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## EBOLA VIRUS: WHERE DOES IT COME FROM AND WHERE IS IT GOING?

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### I. INTRODUCTION

Much has been written in the past five years about the natural history of the filoviruses, but most of this has been highly speculative, only faintly grounded in fact. The mystery about the reservoir host(s) and the means by which index human cases have become infected has been played up by the press/media into a metaphor for “The Ultimate Pathogen” (Kilbourne, 1983, 1996) or “The Andromeda Strain” (Crichton, 1969). Behind this press/media metaphor, however, is the fact that the filoviruses are dangerous on a global scale; we would like to explain this assertion and try to move discussion from the world of science fiction to the world of scientific inquiry. The questions of where the filoviruses came from, that is, where they exist in nature, and where they may be going, where they might emerge again as a public health problem, are the same questions that were once asked about yellow fever virus, measles virus, and other viruses. The difference is that, even now at the end of the twentieth century, the family *Filoviridae* is the only virus family containing human pathogens for which we do not have even an approximate answer.

This chapter is concerned primarily with the point in the natural history of the filoviruses where they are transmitted from their unknown reservoir host(s), perhaps even through an equally unknown intermediary host(s), to the index human host. To explore this point of zoonotic transmission, however, it is also necessary to consider events one step back and one step forward. Since we do not know the reservoir host(s), consideration here must be speculative, but since we do know a great deal about the circumstances of human-to-human trans-

mission and nosocomial and community-acquired disease, consideration here will be based on data and experience. It is our feeling that focus on this point in the natural history of the filoviruses will help demystify other aspects of their natural history, such as those that have to do with disease pathogenesis, epidemiology, and clinical medicine.

We begin with a brief examination of the known filovirus disease outbreaks, searching in the facts surrounding these outbreaks for clues about reservoir host(s) and viral natural history. We proceed by asking the same questions in regard to episodes in hospitals caring for filovirus hemorrhagic fever patients. Then we beg the same questions in regard to what we know about the viruses and the infections they cause (in cell cultures, in experimental animals, in humans). We extend the perspective to the ecological and epidemiological characteristics of filovirus infections, and further extend this to recent field studies aimed at finding the reservoir host(s). Then we move on to a synthesis of the principles underpinning the natural history of zoonotic viruses and how they may apply to the filoviruses specifically. Finally, from an international public health perspective, we restate why we regard the filoviruses with such suspicion and why we think certain research and resources are needed to meet present international public health needs.

## II. FILOVIRUS HEMORRHAGIC FEVER OUTBREAKS AND EPIDEMICS

### A. Lessons from the Point of Zoonotic Transmission to Humans

The virus family *Filoviridae* only became known to medical science in 1967, although there has been speculation that the viruses have been around for at least as long as to have caused one of the plagues of Athens (Olson *et al.*, 1996). In 1967, African green monkeys (*Cercopithecus aethiops*) brought Marburg virus from Africa to Europe, resulting in 31 human cases (including six secondary cases) and seven deaths among workers handling the monkeys or their tissues (Siegert *et al.*, 1967); Smith *et al.*, 1967; Kissling *et al.*, 1968, 1970; Simpson, 1970; Martini and Siegert, 1971). Zoonotic transmission occurred in circumstances where there was very close contact between the monkeys and the humans. Monkeys were handled without substantial biocontainment equipment or practices; removal of kidneys and handling of cell cultures prepared from them was done with only rudimentary protocols to prevent bacterial contamination. In reviewing events before the point of zoonotic transmission, it was realized that many of the monkeys recently shipped from Uganda had died of a hemorrhagic disease. However, no specific antibodies were found in sera from monkeys subsequently captured in Uganda in the area where the monkeys had originated, and the source of the virus remains unknown. Because in later studies, all African green monkeys experimentally inoculated with the virus died acutely, it was postulated that monkeys might not be involved in the Marburg virus reservoir host cycle in nature. It was also apparent that the capacity of the virus for human-to-human spread was likely limited; this was evidenced by the low secondary attack rate among the many people exposed and the absence of

tertiary cases. In fact, the human disease disappeared once the monkeys were eliminated. Since 1967, Marburg virus has reappeared only a few times, in Africa, in limited circumstances (Gear *et al.*, 1975; Conrad *et al.*, 1978; Smith *et al.*, 1982; Johnson *et al.*, 1996) (Table I).

Ebola virus did not appear on the scene until 1976, at which time two epidemics occurred, one in Zaire (now Democratic Republic of Congo), the other in the Sudan, together involving more than 550 cases and more than 430 deaths [Johnson *et al.*, 1977, 1978; Bowen *et al.*, 1977; World Health Organization (WHO), 1978a,b] As of the time of writing in early 1997, approximately 18 Ebola virus disease episodes have been identified, caused by four genetically distinct viruses, presenting an ever increasing geographic range within Africa (Heymann *et al.*, 1980; Teepe *et al.*, 1983; Baron *et al.*, 1983; WHO, 1979, 1982, 1995a–d, 1996, 1997) (Table I). There have also been two episodes of infection of scientists who were working on filoviruses in the laboratory, one involving Marburg virus and one the Sudan subtype of Ebola virus (Emond *et al.*, 1977; Nikiforov *et al.*, 1994); although no further transmission occurred in either episode, disease spread is certainly possible if this were to occur in circumstances where the diagnosis was missed.

The zoonotic source of the major epidemics of Ebola hemorrhagic fever, in northern Zaire and southern Sudan in 1976 and in Kikwit, Zaire, in 1995, has never been determined. Index human cases were in close contact with tropical forest ecosystems, but despite organized nonhuman specimen collecting expeditions and state-of-the-art laboratory technology for virus isolation (supplemented with viral antigen and viral RNA detection methods from 1995 onward), no trace of the zoonotic source of the virus has yet been found. The identification of the primary human index cases has been determined with high probability; however, attempts to backtrack the virus further, to the zoonotic contact point, have failed.

In the case of (a) the original Marburg hemorrhagic fever outbreak in Europe, (b) the disease outbreaks in monkeys in primate import quarantine facilities in the United States in 1989–1990 and 1996 caused by the Reston subtype of Ebola virus, (c) the single human case in the Côte d'Ivoire in 1994 caused by the Côte d'Ivoire subtype of Ebola virus, and (d) the outbreaks of disease in Gabon in 1994 and 1996 caused by the Zaire subtype of Ebola virus, the point of zoonotic transmission involved close association between nonhuman primates and primary human index cases [Siegert *et al.*, 1967; Jahrling *et al.*, 1990; Centers for Disease Control (CDC), 1990a,b, 1996; Peters *et al.*, 1991a; LeGuénno *et al.*, 1995; Simpson, 1995; WHO, 1996, 1997; LeGuénno, 1996; Georges *et al.*, 1996; Tukei, 1996; Amblard *et al.*, 1997]. However, attempts to backtrack from the implicated primates in these episodes have also failed to reveal a true reservoir host. In these episodes, most investigators have surmised that the nonhuman primates involved likely became infected from the same still mysterious reservoir host(s) that in other episodes seem to have exposed humans directly. This notion follows also from some evidence that transmission of filoviruses from monkey to monkey is not very efficient and that close contact among monkeys is needed to accomplish transmission just as it is in humans (e.g., antibody was not found in free-living chimpanzees in contact with those that died in the Taï Forest, Côte d'Ivoire, in 1994). Further, this notion is

**TABLE I Filovirus Hemorrhagic Fever Cases, Outbreaks, and Epidemics**

<b>Virus</b>	<b>Year</b>	<b>Location</b>	<b>Cases (% mortality)</b>	<b>Circumstances of human infection</b>
Marburg	1967	Germany and Yugoslavia	31 (23%)	Imported monkeys from Uganda were the source of the virus; humans became infected by contact with the monkeys or kidney cell cultures prepared from them; there were six secondary cases and no tertiary cases.
Marburg	1975	Zimbabwe/ South Africa	3 (33%)	Unknown origin. A traveler was infected in Zimbabwe and died in Johannesburg, South Africa; secondary cases occurred in a companion and a nurse who were infected while providing patient care.
Ebola (subtype Zaire)	1976	Zaire	318 (88%)	Unknown origin. Yambuku and surrounding area. Spread occurred by close contact and by use of contaminated needles and syringes in hospitals and clinics.
Ebola (subtype Sudan)	1976	Sudan	284 (53%)	Unknown origin. Nzara, Maridi, and surrounding area. Spread was thought to be mainly by close contact. Nosocomial transmission and infection of medical care personnel were prominent.
Ebola (subtype Sudan)	1976	England	1 (0%)	Laboratory infection by needle-stick; no secondary spread.
Ebola (subtype Zaire)	1977	Zaire	1 (100%)	Unknown origin. Tandala. Single case in missionary hospital. Other sporadic cases may have occurred nearby at the same time.
Ebola (subtype Sudan)	1979	Sudan	34 (65%)	Unknown origin. Nzara. Recurrent outbreak at the same site as the 1976 Sudan epidemic.
Marburg	1980	Kenya	2 (50%)	Unknown origin. Index case infected in western Kenya, but died in Nairobi; a physician who was secondarily infected survived.
Marburg	1987	Kenya	1 (100%)	Unknown origin. Expatriate traveling in western Kenya.
Ebola (subtype Reston)	1989	United States	4 (0%)	Introduction of virus into quarantine facilities in Virginia, Texas, and Pennsylvania, by imported monkeys from the Philippines. Four humans were asymptotically infected.
Marburg	1990	Russia	1 (0%)	Laboratory infection; no secondary spread.
Ebola (subtype Reston)	1990	United States	0 (0%)	Introduction of virus into same quarantine facilities in Virginia and Texas, from monkeys derived from the same export facility in the Philippines as in 1989. No human infections were identified.

