and (ii) to accelerate the loss of preformed ccc DNA. At the same time, the long-term benefits of antiviral therapy need to be vigorously investigated, particularly with reference to the timing of treatment initiation. The rather dire outlook for chronic HBV infections gained from these recent studies should bring a heightened sense of urgency for those working to prevent the consequences of these infections, which afflicts hundreds of millions of people worldwide.

References

19 Dandri, M. et al. (2000) Increased hepatocyte turnover and inhibition of woodchuck hepatitis B virus replication by adefovir in vitro do not lead to reduction of the closed circular DNA. Hepatology 32, 139–146

Ebola virus ecology: a continuing mystery

Heinz Feldmann1,2, Victoria Wahl-Jensen1,2, Steven M. Jones1,3 and Ute Ströher1,2

1Special Pathogens Program, National Microbiology Laboratory, Health Canada, Winnipeg MB R3E 3R2, Canada
2Department of Medical Microbiology, University of Manitoba, Winnipeg MB R3E 0W3, Canada
3Department of Immunology, University of Manitoba, Winnipeg MB R3E 0W3, Canada

Marburg and Ebola viruses emerged approximately three decades ago, but the reservoir(s) of these zoonotic pathogens and the routes of primary transmission to human and nonhuman primates remains a mystery. Recent outbreaks have been associated with multiple introductions into the population, indicating the circulation of distinct strains that have evolved in reservoir species that occupy different ecological niches. Identification of

www.sciencedirect.com
the viral reservoir(s) is a research priority, with a high impact on public health and prevention.

Ebola virus (EBOV) is highly pathogenic for human and nonhuman primates (NHPs), with lethality rates of up to 90% for humans and no current pre- and post-exposure treatment options [1]. The emergence and re-emergence in epidemic regions of Africa (Figure 1), the potential for introductions into non-endemic countries and the possible use as a bioweapon make EBOV a worldwide public health concern and counter measurements a priority. The recently reported decline of Central African wildlife, particularly the great apes [2,3], has further extended the threats posed by this virus to include fear of extinguishing one of the world’s largest populations of gorillas and chimpanzees.

Elusive reservoirs for filoviruses

The reservoirs of EBOV and Marburg virus (MARV), members of the family Filoviridae, have continually proven to be elusive. As classical zoonotic agents, these viruses probably persist in an animal species (or several) that becomes the source of direct transmission to human and NHPs, or to an interim amplifying host, although this is unlikely. Despite extensive efforts to ascertain the reservoir in nature, beginning with the discovery of EBOV in 1976 [1], nonhuman vertebrate hosts or arthropod vectors have not been identified [4–6]. Morvan et al. [7] reported detection of Zaire ebolavirus (ZEBOV) RNA in organ tissues of rodents and shrews captured in the Central African Republic, suggesting that a reservoir exists within small terrestrial mammals living in peripheral forest areas. Unfortunately, confirmation of such a reservoir by serology, antigen detection or virus isolation has not been achieved. Interestingly, Swanepoel et al. [8] demonstrated that experimentally infected wild African fruit and insectivorous bats support replication and circulation of high titers of EBOV without becoming ill. Bats had previously been discussed as potential infectious sources for the EBOV outbreaks in Sudan [9] and the MARV infections in Kenya [10,11] where the index cases could be linked to a bat-infested cotton factory or a cave, respectively (Figure 2). Because persistently infected hosts are frequently (although not necessarily) associated with zoonotic diseases, chronic infection in bats or other small animal species might be considered a mechanism that regulates the survival of filoviruses in nature.

The epizootics caused by Reston ebolavirus (REBOV), the only filovirus to be Asian in origin, have raised the possibility that NHPs might be a reservoir [12] (Figure 2). This appears to be unlikely, at least for the African filoviruses, which are highly pathogenic for NHPs – a feature that is generally incongruous with the concept of a reservoir host. If not the reservoir, monkeys could be indicator hosts for filovirus circulation. This is supported by deaths in monkey species that occurred before human cases, as described for the outbreak of Ivory Coast ebolavirus in the Tai Forest [13], several of the ZEBOV outbreaks occurring after 1996 in Gabon [1,3], and the recent outbreak of ZEBOV in the Republic of the Congo (RC) [14] (Figure 2). The paper by Leroy et al. [3] confirms this concept but, more importantly, indicates for the first time multiple introductions of ZEBOV from an unknown reservoir into wildlife, which then serve as the source for human infection.

