbert JB, Reed DS, Feldmann F, Grolla A, Stroher U, Fritz EA, Hensley LE, Jones SM, et al (2008) Vesicular stomatitis virus-based vaccines protect nonhuman primates against aerosol challenge with Ebola and Marburg viruses. *Vaccine* 26: 6894-6900
- Geisbert TW, Hensley LE, Jahrling PB, Larsen T, Geisbert JB, Paragas J, Young HA, Fredeking TM, Rote WE, Vlasuk GP (2003) Treatment of Ebola virus infection with a recombinant inhibitor of factor VIIa/tissue factor: a study in rhesus monkeys. *Lancet* 362: 1953-1958
- Georges AJ, Leroy EM, Renaut AA, Benissan CT, Nabias RJ, Ngoc MT, Obiang PI, Lepage JP, Bertherat EJ, Benoni DD, et al (1999) Ebola hemorrhagic fever outbreaks in Gabon, 1994-1997: epidemiologic and health control issues. *J Infect Dis* 179 (Suppl 1): S65-S75
- Groseth A, Stroher U, Theriault S, Feldmann H (2002) Molecular characterization of an isolate from the 1989/90 epizootic of Ebola virus Reston among macaques imported into the United States. *Virus Res* 87: 155-163
- Heymann DL (2004) The international response to the outbreak of SARS in 2003. *Philos Trans R Soc Lond B Biol Sci* 359: 1127-1129
- Heymann DL, Weisfeld JS, Webb PA, Johnson KM, Cairns T, Berquist H (1980) Ebola hemorrhagic fever: Tandala, Zaire, 1977-1978. *J Infect Dis* 142: 372-376
- Huggins J, Zhang ZX, Bray M (1999) Antiviral drug therapy of filovirus infections: S-adenosylhomocysteine hydrolase inhibitors inhibit Ebola virus in vitro and in a lethal mouse model. *J Infect Dis* 179 (Suppl 1): S240-S247
- Jaax NK, Davis KJ, Geisbert TJ, Vogel P, Jaax GP, Topper M, Jahrling PB (1996) Lethal experimental infection of rhesus monkeys with Ebola-Zaire (Mayinga) virus by the oral and conjunctival route of exposure. *Arch Pathol Lab Med* 120: 140-155
- Jahrling PB, Geisbert TW, Dalgard DW, Johnson ED, Ksiazek TG, Hall WC, Peters CJ (1990) Preliminary report: isolation of Ebola virus from monkeys imported to USA. *Lancet* 335: 502-505
- Jahrling PB, Geisbert TW, Geisbert JB, Swearingen JR, Bray M, Jaax NK, Huggins JW, LeDuc JW, Peters CJ (1999) Evaluation of immune globulin and recombinant interferon-alpha2b for treatment of experimental Ebola virus infections. *J Infect Dis* 179 (Suppl 1): S224-234
- Johnson E, Jaax N, White J, Jahrling P (1995) Lethal experimental infections of rhesus monkeys by aerosolized Ebola virus. *Int J Exp Pathol* 76: 227-236
- Johnson ED, Johnson BK, Silverstein D, Tukei P, Geisbert TW, Sanchez AN, Jahrling PB (1996) Characterization of a new Marburg virus isolated from a 1987 fatal case in Kenya. *Arch Virol Suppl* 11: 101-114
- Johnson KM, Lange JV, Webb PA, Murphy FA (1977) Isolation and partial characterisation of a new virus causing acute haemorrhagic fever in Zaire. *Lancet* 1: 569-571
- Keeling MJ, Grenfell BT (1997) Disease extinction and community size: modeling the persistence of measles. *Science* 275: 65-67
- Khan AS, Tshioko FK, Heymann DL, Le Guenno B, Nabeth P, Kerstiens B, Fleerackers Y, Kilmarx PH, Rodier GR, Nkuku O, et al (1999) The reemergence of Ebola hemorrhagic fever, Democratic Republic of the Congo, 1995. Commission de Lutte contre les Epidémies à Kikwit. *J Infect Dis* 179 (Suppl 1): S76-S86
- Kissling RE, Robinson RQ, Murphy FA, Whitfield SG (1968) Agent of disease contracted from green monkeys. *Science* 160: 888-890
- Kuhn JH (2008). *Filoviruses: A compendium of 40 years of Epidemiological, Clinical, and Laboratory Studies*. Wien, NewYork: Springer.