Ebola (subtype Reston)	1992	Italy	0 (0%)	Introduction of virus into quarantine facilities in Siena, by monkeys derived from the same export facility in the Philippines as involved in the U.S. episodes in 1989. No human infections were identified.
Ebola (subtype presumably Zaire)	1994	Gabon	44 (63%)	Unknown origin. Minkebe, Makokou area. Outbreak in gold-mining camps, in deep rain forest, previously thought to be yellow fever, identified retrospectively by serology in 1996.
Ebola (subtype Côte d'Ivoire)	1994	Côte d'Ivoire	1 (0%)	Ethologist became ill after conducting an autopsy on a wild chimpanzee in the Taï Forest; repatriated to Switzerland, recovered. Diagnosis by virus isolation and serology. The troop of chimpanzees had been decimated by a hemorrhagic disease in 1992 and 1994.
Ebola (subtype presumably Côte d'Ivoire)	1995	Liberia/Côte d'Ivoire	1 (0%)	Liberian refugee became ill, was hospitalized and recovered; serological diagnosis only (IgM, IgG antibodies).
Ebola (subtype Zaire)	1995	Zaire	317 (78%)	Unknown origin. Epidemic in Kikwit, traced to an index case who worked in the forest adjoining the city. The epidemic spread through families and hospital, and was terminated with the help of an international team.
Ebola (subtype Zaire)	1996	Gabon	37 (57%)	Mayibout area. A chimpanzee found dead in the forest was butchered and eaten; 19 primary cases occurred in the people who did the butchering, secondary cases occurred in family members.
Ebola (subtype Zaire)	1996	Gabon	60 (75%)	Unknown source. Booué area, with transport of patients to Libreville. Index case was a hunter who lived in a forest camp. Community spread occurred mostly by close contact with patients. A dead chimpanzee found in the forest at the time was implicated.
Ebola (subtype Zaire)	1996	South Africa	2 (50%)	A medical professional traveled from Gabon to Johannesburg, South Africa, after having treated Ebola-infected patients; he was hospitalized and a nurse became infected and died.
Ebola (subtype Reston)	1996	United States	0 (0%)	Introduction of virus into quarantine facility in Texas, from monkeys derived from the same export facility in the Philippines as in 1989. No human infections were identified.
Ebola (subtype Reston)	1996	Philippines	0 (0%)	Unknown origin. Same export facility in the Philippines was involved as in 1989. No human infections were identified.

consistent with the fact that the monkey species which have been studied experimentally have been far too susceptible to the lethal consequences of infection, and too likely to quickly burn out as host populations, to be able to perpetuate the viruses in nature over the long course. At a minimum, if a particular species of monkey were the reservoir host of Ebola virus, one would expect evidence of mortality such as seen with yellow fever in howler monkeys in the rain forests of Central and South America.

Although we really do not know the time or circumstances favoring Ebola transmission in its natural reservoir host niche, it is of interest that in the rainy seasons of 1976–1977 and then again in 1994–1997, circumstances have favored at least enough viral activity to have led to spread to humans living or working in the rain forest (Monath, 1996). These circumstances, of course, remind one of typical arthropod-borne and rodent-borne virus transmission cycles, with their annual periodicity and longer secular trends. In turn, this reminder might lead one to speculate that the four subtypes of Ebola virus represent adaptations to related reservoir hosts in similar habitats, much as we see with the hantaviruses and their rodent hosts.

The fact that the Ebola virus subtypes (i.e., serotypes, genotypes) which have caused human disease episodes have been different from one another (Zaire, Sudan, and Côte d'Ivoire subtypes and Gabon isolates which are not quite identical to Zaire isolates) makes it clear that a common source human-to-human transmission chain extending across sub-Saharan Africa is not the case; rather, virus subtypes lodged at or near each site of the recent human disease episodes have been responsible (Fisher-Hoch *et al.*, 1992a; Sanchez *et al.*, 1993; Georges Courbot *et al.*, 1997a,b). Indeed, Marburg and Ebola viruses are so very different from one another and the four recognized subtypes of Ebola virus are different enough from one another that we should consider each independently in regard to possible reservoirs and probable evolutionary pressures (Sanchez *et al.*, 1996). This being the case, it seems that it will be necessary to study each geographic site separately, being careful not to meld information too casually.

There seems to have been increasing incidence of Ebola hemorrhagic fever in western Africa over the past few years, evident as large and small outbreaks involving an ever-increasing geographic range. It has been said that this is just a matter of increasing recognition of human cases because of publicity, rather than a true emergence phenomenon. However, it seems more likely that epidemics such as that in Kikwit, Zaire, in 1995 would not have been overlooked earlier and that there really is an increasing incidence (CDC, 1995a–c; WHO, 1995a–d; Peters, 1996a). Because the reservoir host(s) is not known, ecological, environmental, and human behavioral changes that might have increased the opportunities for emergence in recent years are still matters of speculation, matters needing more study. Nevertheless, observations in these recent outbreak settings are all we have, so we must proceed from this point. One question in this regard is whether we should focus our attention on urban human-to-human transmission episodes (with particular focus on tracking back to index cases) or orient all research enterprise on the feral niche(s) where the viruses are perpetuated independently of human involvement.

## B. Lessons from Disease Episodes in Hospitals Caring for Filovirus Hemorrhagic Fever Patients

It should be noted that the recent filovirus outbreaks, including the Ebola epidemic in Kikwit, Zaire, 1995, and the first epidemic in Mayibout, Gabon, 1996, were controlled with relatively simple measures (Muyembe-Tamfun and Kipasa, 1995; Georges *et al.*, 1996; Calain, 1996; Amblard *et al.*, 1997; Ivker, 1997; WHO, 1997). Indeed, the virus may never have spread within hospitals and communities, even communities such as Kikwit and Mayibout, if cultural attitudes and economic resources had favored the routine use of simple sanitary measures, strict barrier-nursing practices, and patient isolation. The terrible consequences that can follow such indifference are clear, yet this is a separate matter from the circumstance that brings the first human case, the index case, from the remote site of zoonotic exposure to the crowded sites of human-to-human transmission, starting in the caregiving family, proceeding to the hospital, and then extending to the community at large.

Within poorly equipped African hospitals and clinics, the larger Ebola hemorrhagic fever outbreaks have had a strong iatrogenic amplifier effect, whether this be from the reuse of syringes and needles as was the case in northern Zaire in 1976 or the lack of hygiene and the reality of close patient contact as was the case in Kikwit, Zaire, in 1995 (Johnson *et al.*, 1977, 1978; WHO, 1978a,b; Pattyn, 1978; Baron *et al.*, 1983; Khan *et al.*, 1996). Indeed, it has been stated, "In Africa, hospitals cause Ebola."

Often, after initial outbreaks, hospitals have been closed, not by design but simply by fearful mortality among medical staff. In several instances, transmission has then declined and outbreaks have ended (Johnson *et al.*, 1977, 1978; WHO, 1978a,b; Khan *et al.*, 1996). Secondary attack rates outside the hospital have been about 5–15%, insufficient in most circumstances to sustain the outbreak. Factors contributing to the low secondary attack rate in villages include fundamental characteristics of filovirus infections per se, traditional shunning of the sick, modification of burial rituals once risk to family caregivers has been recognized, and other yet uncertain factors.

The hospital may also be a key to limiting the spread of the virus in the community. In the city of Kikwit, Zaire (population 300,000), where fewer traditional family-based caregiving practices may have been in place than in rural areas and traditional villages, the transport of patients to the hospital early in the course of their illness may have limited virus spread (Muyembe-Tamfun *et al.*, 1996). The renovation of the Kikwit General Hospital (300 beds, plus a 60-bed maternity unit) in the years before the outbreaks in 1995 may have added to this trend of bringing patients to hospital early in their course of illness.

In regard to the question, "Where is Ebola going?" (i.e., "What will be the outcome of future Ebola episodes?"), it might be hoped that lessons learned in Kikwit, Zaire, 1995, would last. However, this remains to be seen. Some investigators have suggested that as early as 1 year after the epidemic, behaviors and practices had reverted to usual ways (Khan *et al.*, 1996). This is especially distressing because experiences in Kikwit in 1995 provided such a good test of the concept that simple sanitary measures, strict barrier-nursing practices, and



patient isolation could suffice to (a) terminate the viral transmission chain, (b) provide health care provider safety, and (c) provide adequate patient care.