Figure 1. Filovirus outbreaks in Central Africa. Reported outbreaks of hemorrhagic fever caused by Marburg (MARV) and Ebola (EBOV) viruses are indicated with the corresponding year. Countries affected by the outbreaks are specifically named and the borders are highlighted in bold. The natural vegetation zones are presented in different underlying colours.

www.sciencedirect.com
Genetic stability of filoviruses

Determination of the nucleotide sequence of the glycoprotein gene derived from clinical specimens of EBOV patients from outbreaks in Gabon and RC from 2001 to 2003 indicated the co-circulation of different virus strains. By contrast, sequences derived from genes that were isolated from patients involved in a distinct epidemic chain of an outbreak were conserved [3]. Genomic conservation among sequences derived from clinical specimens was reported previously from the ZEBOV outbreaks in Kikwit, the Democratic Republic of the Congo (DRC) [15], and Gabon [16], which are both thought to be caused by single EBOV introductions into the human species. Bats have been discussed as potential sources for human infection in at least three outbreaks but direct evidence is missing. Abbreviations: African green, *Cercopithecus aethiops*; chimpanzee, *Pan troglodytes*; Cynomolgus macaque, *Macaca fascicularis*; DRC, Democratic Republic of the Congo; duiker, *Cephalophus spp*.; gorilla, *Gorilla gorilla*, RC, Republic of Congo.

Challenges in identification of the reservoir

The report by Leroy et al. [3] has inspired the field to again question the reservoir and persistence of EBOV in nature and, consequently, the route of primary transmission to wildlife, particularly primates. Filoviruses appear to be endemic in Central Africa in an area between the 10th parallel north and south of the equator (Figure 1) [1]. Because of past efforts to identify the reservoir, it can be assumed that the reservoir is either a rare species or that transmission within the reservoir species itself is less efficient. The Durba MARV outbreak strongly pointed towards the gold mine (mentioned previously) as the source of infection, thereby offering renewed confidence in the search for the reservoir, but as with the Tai Forest
project starting in 1995 [18], all optimism quickly vanished. The area on both sides of the border between Gabon and RC where the recently reported ZEBOV outbreaks occurred (Figure 1) is another well-defined place to look for the reservoir, and although hope is renewed [3], without new strategies and enhanced diagnostic tools success remains questionable.

Detection of virus-specific antibodies is a common approach for determining the contact of a virus with an animal species, however, lack of specific reagents for the majority of wild animal species has limited its use. Nucleic acid and antigen detection as well as virus isolation are alternative approaches. RT-PCR (reverse transcriptase-polymerase chain reaction) is powerful but prone to contamination and firm conclusions should only be made when data are confirmed using an independent approach. Antigen detection (e.g. ELISA) and virus isolation are time-consuming, labour intensive (immunohistochemistry) and less sensitive when compared with other methodology and might need high bio-containment (virus isolation) facilities; therefore, they are less practical in surveillance studies. However, a recently developed antibody-phage indicator assay, designed to be simple and economical in the field, has the potential to overcome limitations involved in the detection of virus-specific antibodies from a variety of animal species [19]. An alternative approach to testing a large variety of wild animal species in less-defined geographical areas would be the use of sentinel animals placed in known areas of virus activity. This approach has been successfully used for the surveillance of West Nile virus and other arbovirus infections [20,21]. It might appear strange to apply this strategy for filoviruses, but in places such as the gold mine in Watsa and Durba and the Mt. Elgon cave in Kenya, which have been implicated in the transmission of filoviruses to humans [10,11,17], it might help to define a focus for subsequent intense searches for the reservoir.