- Kuiken T, Fouchier R, Rimmelzwaan G, Osterhaus A (2003) Emerging viral infections in a rapidly changing world. *Curr Opin Biotechnol* 14: 641-646
- Kuiken T, Leighton FA, Fouchier RA, LeDuc JW, Peiris JS, Schudel A, Stohr K, Osterhaus AD (2005) Public health. Pathogen surveillance in animals. *Science* 309: 1680-1681
- Kunz C, Hofmann H, Kovac W, Stockinger L (1968) [Biological and morphological characteristics of the "hemorrhagic fever" virus occurring in Germany] Biologische und morphologische Charakteristika des Virus des in Deutschland aufgetretenen "Hamorrhagischen Fiebers". *Wien Klin Wochenschr* 80: 161-162 passim.
- Lahm SA, Kombila M, Swanepoel R, Barnes RF (2007) Morbidity and mortality of wild animals in relation to outbreaks of Ebola hemorrhagic fever in Gabon, 1994-2003. *Trans R Soc Trop Med Hyg* 101: 64-78
- Lamunu M, Lutwama JJ, Kamugisha J, Opio A, Namboze J, Ndayimirije N, Okware S (2004) Containing a hemorrhagic fever epidemic: the Ebola experience in Uganda (October 2000-January 2001). *Int J Infect Dis* 8: 27-37
- Legrand J, Grais RF, Boelle PY, Valleron AJ, Flahault A (2007) Understanding the dynamics of Ebola epidemics. *Epidemiol Infect* 135: 610-621
- Leroy EM, Baize S, Mavoungou E, Apetrei C (2002a) Sequence analysis of the GP, NP, VP40 and VP24 genes of Ebola virus isolated from deceased, surviving and asymptotically infected individuals during the 1996 outbreak in Gabon: comparative studies and phylogenetic characterization. *J Gen Virol* 83: 67-73
- Leroy EM, Kumulungui B, Pourrut X, Rouquet P, Hassanin A, Yaba P, Delicat A, Paweska JT, Gonzalez JP, Swanepoel R (2005) Fruit bats as reservoirs of Ebola virus. *Nature* 438: 575-576
- Leroy EM, Rouquet P, Formenty P, Souquiere S, Kilbourne A, Froment JM, Bermejo M, Smit S, Karesh W, Swanepoel R, et al (2004) Multiple Ebola virus transmission events and rapid decline of central African wildlife. *Science* 303: 387-390
- Leroy EM, Souquiere S, Rouquet P, Drevet D (2002b) Re-emergence of ebola hemorrhagic fever in Gabon. *Lancet* 359: 712
- Ligon BL (2005) Outbreak of Marburg hemorrhagic fever in Angola: a review of the history of the disease and its biological aspects. *Semin Pediatr Infect Dis* 16: 219-224
- Lipsitch M, Cohen T, Cooper B, Robins JM, Ma S, James L, Gopalakrishna G, Chew SK, Tan CC, Samore MH, et al (2003) Transmission dynamics and control of severe acute respiratory syndrome. *Science* 300: 1966-1970
- Martina BE, Osterhaus AD (2007). *Wildlife and the risk of vector-borne viral diseases*. Wageningen, The Netherlands: Wageningen Academic Publishers.