The data from Kikwit, Zaire, are clear in this regard: whereas 76 medical staff were infected during the first weeks of the epidemic, after the institution of sanitary measures, barrier nursing, and patient isolation, although medical staff still entered the ward to care for patients, only one health care worker became infected (Calain, 1996). It should be noted that during the epidemic in Kikwit, strict barrier-nursing precautions included the use of a double gown (with impervious plastic lining), double gloves, boots, goggles, and mask (usually a HEPA-filtered mask) (Peters *et al.*, 1991b; CDC, 1995d; CDC/NIH, 1993). The view that adequate hygiene, strict barrier-nursing practices, and patient isolation can limit the spread of Ebola virus to medical staff is also supported by the experience in Mayibout, Gabon, in 1996, where 32 patients were cared for without any infections in medical staff (LeGuanno, 1996).

### III. THE FILOVIRUSES AND BASIC ASPECTS OF FILOVIRUS INFECTIONS

#### A. Lessons from Physical and Molecular Characteristics of the Filoviruses

Filovirus virions are enveloped and pleomorphic, appearing filamentous or bacilliform, or “U”-shaped, or “6”-shaped, or circular. Particles have a uniform diameter of 80 nm but vary greatly in length (up to 14,000 nm; however, virions recovered from the peak-infectivity band after gradient centrifugation are more uniform—Ebola virions are about 1000 nm and Marburg virions about 800 nm in length) (Murphy *et al.*, 1978; Regnery *et al.*, 1981; Geisbert and Jahrling, 1995). Virions are covered by surface peplomers, about 7 nm in length, spaced at 10-nm intervals. Inside the virion envelope is a helical nucleocapsid about 50 nm in diameter with a helix periodicity of about 5 nm. Virus infectivity is rather stable at less than 20°C, but infectivity is rapidly destroyed at 60°C. Infectivity is sensitive to lipid solvents,  $\beta$ -propiolactone, formaldehyde, hypochlorite, quarternary ammonium and phenolic disinfectants, and UV- and  $\gamma$ -irradiation (Murphy *et al.*, 1995; Feldmann and Klenk, 1996).

The genomes of Marburg and Ebola viruses are the largest thus far identified for any nonsegmented negative-strand RNA virus (19.1 kb); they contain seven linearly arranged genes that are organized in the same way as are the genes of rhabdoviruses and paramyxoviruses (gene order 3' NP-VP35-VP40-GP-VP30-VP24-L 5') (Sanchez *et al.*, 1992, 1993; Feldmann *et al.*, 1992, 1993, 1996a,b). An unusual characteristic of the genome organization of the filoviruses is the presence of gene overlaps, that is, short regions (17–20 bases) where the transcription start site of the downstream gene overlaps the transcription stop site of the upstream gene (Sanchez *et al.*, 1993, 1996). The proteins of Ebola and Marburg viruses are quite similar, despite the level of divergence of their nucleotide sequences and their lack of serological cross-reactivity.

Thus, it would seem that although the basic physical and molecular characteristics of filovirus virions are interesting, and although their morphology does seem to add to the sense of mystery that has been fomented by the press/media, there is nothing that would really isolate these viruses from other human

and animal viruses, nothing that would point to some unique habitat or reservoir host. On the other hand, some of the proteins of the filoviruses do lead us to wonder about the evolution of these viruses and their development of particular functional attributes that may have furthered their survival in their yet unknown habitat(s) and reservoir host(s).

For example, study of the Ebola glycoproteins has yielded several tantalizing findings, none of which has been fully evaluated (Feldmann *et al.*, 1994; Sanchez *et al.*, 1996). The glycoprotein genes of Marburg and the three Ebola subtypes differ significantly: the single Marburg glycoprotein is encoded in a single open reading frame, whereas the two virion glycoproteins of Ebola viruses are encoded in two reading frames and are expressed through transcriptional editing and translational frame-shifting. The Ebola *virion* glycoprotein ( $M_r$  120,000–170,000) forms the surface peplomers, whereas the second glycoprotein ( $M_r$  60,000), made in large amounts, is secreted extracellularly (Sanchez *et al.*, 1996). The participation of this soluble glycoprotein in the pathogenesis of Ebola disease in humans and experimental animals remains unknown; it may serve as some sort of immune decoy that minimizes the immune response to the virus. If so, does this point to any particular virus–host relationship, any particular reservoir host?

Filovirus glycoproteins are heavily glycosylated (carbohydrate may constitute one-third of the weight of the molecule; Feldmann *et al.*, 1993; Peters *et al.*, 1994), and it has been hypothesized over many years that viruses that exhibit this quality may better escape host defenses. Virions and virion budding sites on the plasma membranes of infected cells may not present T-cell recognition signals and may not present optimal targets for the efferent limb of the immune response once sensitization has occurred (i.e., they may not be targets for antibody action, macrophage action, and killer T-cell action).

One motif in the glycoproteins of Ebola and Marburg viruses has a high degree of sequence similarity with a putative immunosuppressive motif in the glycoproteins of oncogenic retroviruses. The retrovirus peptide has been shown to inhibit lymphocyte blastogenesis, decrease monocyte chemotaxis and macrophage infiltration, and inhibit the activity of natural killer cells (Cianciolo *et al.*, 1985; Kadota *et al.*, 1991; Burkreyev *et al.*, 1993; Volchkov *et al.*, 1995; Becker, 1995). It is not clear whether this conserved motif is important in the pathogenesis of filovirus hemorrhagic fever. Again, does this characteristic point to any particular virus–host relationship, any particular reservoir host?

## B. Lessons from Genetic and Phylogenetic Properties of the Filoviruses

When Marburg and Ebola viruses were first discovered, it was thought that they might be rhabdoviruses (Pattyn, 1978). However, as characterization work proceeded and as genomic sequencing was initiated, it became clear that the viruses deserved the formation of a new family, the family *Filoviridae* (Murphy *et al.*, 1978; Kiley *et al.*, 1982, 1988). Later, when it was realized that the member viruses of the family *Filoviridae* exhibit phylogenetic characters (conserved domains in nucleoprotein and polymerase genes) in common with members of the families *Rhabdoviridae* and *Paramyxoviridae*, the three families were brought together in the order *Mononegavirales* (Muhlberger *et al.*, 1992; Murphy *et al.*,

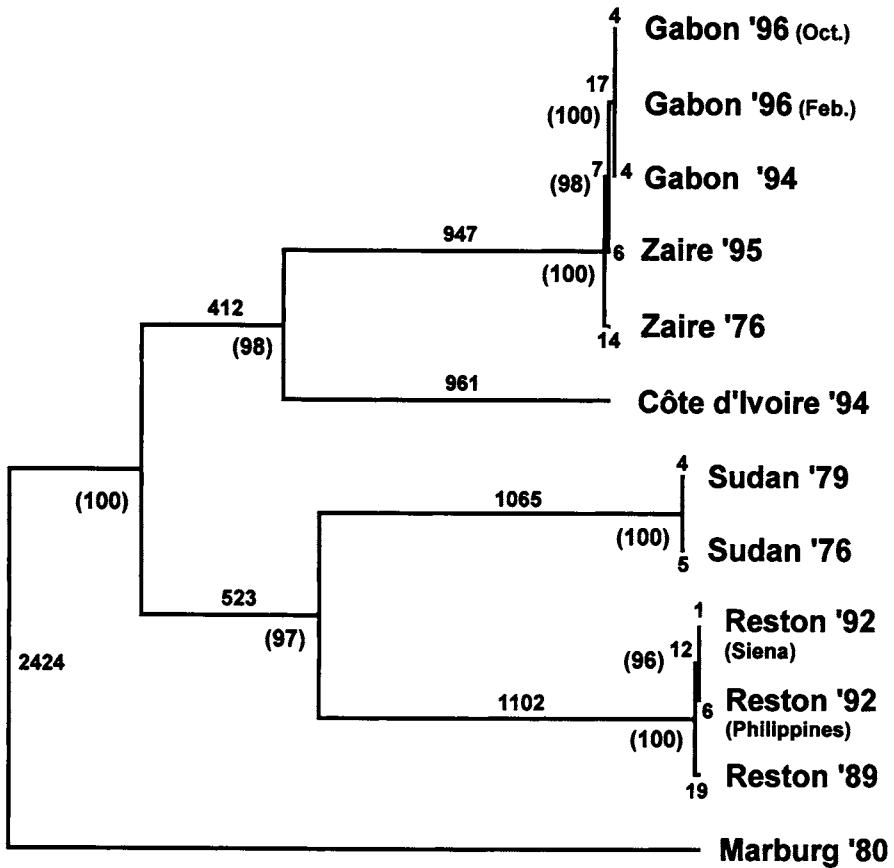
1995). The similarities in these viruses that led to these taxonomic constructions suggest the possibility that more tractable, less hazardous substitute viruses might be chosen as stalking horses in searching for antiviral compounds and cytokines. Indeed, both vesicular stomatitis virus and respiratory syncytial virus have been used to identify compounds active against Ebola virus (Huggins *et al.*, 1996). The finding of compounds effective against Ebola virus and respiratory syncytial virus is particularly intriguing: the importance of the latter as a pediatric pathogen throughout the world might favor the commitment of the financial resources needed to bring development of candidate compounds to commercial reality. Given the minimal activity of the only three therapeutic modalities that have been tried so far, namely, hyperimmune horse serum, convalescent human plasma (and whole blood), and interferon- $\alpha$ , antiviral drug development is a high priority.