**Efforts to control epizootic outbreaks**

There has been a long-standing discussion about the need for vaccines for EBOV and other rarely occurring viral hemorrhagic fever agents. The rare appearance of EBOV hemorrhagic fever in the past and the remote locations of the outbreaks did not favour vaccine development, particularly because this would require industrial support despite a lack of market for the vaccine. This view has totally changed with the existing threat from bioterrorism. The increasing threat by EBOV to extinguish endangered great ape populations in Central Africa [2,3] might intensify calls for the development of pre-exposure prophylaxes. In the past, several vaccine strategies have been successful in providing protection to rodents against EBOV, however, almost all were universally unsuccessful in protecting NHPs [22]. The first vaccine to have proven efficacy against EBOV challenge in NHPs was a DNA prime—adenovirus boost approach, which was further developed into an accelerated method using a single dose of the recombinant adenovirus [23]. Previously, Hevey et al. [24] were successful in protecting NHPs against a lethal MARV challenge using an alphavirus replicon strategy. More recently, a new vaccine strategy using live attenuated recombinant vesicular stomatitis virus (VSV) has been successful in both rodent and NHP models of EBOV infection [25]. VSV-based vaccines have the potential of being administered by the intranasal and oral routes. Therefore, this vaccine might offer some hope of protecting wild animals, such as the great apes, for which vaccines that need to be injected are not practical, whereas baiting of food might be a realistic alternative (S. Jones et al., abstract: Replicating Vectors for Vaccine Development, VRC Symposium on Viral Hemorrhagic Fevers, Bethesda, USA, October 2003).

**Concluding remarks**

We now know much more about transmission of EBOV and the pathogenesis of the disease, however, it remains difficult to judge how close we are to identification of the reservoir. The implementation of appropriate projects is difficult given the remote endemic areas, understandable sensitivity of the population in these locations, biosafety concerns, and funding for such endeavours. Foremost, we need additional serosurveys in humans and animals to more clearly define endemic areas. Many of the older studies were based on serological test systems that are not completely reliable. Those studies should be confirmed and complemented using more reliable techniques that have been developed during the past few years [19]. The identification of the natural reservoir is an important key to understanding the ecology of filoviruses and might help to develop strategies for prevention. In this respect, new working hypotheses need to be formulated in cooperation with investigators from different fields, such as virology, infectious diseases, geography, geology, meteorology, botany and anthropology. A good start was made recently using ecologic niche modeling of outbreaks and sporadic cases. This study revealed different distributions for filoviruses in Afrotropics, with EBOV more likely to occur in the humid rain forests of Central and Western Africa and MARV in the drier and more open areas of Central and East Africa [26] (Figure 1). Pre-existing field sites (e.g. Tai Forest, Cote d’Ivoire; Watsa and Durba, DRC; Gabon, DC) should be used for more focused searches that could include the use of sentinel animals. One should also keep in mind that more than one reservoir, perhaps even amplifying hosts, might be involved in transmission to human and NHPs. Closer surveillance of affected animal populations, such as the great apes, and experimental studies in potential reservoir species [8] should be initiated and/or intensified to better understand virus persistence and transmission. Because aerosol transmission is of concern in the case of filovirus infections, the potential of this route for animal-to-human and human-to-human transmission must be more clearly defined. This could be done by adequate epidemiological studies and appropriate experiments in suitable animal models.

**References**

Recent work has signaled the beginning of the comparative genomics era for analysis of the important pathogen *Pseudomonas aeruginosa*. Comprehensive systematization of microorganisms requires extensive genome-wide comparisons of conserved and variable regions located in the genomes, determination of expression profiles, gene complements and correlation of phenotypic characteristics. An important stimulus to such systematic studies is expected from decoding and comparison of full genome sequences.

Advances in automatic DNA sequencing techniques and the whole-genome shotgun strategy have resulted in a tremendous increase in the amount of available genome data. To date, greater than 185 whole genome sequences and their annotations have been published, and at least 1050 genome sequencing projects are ‘in progress’ (http://www.genomesonline.org/). Among publicly available genomes, approximately 90% of the genome sequences are from microbes. These valuable data provide good subjects for experimental studies and functional analysis. Comparative genomics has become more and more attractive, especially between two close species. By performing pairwise genome comparisons, information about the genome structure, evolving patterns and also processes that influence genomic design can be revealed [1]. For example, by comparison of the genome organizations of *Mycoplasma genitalium* and *Mycoplasma pneumoniae*, a conserved gene order was discovered [2]. A comparison of the genomes of *Chlamydia trachomatis* and *Chlamydia*

---

**Comparative genomic analysis of *Pseudomonas aeruginosa* virulence**

**Donald E. Woods**

Department of Microbiology and Infectious Diseases, Faculty of Medicine, University of Calgary Health Sciences Centre, 3330 Hospital Drive, NW, Calgary, Alberta, Canada T2N 4N1

---


Available online 24 August 2004