- Morikawa S, Saijo M, Kurane I (2007) Current knowledge on lower virulence of Reston Ebola virus (in French: Connaissances actuelles sur la moindre virulence du virus Ebola Reston). *Comp Immunol Microbiol Infect Dis* 30: 391-398

- Okware SI, Omaswa FG, Zaramba S, Opio A, Lutwama JJ, Kamugisha J, Rwaguma EB, Kagwa P, Lamunu M (2002) An outbreak of Ebola in Uganda. *Trop Med Int Health* 7: 1068-1075
- Osterhaus A (2001) Catastrophes after crossing species barriers. *Philos Trans R Soc Lond B Biol Sci* 356: 791-793
- Pourrut X, Kumulungui B, Wittmann T, Moussavou G, Delicat A, Yaba P, Nkoghe D, Gonzalez JP, Leroy EM (2005) The natural history of Ebola virus in Africa. *Microbes Infect* 7: 1005-1014
- Rec WE (1992) Viral haemorrhagic fever in imported monkeys. *Wkly Epidemiol Rec* 67: 142-143
- Rec WE (2005) Outbreak of Ebola haemorrhagic fever in Yambio, south Sudan, April-June 2004. *Wkly Epidemiol Rec* 80: 370-375
- Rodriguez LL, De Roo A, Guimard Y, Trappier SG, Sanchez A, Bressler D, Williams AJ, Rowe AK, Bertolli J, Khan AS, *et al* (1999) Persistence and genetic stability of Ebola virus during the outbreak in Kikwit, Democratic Republic of the Congo, 1995. *J Infect Dis* 179 (Suppl 1): S170-S176
- Roels TH, Bloom AS, Buffington J, Muhungu GL, Mac Kenzie WR, Khan AS, Ndambi R, Noah DL, Rolka HR, Peters CJ, *et al* (1999) Ebola hemorrhagic fever, Kikwit, Democratic Republic of the Congo, 1995: risk factors for patients without a reported exposure. *J Infect Dis* 179 (Suppl 1): S92-S97
- Rollin PE, Williams RJ, Bressler DS, Pearson S, Cottingham M, Pucak G, Sanchez A, Trappier SG, Peters RL, Greer PW, *et al* (1999) Ebola (subtype Reston) virus among quarantined nonhuman primates recently imported from the Philippines to the United States. *J Infect Dis* 179 (Suppl 1): S108-S114
- Sanchez A, Rollin PE (2005) Complete genome sequence of an Ebola virus (Sudan species) responsible for a 2000 outbreak of human disease in Uganda. *Virus Res* 113: 16-25
- Schou S, Hansen AK (2000) Marburg and Ebola virus infections in laboratory non-human primates: a literature review. *Comp Med* 50: 108-123
- Siegert R, Shu HL, Slenczka W, Peters D, Muller G (1967) [On the etiology of an unknown human infection originating from monkeys] Zur Aetiologie einer unbekannteren, von Affen ausgegangenen menschlichen Infektionskrankheit. *Dtsch Med Wochenschr* 92: 2341-2343
- Slenczka W, Klenk HD (2007) Forty years of marburg virus. *J Infect Dis* 196 (Suppl 2): S131-S135
- Smith DH, Johnson BK, Isaacson M, Swanapoel R, Johnson KM, Killey M, Bagshawe A, Siongok T, Keruga WK (1982) Marburg-virus disease in Kenya. *Lancet* 1: 816-820
- Snowden FM (2008) Emerging and reemerging diseases: a historical perspective. *Immunol Rev* 225: 9-26
- Sullivan NJ, Geisbert TW, Geisbert JB, Shedlock DJ, Xu L, Lamoreaux L, Custers JH, Popernack PM, Yang ZY, Pau MG, *et al* (2006) Immune protection of nonhuman primates against Ebola virus with single low-dose adenovirus vectors encoding modified GPs. *PLoS Med* 3: e177
- Sullivan NJ, Geisbert TW, Geisbert JB, Xu L, Yang ZY, Roederer M, Koup RA, Jahrling PB, Nabel GJ (2003) Accelerated vaccination for Ebola virus haemorrhagic fever in non-human primates. *Nature* 424: 681-684
- Swanapoel R, Leman PA, Burt FJ, Zachariades NA, Braack LE, Ksiazek TG, Rollin PE, Zaki SR, Peters CJ (1996) Experimental inoculation of plants and animals with Ebola virus. *Emerg Infect Dis* 2: 321-325
- Swenson DL, Wang D, Luo M, Warfield KL, Woratanadham J, Holman DH, Dong JY, Pratt WD (2008) Vaccine to confer to nonhuman primates complete protection against multistrain Ebola and Marburg virus infections. *Clin Vaccine Immunol* 15: 460-467
- Timen A, *et al* (2008) Dutch tourist infected with Marburg virus. *ProMed mail*.