Phylogenetic analysis of the filoviruses shows clear separation of Marburg virus from the Ebola subtypes; however, each Ebola subtype shows a nearly equal difference from each of the others. The nucleotide sequence difference between the Ebola subtypes is about 47%, whereas the difference between Marburg and the Ebola viruses is about 72% (Sanchez *et al.*, 1992, 1993; Georges Courbot *et al.*, 1997a,b) (Fig. 1).

The degree of stability of filovirus sequences overall and the absence of genetic variability among Ebola virus isolates obtained within an outbreak match the character of other member viruses of the order *Mononegavirales*. Such genomic stability and the phenotypic (serotypic, pathotypic, geotypic) stability that follows on it, has typified wild-type measles virus isolates over the years and street rabies virus isolates from particular reservoir host niches (e.g., raccoons throughout the eastern United States). The Darwinian stabilizing influences operating here are not really understood but must involve constraints on multigenic viral replicative functions as well as constancy in environmental selective pressures (Holland, 1993). The degree of this genomic stability was made strikingly evident when isolates from the epidemic in Kikwit, Zaire, 1995, were compared with those from Yambuku, Zaire, 1976, and from several sites in Gabon, 1996 (sequences of glycoprotein genes of isolates from these disparate sources differed by less than 1.5%; Sanchez *et al.*, 1996; Georges Courbot *et al.*, 1997a,b; Amblard *et al.*, 1997; Nichol and Ksiazek, 1997).

In begging the question of the habitat(s) and reservoir host(s) of the filoviruses in nature, it is these genetic differences rather than the similarities that seem most important. As noted above, these differences are enough to suggest geographic isolation of each virus in or near the site where human (and nonhuman primate) disease occurred and where the virus was isolated. That is, these differences make it clear that a common source human-to-human transmission chain extending across sub-Saharan Africa is not the case. Only in epidemics does a single invariant viral genotype occur. Again, this observation suggests that we will have to investigate every Ebola virus emergence individually, building, we hope, on clues gathered in the first sites, such as the Taï Forest in Côte d'Ivoire, to be studied in comprehensive fashion (Formenty *et al.*, 1996; LeGuanno, 1996).

The genetic differences between the Ebola virus subtypes also raise the question of when and where we might encounter other variants: the progenitors



**FIGURE 1** Phylogenetic tree showing relationships among the Ebola viruses. The entire coding region for the glycoprotein gene of the viruses shown was used in maximum parsimony analysis, and a single most parsimonious tree was obtained. Numbers in parentheses indicate bootstrap confidence values for branch points and were generated from 500 replicates (heuristic search). Branch length values are also shown. From Sanchez *et al.* (1996) and Georges Courbot *et al.* (1997), with permission.

representing the incremental steps in the evolution of this group of viruses, the evolutionary progeny of continuing selective pressures, the variants that evolve to invade different niches, different host species, and different organs. The emergence of the Reston subtype of Ebola virus from macaques in the Philippines, with its unusual and unexpected pathogenicity pattern (in nonhuman primates versus humans), certainly begs this question.

Most particularly, the question of mutability of the Ebola virus genome has led to public concern, fueled by the press/media, that the virus(es) will somehow acquire new means and patterns of transmission. The error-prone nature of RNA virus polymerases are usually mentioned when this notion is discussed. However, there is much more to acquiring a new phenotype than the incorporation of the occasional polymerase error. The probability that a random polymerase error will result in a new Ebola virus phenotype, say, a virus that is regularly transmitted by aerosol from severe respiratory tract infection, must be

small given the amount of human disease seen to date without emergence of such a virus. It is difficult to assess whether it could happen at all, given our rudimentary understanding of the Ebola genome, the small amount of experimental work done on the whole question of aerosol transmissibility, and the early stages of our explanation of the significance of the quasi-species concept for pathogenesis and evolution of RNA viruses. We must “never say never,” but at least changes in viral transmission patterns are not common, except when humans intervene.

### **C. Lessons from the Biological Nature of Filovirus Infections in Cell Culture**

Is there relevant biological information from the virology laboratory that might help in pointing the way to the filovirus reservoir host(s)? The filoviruses are readily isolated in Vero cells (Vero is a continuous African green monkey cell line). The viruses can also be grown easily in several other mammalian cell cultures, with or without cytopathic effect; however, the viruses have not been successfully propagated in reptilian, amphibian, or mosquito cells (van der Groen *et al.*, 1978; Swanepoel *et al.*, 1996a,b). Although there are several dramatic and often-discussed correlations between viral growth in cell culture and *in vivo*, these have often been misleading. The one generalization that has most often proved true is that viruses which fail repeatedly to grow in cell cultures from certain hosts usually do not infect the same hosts *in vivo*. Thus, such studies of filoviruses must be done, must be interpreted from the perspective of searching for the reservoir host(s), and must not be prejudiced toward a mammalian host or to drive experimental laboratory and field investigations only toward mammalian host candidates.

The filoviruses, in cell culture systems (and in experimental animal host systems), have been difficult to neutralize with convalescent sera and have been resistant to the antiviral effects of interferon- $\alpha$ . These properties are by no means unique to the filoviruses, but they are somewhat unusual among human pathogens. Moreover, they are shared most prominently with viruses such as Lassa virus and lymphocytic choriomeningitis virus (arenaviruses), which are perpetuated in nature via persistent infection of specific rodent hosts.

There has even been speculation that the filoviruses may be plant viruses and/or that plants may play a role in their maintenance. Indeed, infection of plant cells in culture has been tried, but without success (Swanepoel *et al.*, 1996a,b). Of course the idea of involvement of plants cannot be ruled out by such arbitrary experimentation or on theoretical grounds, but in this case consideration of known unique molecular and replicative properties of plant viruses makes us think that this is a very unlikely possibility.

Taken together, cell culture data might bias one toward a mammalian reservoir for the filoviruses. On further reflection and consideration of known properties of many of the viruses of birds, reptiles, amphibians, arthropods, etc., however, the data do not permit definitive conclusions or limiting predictions.

### **D. Lessons from Clinical and Pathological Characteristics of Filovirus Infections in Humans**

An old truism states, “Understanding the nature of a disease in the individual patient is a key to understanding the nature of the disease in the population.”

This truism must stem from diseases where there is one host species, one host population involved. However, with recognition of the extra complexity added by multispecies zoonotic transmission cycles, especially an unknown zoonotic transmission cycle, precise lessons seem hard to come by. Nevertheless, a review of the clinical and pathological nature of filovirus infections is warranted.

Marburg and Ebola virus subtypes Zaire, Sudan, and Côte d'Ivoire cause severe hemorrhagic fever in humans—"the evolution of disease often seems inexorable and invariable" (Piot *et al.*, 1978). Following an incubation period of usually 4 to 10 days (extreme range 2 to 21 days for infection by the Zaire subtype of Ebola virus), there is an abrupt onset of illness with initial nonspecific symptoms including fever, severe frontal headache, malaise, and myalgia. Early signs include bradycardia and conjunctivitis, and there may be a macropapular rash most readily evident on white skin (Pattyn, 1978; Peters *et al.*, 1994, 1996; Khan *et al.*, 1996). Deterioration over the following 2 to 3 days is marked by pharyngitis, nausea, and vomiting, progressing to hematemesis and melena. There is prostration and bleeding which is manifested as petechiae, ecchymoses, uncontrolled bleeding from venepuncture sites, and postmortem evidence of visceral hemorrhagic effusions. Death usually occurs 6 to 9 days after onset of clinical disease (range 1 to 21 days). Abortion is a common consequence of infection, and infants born to mothers dying of infection are fatally infected. Convalescence is slow and marked by prostration, weight loss, and often amnesia for the period of acute illness.

In filovirus infections of humans, there is infection of macrophages and endothelial cells throughout the body and infection of the parenchyma of multiple organs, especially the liver and spleen. The infection of these tissues is devastating, with swelling, hemorrhage, and focal necrosis (Murphy *et al.*, 1978; Dietrich *et al.*, 1978; Zaki *et al.*, 1996a,b). Disseminated intravascular coagulation is one of the mechanisms by which the patient is compromised. Destruction of lymphoreticular tissues may be partially responsible for the common absence of an effective immune response. Virus shedding from infected humans occurs from all body surfaces and orifices, including the skin and mucous membranes, and especially from hemorrhagic diatheses (Schnittler *et al.*, 1993; Zaki *et al.*, 1996a,b; Feldmann *et al.*, 1996a).

Of course, there is no way to extend these clinical and pathological observations to predict the nature of infection in the unknown reservoir host(s), but given the systemic nature of infection in humans and the similarity of this pattern of infection in susceptible experimental animals, especially nonhuman primates, it seems likely that if the reservoir host(s) is a mammal, its infection might also involve viral entry via the body surface (mucous membranes of the oro-naso-pharynx or eye, and/or breaks in the skin), and might also require hematogenous spread, systemic organ/tissue infection, and shedding via blood, mucosal surfaces, and the respiratory tract. This pattern of infection is common among other member viruses of the order *Mononegavirales*, such as measles virus, canine distemper virus, mumps virus, Newcastle disease virus in birds, and Sendai virus in mice. In other words, it might be more likely that filovirus infection of a mammalian reservoir host would not be superficial, involving primarily only the respiratory epithelium or intestinal epithelium. This hypothesis can be extended to avian, reptilian, and amphibian candidate reservoir hosts, so its predictive value is not too remarkable.

### E. Lessons from the Pathogenetic Characteristics of Filovirus Infections in Experimentally Infected Animals

Another old truism might be stated, "Understanding the nature of a disease in an experimental animal model can hold the key to understanding its nature in its definitive host." In the case of the filoviruses, the same problem exists as in attempting to extend observations from human to laboratory animal infections; until we identify the reservoir host(s) of the filoviruses, we cannot know whether any of the experimental animals that have been studied bring us any closer to understanding the nature of infection in the reservoir host(s).

Nevertheless, many experimental animals have been studied. All of the usual laboratory mammalian species have been inoculated with Marburg and the Zaire subtype of Ebola virus (Kissling *et al.*, 1970; Murphy *et al.*, 1971; Murphy, 1978; Pokhodyaev *et al.*, 1991; Pereboeva *et al.*, 1993; Ryabchikova *et al.*, 1993, 1996; Murphy and Nathanson, 1996; Peters, 1996b). There have been fewer studies of the Sudan and Reston subtypes and none yet reported of the Côte d'Ivoire (Fisher-Hoch *et al.*, 1992a; Geisbert *et al.*, 1992). Several species of monkeys, mice, guinea pigs, and hamsters are highly susceptible to Marburg virus and the Zaire subtype of Ebola virus, with infection usually ending in death. The Sudan subtype of Ebola virus often causes a self-limited infection in mice, guinea pigs, and the same species of monkeys.

In rhesus monkeys (*Macaca mulatta*), cynomolgus monkeys (*Macaca fascicularis*), African green monkeys (*Cercopithecus aethiops*), and baboons (*Papio* spp.) inoculated with Marburg virus or the Zaire subtype of Ebola virus, the incubation period is 4 to 6 days, during which time virus replicates to high titer in the reticuloendothelial system (including lymph nodes and spleen), endothelium, liver, and lungs. With the onset of clinical disease, there is severe necrosis of these target organs, which is most evident in liver, and there is interstitial hemorrhage, which is most evident in the gastrointestinal tract (Murphy *et al.*, 1971; Murphy, 1978; Bazhutin *et al.*, 1992; P'yomkov *et al.*, 1995; Luchko *et al.*, 1995; Jaax *et al.*, 1996).

One observation in particular made during the episode of disease in monkeys in the quarantine facility at Reston, Virginia, in 1989, deserves further attention: infection of many macaques (*Macaca fascicularis*) by what turned out to be the Reston subtype of Ebola virus was characterized by the usual clinical signs and histopathological lesions, as noted above (Dalgard *et al.*, 1992; Hayes *et al.*, 1992; Jaax *et al.*, 1995; Jahrling *et al.*, 1996a). However, in addition there were inordinate amounts of respiratory and nasal secretions. These secretions contained over  $10^6$  plaque-forming units (pfu)/ml of Ebola/Reston virus, and no other viruses or bacteria were found. Given the concern over Ebola transmission by aerosol, this observation needs to be followed up (C. J. Peters, personal communication, 1997).

Thus, there is considerable similarity in the way filoviruses attack humans and certain nonhuman primates. As in other successful infections, the filoviruses have the capability to adapt to experimental animal species with which they have not likely had experience in nature. They are successful in gaining entry, escaping innate resistance factors, finding receptors on specific cells in several organs, finding routes and cellular substrates for systemic infection, overcoming

acquired resistance factors such as the host immune response, and assuring shedding and continuation of the virus life cycle. As is the case in human infection, it must be that within this complex systemic pattern of infection, the filoviruses outmaneuver the specific host defense mechanisms of experimental animals by (a) their speed, as animals often die before it might be expected that an effective primary specific inflammatory/immune response would be elicited, and (b) their tropism(s), as the early reticuloendothelial and lymphoid tropisms likely minimize the response that might be elicited otherwise.

Thus, all in all, the same thing might be said from the lessons of filovirus infections in experimentally infected mammals as about infection in humans. Infection in a mammalian reservoir host might be systemic, peracute, and very productive of contagion, but such a hypothesis means little in the absence of direct knowledge.

Two species of insectivorous and one species of fruit-eating bats have been found to support the growth of Ebola virus very well (Swanepoel *et al.*, 1996b): some bats were found to contain virus in their tissues and blood for as long as three weeks. Once again, there is the caveat that many viruses have been isolated from bats without evidence that these animals participate in the maintenance of the virus life cycle (e.g., St. Louis encephalitis virus, Japanese encephalitis virus, chikungunya virus, Rift Valley fever virus, Toscana virus) (American Committee on Arthropod-Borne Viruses, 1985). Nevertheless, bats are an attractive candidate to be the filovirus reservoir host. Most species of bats are migratory and so could account for the seasonality of Ebola virus appearances, and several other member viruses of the order *Mononegavirales* have bats as a primary or secondary reservoir host (e.g., rabies and other lyssaviruses such as the newly identified Australian bat lyssavirus; several other rhabdoviruses such as Mt. Elgon bat virus and Kern Canyon virus; Australian equine morbillivirus) (Murphy *et al.*, 1995; Murray *et al.*, 1995; Young *et al.*, 1996; Fraser *et al.*, 1996). Clearly, the possibility that certain bats may serve as the reservoir host of filoviruses will not be answered easily.

There is no evidence for latency or persistence in any filovirus infection in any experimental animals that have been studied (or in humans for that matter) (Fisher Hoch *et al.*, 1992b; Khan *et al.*, 1996). Occasional cases of subacute uveitis and orchitis have been observed in humans, but these have only reflected a short-term persistence of virus in tissues that are relatively protected from the acute inflammatory/immune response. Neither is there evidence that subclinical or silent productive infections play any important role in experimental animal models.

Evidence that there is a range of temperate virus strains in nature that might complicate our understanding of the pathogenesis and pathology of infections caused by the filoviruses is also lacking. We have no way of knowing whether the range in virulence from the "hottest" known Ebola virus, that is, the Zaire subtype, to the "coolest," that is, the Reston subtype, represents the full spectrum of biotypes/pathotypes in nature. The Reston subtype of Ebola virus may be temperate in humans, but it is quite virulent in nonhuman primates (Jahrling *et al.*, 1996a). If there is a more complex interplay in nature between the filoviruses than we know about and if there are undiscovered less virulent subtypes, then we might easily go off on the wrong tangent in our speculations. Perhaps



as long as we remind ourselves that “unnatural” virus–host pairings (e.g., Ebola virus Zaire subtype in humans or experimentally infected monkeys) may be more pathogenic than natural virus–host (reservoir host) pairings, and that for every generalization there is an exception, we can keep an open mind about the lessons from experimental pathology.

A limited number of nonmammalian species, such as pigeons, frogs, geckos, snakes, leafhoppers, spiders, and so forth, have been inoculated with Ebola virus, in every case with negative outcome (Swanepoel *et al.*, 1996a,b). Ebola virus replication has not been demonstrated after intrathoracic inoculation of several species of mosquitoes (Turell *et al.*, 1996; Swanepoel *et al.*, 1996a,b); however, these negative results may have too casually dismissed all focus on the possible role of arthropods in filovirus transmission. The arthropod host specificity of most arboviruses is quite narrow, and there are many, many different arthropods that could be considered candidate filovirus hosts. Few exotic arthropods have been tested: the many species of biting flies, midges, mites, cimicid bugs, spiders, scorpions, etc., would each require particular wrinkles in experimental design. As in the case of any wild, exotic candidate animal host, it is important that adequate attention be given in such studies to the identification of specimens and the taxonomic system by which they are identified. Attention must also be paid to the archiving of data from such studies, via publication and public database development.