- Towner JS, Khristova ML, Sealy TK, Vincent MJ, Erickson BR, Bawiec DA, Hartman AL, Comer JA, Zaki SR, Stroher U, *et al* (2006) Marburg virus genomics and association with a large hemorrhagic fever outbreak in Angola. *J Virol* 80: 6497-6516
- Towner JS, Pourrut X, Albarino CG, Nkoghe CN, Bird BH, Grard G, Ksiazek TG, Gonzalez JP, Nichol ST, Leroy EM (2007a) Marburg virus infection detected in a common African bat. *PLoS ONE* 2: e764
- Towner JS, Rollin PE, Bausch DG, Sanchez A, Crary SM, Vincent M, Lee WF, Spiropoulou CF, Ksiazek TG, Lukwiya M, *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: 4330-4341
- Towner JS, Sealy TK, Khristova ML, Albarino CG, Conlan S, Reeder SA, Quan PL, Lipkin WI, Downing R, Tappero JW, *et al* (2008) Newly discovered ebola virus associated with hemorrhagic fever outbreak in Uganda. *PLoS Pathog* 4: e1000212
- Towner JS, Sealy TK, Ksiazek TG, Nichol ST (2007b) High-throughput molecular detection of hemorrhagic fever virus threats with applications for outbreak settings. *J Infect Dis* 196 (Suppl 2): S205-S212
- Vastag B (2004) Ebola vaccines tested in humans, monkeys. *JAMA* 291: 549-550
- Vynnycky E, Trindall A, Mangtani P (2007) Estimates of the reproduction numbers of Spanish influenza using morbidity data. *Int J Epidemiol* 36: 881-889
- Walsh PD, Biek R, Real LA (2005) Wave-like spread of Ebola Zaire. *PLoS Biol* 3: e371
- Warfield KL, Olinger G, Deal EM, Swenson DL, Bailey M, Negley DL, Hart MK, Bavari S (2005) Induction of humoral and CD8+ T cell responses are required for protection against lethal Ebola virus infection. *J Immunol* 175: 1184-1191
- WHO (1978) Ebola haemorrhagic fever in Sudan, 1976. Report of a WHO/International Study Team. *Bull World Health Organ* 56: 247-270
- Yaddanapudi K, Palacios G, Towner JS, Chen I, Sariol CA, Nichol ST, Lipkin WI (2006) Implication of a retrovirus-like glycoprotein peptide in the immunopathogenesis of Ebola and Marburg viruses. *Faseb J* 20: 2519-2530
- Zaki SR, Goldsmith CS (1999) Pathologic features of filovirus infections in humans. *Curr Top Microbiol Immunol* 235: 97-116
- Zaki SR, Shieh WJ, Greer PW, Goldsmith CS, Ferebee T, Katshitschi J, Tshioko FK, Bwaka MA, Swanapoel R, Calain P, *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-S47
- Zampieri CA, Sullivan NJ, Nabel GJ (2007) Immunopathology of highly virulent pathogens: insights from Ebola virus. *Nat Immunol* 8: 1159-1164