## **F. Lessons from the Characteristics of Various Other Viruses That Make Them Successful Pathogens**

Over the years, certain viral characteristics have been judged in regard to their contribution to the overall “success” of particular viral pathogens. It might be of interest to judge the filoviruses in this regard, hoping thereby to find clues to their reservoir host(s). Characteristics of concern pertain partly to Darwinian forces favoring competitive survival (survival of the fittest) and partly to our sense of anthropocentric forces favoring shared survival [i.e., pertaining to the oft-stated notion that the successful pathogen should drift toward commensalism in its relationship with its natural host(s)] (Holland, 1993). However, every characteristic that may be judged as advantageous in defining a successful virus, perhaps even “The Ultimate Pathogen” (Kilbourne, 1983), also calls to mind examples where an opposite character is favored by other successful pathogens (Nathanson and Murphy, 1996). The following are some characteristics and a judgment of their importance to the success of the filoviruses:

1. *Capacity of the virus to grow rapidly:* Some of the most successful pathogens complete their life cycle in their reservoir hosts very quickly (e.g., Venezuelan equine encephalitis virus, Rift Valley fever virus, vesicular stomatitis virus, influenza virus, paramyxoviruses). The survival advantage here may involve the need for transmission via a fleeting intermediate host, for example, a mosquito that is active for only a short period seasonally, or the need for assuring transmission before host immunity intervenes. The filoviruses do grow rapidly, as indicated by their characteristic growth dynamics in cell cultures as well as their behavior in infected humans and monkeys. Does this characteristic carry over into the reservoir host(s)? Does this characteristic point to an arthro-

pod host or a host present in very large numbers, such as a rodent host, where rapid viral transmission favors staying ahead of host immunity and population immunity?

2. *Capacity of the virus to grow to high titer:* Capacity to grow to high titer is a corollary of the capacity of a virus to grow rapidly and is especially important in the life cycle of arboviruses. Vertebrate host viremia, dependent on productive viral growth in tissues, is necessary for the transmission of virus to an arthropod seeking a blood meal; because blood meal volumes are so small, high viremia likely represents a survival advantage to the virus. Enteric viruses also commonly grow to high titers, in this case so as to favor fecal contamination and the success of the fecal–oral transmission cycle. We know that the filoviruses grow to very high titers in humans and experimental animals; does this point to, as noted above, an arbovirus life cycle? Does this point to a fecal–oral transmission cycle?

3. *Capacity of the virus to be shed quickly:* Some successful pathogens grow quickly, as noted above, but have other mechanisms that increase shedding (e.g., rotaviruses, other enteric viruses, many respiratory viruses). Efficient shedding may be favored by short-lived clinical/physiological qualities of infection, such as diarrhea or productive coughing with catarrh. The filoviruses are shed quickly, but seemingly not in specifically produced body fluids (although in the setting of the primate quarantine facility where the Reston subtype of Ebola virus emerged, spread via respiratory secretions/excretions seems to have represented an exception). All in all, is it reasonable to predict that filoviruses do not employ in nature life cycles like those of the diarrhea viruses or the strict respiratory viruses?

4. *Capacity of the virus to replicate in certain key tissues that favor transmission:* Many successful pathogens employ specific tissues for shedding; for example, many poxviruses, although causing multiorgan systemic infection, are transmitted only after infecting skin epithelium, where they cause a virus-laden exanthem which is infectious by contact or by fomite (even in some cases by mechanical carriage by arthropods). Rabies virus, although neurotropic through most of its infection path, is transmitted in nature via virus shed from salivary gland epithelium. Human immunodeficiency virus (HIV), also systemic in its infection pattern, is quite lymphoreticular in its tropism, but transmission nearly always involves sexual contact or blood contact (sharing needles among intravenous drug users, blood contagion at birth, formerly blood transfusion and certain blood products). In humans and experimentally infected monkeys, the filoviruses are shed from the respiratory tract, skin, and mucous membranes, and especially from blood and blood-contaminated body fluids. Transmission has only occurred via close contact except in the unusual circumstances in monkey quarantine facilities where aerosol transmission has been evident (Peters *et al.*, 1991a,b; Jaax *et al.*, 1995). Do these comparisons point to filovirus transmission in nature only by close contact? Do they point away from transmission involving unusually restricted sites such as salivary glands? Do they point to a separation between major sites of virus replication and sites of virus shedding?

5. *Capacity of the virus to be shed even in the face of rising host immunity:* The capacity for viral shedding follows on the capacity of certain viruses to

evade host defenses and establish persistent infection. This characteristic is related to the capacity of certain viruses to be transmitted congenitally and others to be shed chronically. Often this pattern of infection involves a particular immunopathological interaction of the virus and the host immune system, and often it involves a sequestration of infection in immunologically privileged tissue sites, such as the kidney, salivary glands, and sexual organs. Often this pattern of infection is unique to one (usually the reservoir) but not all host species. The long-term shedding of arenaviruses and hantaviruses in reservoir host urine and saliva, as contrasted with the acute, self-limiting course of infection, with modest, short-lived shedding in humans, is exemplary. The recrudescence of herpesviruses from ganglionic neurons and the long-term shedding of hepatitis B and C viruses by carriers are also models. The filoviruses do not seem to fit this category: in humans, nonhuman primates, and in all other experimental animals that have been studied, no persistent infection has been found. Thus, unless the behavior of the filoviruses in their reservoir host(s) is quite different, this would not seem to be a priority issue for immediate research.

6. *Capacity of the virus to survive after being shed:* Viral survival after shedding is usually an intrinsic quality of the virion, pertaining to its resistance to heat and other physical insults, solvents and other chemical insults, irradiation, etc. The range in environmental stability/instability among all human pathogenic viruses is very wide, indeed. Many but not all viruses that employ the fecal-oral transmission cycle are intrinsically "tough" (e.g., polioviruses, parvoviruses, and reoviruses are very resistant to environmental insults, whereas coronaviruses and toroviruses are not); many but not all viruses that are transmitted by the respiratory route or by other direct means are "fragile" (e.g., rhinoviruses, caliciviruses, and adenoviruses are rather resistant, whereas orthomyxoviruses, paramyxoviruses, and morbilliviruses are not). Further, most hepatitis viruses are rather "tough," having first to resist degradation in the intestine, and most arthropod-borne viruses are "fragile," never having to survive outside their vertebrate and arthropod hosts. Here, further information is needed about filovirus environmental stability. Anecdotes about Ebola virus surviving for months in blood at ambient temperature in the Kikwit hospital must be supported by controlled laboratory study. The evidence that we do have indicates that the filoviruses are rather average in stability (Cheprunov *et al.*, 1995; Belanov *et al.*, 1996). That is, the filoviruses are stable enough to represent the particular risk that has been evident in nosocomial contact transmission episodes and contaminated needle transmission episodes (even when syringe and needle have been held at room temperature for some time), but not enough to represent risk of remote environmental spread. The latter point is complemented by the knowledge that viruses which are most like the filoviruses (i.e., the member viruses of the order *Mononegavirales*) are not transmitted in nature via cycles involving long-term survival outside their host(s).

#### **IV. ECOLOGICAL AND EPIDEMIOLOGICAL CHARACTERISTICS OF FILOVIRUS INFECTIONS**

In regard to the issue of the natural history of the filoviruses, the perspective of disease ecology begs unique questions: What ecological and population pro-

cesses account for the pattern and likelihood of disease emergence within a particular ecosystem? How can knowledge of spatial population dynamics increase the capacity for predicting the spread of disease? What are the ecological influences on evolutionary processes affecting a pathogen and its host(s) that may account for given patterns in disease and disease resistance? What long-term relationships between host and pathogen may be expected? How may the population dynamics and the reproductive ecology of the host, pathogen, and vector be modeled? How will the pathogen respond to global climate change and changing patterns of land and water use? What is the functional role of disease in ecosystem management? In sum, the perspective of disease ecology focuses on the overall *environment* and seeks to determine the influence of a pathogen, its host, and their interrelationship in the overall environmental situation. We shall attempt to apply this perspective to the question of the natural history of the filoviruses.

The epidemiological perspective is quite different; it begs questions relating directly to the determinants, dynamics, and distribution of the disease in the population at risk. Its focus is on the bases for risk of infection and disease in the *population*, as these are determined by characteristics of the virus, of individual hosts, and the host population. There is overlap in that there also may be emphasis on environmental and ecological factors that affect transmission from one host to another, but, as in the case of the field of disease ecology, the goal of infectious disease epidemiology is to meld understanding of all causative factors into a unified whole.

### **A. Lessons from the Ecological and Epidemiological Characteristics of Filovirus Infections**

Filovirus index cases have often occurred in the tropical rainy season. Ebola hemorrhagic fever episodes in Zaire, Sudan, Côte d'Ivoire, and Gabon have all occurred in or near the end of the rainy season, and all have been associated with tropical forest or the marginal zone between tropical forest and savanna. On the one hand, this puts the search for the viruses in the most biologically diverse of all ecomiches, but, on the other hand, this at least narrows the search area somewhat. In this location, many reservoir host candidates exhibit seasonality in behavior (seasonal breeding, migration, contact with humans or non-human primates and other normal behaviors). Going further, this location may also point to reservoir host candidates with multiyear seasonal behavioral patterns. Does seasonality or multiyear seasonality of filovirus disease episodes point to arthropods? Are the most likely candidates arthropods that are capable of becoming infected and amplifying virus? True arboviruses exhibit marked seasonality in their distribution as evidenced by the distribution of virus in arthropod populations and by the distribution of disease in humans or domestic animals. Are the most likely reservoir candidates rodents, which exhibit marked seasonal breeding and feeding habits and which exhibit multiyear population and behavior differences because of varying food supplies? Are the most likely candidates bats or other seasonally migrating species? Or are the most likely candidates species that exhibit seasonal or multiyear seasonal variations that we do not even know about or recognize as significant in the context at hand?

Further, it must be recognized that within the ecosystems under considera-

tion, the tropical rain forest and bordering savanna, there is great microniche isolation and ecological insularity; that is, there are many, many sites within larger geographical areas in which the filoviruses may invisibly coexist with their reservoir host(s). Many such econiches have never been examined in regard to any virological question, many may not even be known or defined at all. For example, when arbovirologists first studied the tropical rain forests of Africa and South America (Downs, 1973), it was not even understood that the forest canopy, the under-canopy, and each lower level down to ground level each represented a distinct, isolated econiche, each niche filled with different mosquitoes and with different mosquito-borne viruses. It took years of study just to begin to understand the complexity of these econiches. The real question here will be how to simplify the study of tropical rain forest and savanna ecosystems in a way practical enough to fit in with the limited global budget for filovirus field research.

Retrospective ecological studies have been performed at varying time intervals after most of the known filovirus outbreaks, but these studies have been limited when compared to studies of arbovirus ecosystems in tropical forests, as noted above. These classic studies, led in Brazil and Nigeria by O. R. and C. E. Causey and colleagues, as part of the Rockefeller Foundation Virus Program which ran from 1951 to 1970, depended on long-term staffing of field stations on site, with backup from a world-class reference laboratory, the Yale Arbovirus Research Unit, which later became the WHO World Reference Center for Arboviruses (Downs, 1973). The field programs in Brazil and Nigeria led to the identification of more than 100 new arboviruses and defined many of their reservoirs. In contrast, filovirus field studies have been carried out only through brief one-time expeditions to sites where human exposure had occurred, and even then such expeditions have usually been delayed until months or even years after human or nonhuman primate disease episodes. Given the very small number of filovirus field expeditions, their limited scope and scale, and their very narrow focus (i.e., to find one virus), it seems no wonder that there has been so little success. In this regard, the ongoing studies in the Taï Forest in Côte d'Ivoire become extremely important.

Would past arbovirus field programs, such as the Rockefeller Foundation Virus Program, as described above, or the long-running Institut Pasteur programs in several African countries, have recognized filoviruses if they had been present in arthropod or vertebrate specimens? All of these field studies carried out in the 1950s to 1970s were based on the inoculation of specimens (pooled, ground mosquitoes and far fewer animal and bird blood and tissue samples, etc.) into newborn mice. We know that Ebola virus, Zaire subtype, from human specimens, is lethal for this host (van der Groen et al., 1978), but it is not clear whether this virus or other filoviruses would have been identified in the serology-based system designed to identify and classify arboviruses. A few non-arthropod-borne zoonotic viruses were discovered through the Rockefeller Foundation and Institut Pasteur programs, but there is no way to know what the sensitivity of the programs was for such viruses. In any case, filoviruses were not identified in these programs, and perhaps, given the state of biocontainment in the field laboratories serving these programs at the time, it was fortunate that they did not appear (Casals, 1961, 1967; Downs, 1973).

We should examine the basis for the rarity of human infection from the ecological perspective. To do this, we have chosen to divide the subject into four premises, none of which are mutually exclusive and all of which pertain to the strategy that we would employ to search for the reservoir host(s) in the field.

1. *The reservoir host(s) is rare:* There are many species of animals and arthropods that exist in very small populations, often in very limited geographic areas and often in very restricted niches. The adaptations necessary to assure the perpetuation of such species are many, and many remain unknown. We know very little about the viral flora of such species and even less about their overall zoonotic contribution to human viral diseases. The premise that the reservoir host(s) of the filoviruses is rare calls for a very difficult search approach, one based on the exhaustive examination of as many rare species as possible to find and trap. However, given the difficulty of specimen acquisition, testing this premise would put most demands on the field work and least on laboratory resources. Again, given the minimal level of our knowledge of the rare species in the tropical forests and adjoining savanna, there would seem to be minimal opportunity to focus this kind of search and there would be maximal dependence on good luck in finding the right niche.

2. *The reservoir host(s) is rarely infected:* There are many viral infections that seem to be very rare, occurring at a very low incidence in their host population. Most of the viruses of humans and animals that exist in this way do so through long-term or lifelong persistent infection, often with a long incubation period and/or intermittent low-level shedding. In some instances, such as with some of the agents of the transmissible spongiform encephalopathies, we have no idea how the infectious agent exists in nature. The point is that such infectious agents have adapted and have developed probably unique counters to their seemingly high risk of extinction. Considering the filovirus reservoir host(s), this premise also promises a most difficult search strategy. Here, we would focus on common animal and arthropod species, again also considering the possibility of focal distribution of virus within any overall species distribution and again considering the influence of subspeciation, genotypic variation, and toptype variation. Here, the search strategy would involve exhaustive collection of as large a number of candidate species as possible. Here, again, it would seem as if long-term field laboratory resources would be needed on site in Africa, but there would also be a large burden on the laboratory.

3. *The reservoir host(s) rarely comes in contact with humans:* There are many species of animals and arthropods that for many reasons do not come into contact with humans. Some such species are just ignored because they never have seemed valuable, interesting, or dangerous. Arboreal species, solitary species, camouflaged and reclusive species, as well as species not taken for food would be candidates. A new mind-set would be needed, focusing on the sorts of animals and arthropods that one might otherwise ignore, but systematic collection of moderate numbers of specimens from many such species would be a good start. Again, it would seem that a long-term field presence on site would be needed, but the laboratory burden would not be massive.

4. *The reservoir host(s) is not very infective because it rarely sheds virus, or rarely sheds virus in sites where humans are at risk of infection:* Many known

zoonotic viruses are rarely transmitted to humans (or to domestic animals), either because the reservoir host does not shed much virus (or sheds virus intermittently) or because its habits are not conducive to transmission to nonreservoir hosts. For example, many species represent nearly dead-end hosts for rabies virus. Hantaviruses, although often infecting a substantial proportion of their reservoir rodent host populations, cause few human infections because of the behavior of these rodents (e.g., *Peromyscus maniculatus*, the reservoir host of Sin Nombre virus, the etiologic agent of hantavirus pulmonary syndrome, presents risk of human infection when it enters houses seasonally, whereas *Clethrionomys* spp., the reservoir hosts of Puumala virus, the etiologic agent of nephropathia epidemica in Scandinavia, causes human infection rarely because of its reclusive habits). Eastern equine encephalitis virus, although common in its wild bird niche in parts of eastern United States, only rarely causes human disease because of the feeding habits of its mosquito hosts. Pursuing this premise in regard to the filovirus reservoir host(s) also leads to a search strategy rather like that for species that rarely come in contact with humans (or nonhuman primates). Pursuing this premise would also require the systematic collection of moderate numbers of specimens from many such species. Again, it would seem that a long-term field presence on site would be needed, but the laboratory burden would not be overwhelming.

5. *The virus requires a genetic adaptation before transmission can occur to humans:* Genetic adaptation is the premise underpinning viral “species jumping,” the initial crossing of the species barrier. This is not so far from current thinking as one might suppose. The importance of rapid genomic mutation rates and adaptation in RNA viruses has been widely accepted, but it has not been well integrated into our thinking about the natural history of viruses. For example, Ebola virus, Sudan subtype, from human blood or from primary cell culture passage, is infective but not pathogenic for guinea pigs. It requires a few passages before becoming lethal for this new host. Pursuing this premise in regard to the filovirus reservoir host(s) might be the most difficult of all. It would involve much searching in the dark. It would require substantial field collection resources but additionally would require an exceptional scale of manipulative research, much of which would be difficult to tie back to candidate virus–host relationships in nature. Finally, it would dictate the extensive use of polymerase chain reaction (PCR), which would greatly increase overall costs.

On reflection, it is easy to see from the complexity of the above premises why there might continue to be a need for a broad range of candidate specimen collection and testing activities, especially in outbreak settings. The point where such searching becomes redundant or nonproductive, however, is a matter of judgment, not necessarily made clear to all investigators at the same time. In our view, this point has now been reached: we believe that it is time to move beyond episodic collecting in areas near human disease outbreaks to testing the above premises and to incorporating these premises into a comprehensive field/laboratory search enterprise. We believe that it is necessary to reinvent some of the long-term, on-site field strategies that guided the Rockefeller Foundation and Institut Pasteur arbovirus programs, adapting them to the problem at hand. We believe that such a program should also incorporate a basic virology research

element, tying in viral molecular genetic approaches more tightly and bringing such approaches from the laboratory to the field.

## **B. Confounding Role of Serosurvey Data in Trying to Determine Ecological and Epidemiological Characteristics of Filovirus Prevalence**

In several serosurveys, all performed with the indirect fluorescent antibody (IFA) technique, a high prevalence of Ebola antibodies has been found in apparently normal human populations. Given the absence of any confirmatory testing in these serosurveys, and the failure to find concordance when the IFA technique has been run comparatively with various confirmatory tests, it is remarkable that conclusions reached from such surveys still influence our ideas about the epidemiology and natural history of the filoviruses. The usual IFA technique employs acetone-fixed filovirus-infected cells (inactivated by  $\gamma$ -irradiation) as substrate for testing for the presence of antibodies in untreated human or animal serum (Elliott *et al.*, 1993).

As examples of the confusion that the use of the IFA test has caused, consider the fact that in surveys of humans from Africa, Alaska, and Panama, monkeys from Asia, and a variety of animal species obtained worldwide, in the absence of any recognized disease, a high prevalence of antibodies to filoviruses, particularly Ebola virus subtypes, has been reported (Pattyn, 1978; Johnson *et al.*, 1981; Stansfield *et al.*, 1982; van der Walls *et al.*, 1986; Meunier *et al.*, 1987a,b; Gonzalez *et al.*, 1989; CDC, 1990a,b; Peters *et al.*, 1991a; Johnson *et al.*, 1993a,b). This IFA reactivity is not a simple technical artifact; in fact, its cause remains unknown, although there are suspicions that it reflects cross-reactivity from infectious with extremely distantly related viruses such as other member viruses of the order *Mononegavirales*. More specifically, in one recent IFA-based study (admittedly, incorporating some confirmatory testing), it was reported that there is a high prevalence of antibodies in inhabitants of the Congolese basin of western Africa: up to 30% prevalence (Ebola virus, subtype Zaire) was reported in people living in the rain forest (with greater than 20% in the Pygmy ethnic group and 14% in Bantu people living in the same area), and up to 10% prevalence (Ebola virus, subtype Sudan) in people living in the savanna. Antibody to filoviruses was reported to also be present in domestic and wild animals: dogs, pigs, guinea pigs, and monkeys (*Cercopithecus aethiops* and *C. ascanius*) (Gonzalez, 1996).

Clearly, such studies can have an overwhelming influence on our thinking about the reservoir host(s) of the filoviruses. Such studies require independent confirmation, but moreover they should be conducted from their start with gold-standard techniques. What is needed is such a gold-standard test for filovirus antibodies in human and animal sera that would engender the confidence of all investigators in the field, a test that would have the same credibility as the virus neutralization test has had in assaying polio or measles or Japanese encephalitis virus antibodies. Until recently, unfortunately, candidate confirmatory tests have not proved particularly useful: (a) western blot test results have been ambiguous; (b) no viral hemagglutinin has been detected, so there can be no hemagglutination-inhibition test; and (c) very little or no neutralization



by convalescent serum has been identified, so there can be no viral neutralization test.

As of 1997, the best bet for a gold-standard confirmatory test is a particular enzyme-linked immunosorbent assay (ELISA) (Ksiazek *et al.*, 1992). This ELISA is relatively simple to use; it is based on inactivated infected cell lysate as antigen and employs an essential negative control antigen test for every serum specimen tested. Serosurveys, employing this ELISA, carried out on patients, contacts of patients, and others in Kikwit in 1995 indicated an extremely low prevalence of antibodies, affirming that human infections are usually symptomatic. This test has been evaluated for specificity and sensitivity: it has been concordant with virus isolation results when used on sera from humans and monkeys known to have been infected with filoviruses, and it has been negative when used on sera from thousands of humans and monkeys from areas of North America where there never has been any evidence of the presence of a filovirus. Among the latter sera there were some that were reactive by the IFA test, but none exhibited ELISA reactivity. Seropositivity using this ELISA has also been shown to be maintained for a long period after infection: in a small number of human sera collected more than 10 years after infection, antibody has been detectable, whereas IFA results have been equivocal or negative.

## **V. ONGOING AND NEEDED RESEARCH ON FILOVIRUSES IN THE FIELD AND IN THE LABORATORY**

### **A. Ongoing and Needed Field-Based Filovirus Research**

Many filovirus investigators believe that a key feature of any search for the reservoir host(s) of the filoviruses must involve extensive examination of vertebrates near the places in Africa where human cases have occurred. It is argued that it is in such sites where the reservoir host(s) must participate in a transmission cycle, whether this involves direct contact transmission, fomite transmission, arthropod transmission, or whatever. It is argued that vertebrates should be the primary focus of the search; vertebrates might at least serve as sentinels, providing evidence of past experience with the virus(es) by the presence of antibody. It is argued that vertebrates, specifically mammals, are the most likely reservoir hosts, based on results of infection of experimental animals and cultured mammalian cells. It is further argued that arboreal species should be considered leading candidate reservoir hosts, given the recent finding of a dead, Ebola-infected red colobus monkey in the Tai Forest, Côte d'Ivoire, in association with disease in chimpanzees (Formenty *et al.*, 1996; ProMED Internet news item, dated 16 November 1996). Finally, it is argued that migratory bats should be considered important candidate reservoir hosts. There have been anecdotes involving bats in several filovirus disease episodes, and, even though bats are common in tropical Africa, these clues must be followed up. Further, the involvement of bats in the natural history of some of the viruses most closely related to the filoviruses (i.e., some member viruses of the order *Mononegavirales*), along with the capacity of bats to sustain some viral infections for inordinately long periods, adds modest credibility to their candidacy. We believe that in pur-

suings such candidates, sound hypotheses must be established and explored. A sound work plan must be set up and followed so that when initial hypotheses are rejected there is a clear course of action to test the next in priority order. A proper shared information system is crucial in this regard, given the diversity of field projects to be undertaken and the geographic separation of scientists from different institutions from several different countries. The publication of all results will be essential if we are to be able to profit from experiences and findings, even if findings are negative.

Many of the above considerations have been employed in designing the collaborative search by investigators from several institutions for the Ebola virus reservoir host(s) in the forests around Kikwit, Zaire, and in the Taï Forest, Côte d'Ivoire (Ksiazek, 1996; Swanepoel *et al.*, 1996a,b; Formenty *et al.*, 1996). Investigators have started by examining sites that have been connected with the person identified as the index case in these outbreaks. These searches span critical ecological zones such as primary and secondary forest as well as savanna; however, there is a concentration on the rain forest zone because of the evidence mentioned above. As noted above, it has been decided that mammals should be the primary focus of the collections. However, not to be "too smart" about predicting a mammalian reservoir host(s), the investigators are testing any vertebrates that enter traps and are also testing some market animals obtained in nearby towns. The issue of an arthropod as reservoir host has been dealt with by collecting a large variety of species, including mosquitoes, ticks, sandflies, cimicids, spiders, and others.

In Kikwit, Zaire, 1995, a major question was whether to begin specimen collection in the dry season following the epidemic (which had begun in the rainy season) or to wait until the next rainy season. There was no clear choice. In favor of an immediate initiation of the studies was the well-known propensity for most zoonotic viruses to be transmitted intensively only at infrequent intervals; after all, the discovery and last known activity of Ebola virus in Africa had been in 1976–1979. There was always the possibility that a chronically infected reservoir host might still be present or some anomaly of animal species distribution might be noticed. Because most species in the rain forest live less than 1 year immediate action represented the best opportunity to detect the presence of antibodies in sentinel species. Against collecting at that time was the suggestion of rainy season proclivities of Ebola transmission and the likelihood of missing migratory species, intermittently active and short-lived arthropods, or other temporally unique opportunities. Logistics and politics also led to the decision to start immediately, in the dry season: the Zairian political climate was becoming more fragile, the cooperation of the local people was waning, the willingness of other institutions to collaborate was fleeting, the availability of funding was limited to the immediate accounting period, and, most importantly, the investigators hoped to do their work without the ensnaring mud and disruptive tropical downpours of the rainy season. The downside of this decision was addressed by more limited studies done during the next rainy season with a concentration at that time on migratory bats, unexpected species, and certain arthropods.

At the time of this writing in 1997, analysis of the samples collected during the field project is not yet finished. The complex and time-consuming process of

identifying the animals collected occupied many experts for some time; for example, a new shrew species was identified in the course of this work. So far, no evidence of the presence of Ebola virus or antibodies to it have been found in any specimen (T. G. Ksiazek, R. Swanepoel, P. Jahrling, and colleagues, personal communication, 1997).

## B. Ongoing and Needed Laboratory-Based Filovirus Research

There are many laboratory-based experiments that must be done, (a) to complement the above field-based research, (b) to support disease prevention and control activities, and (c) to bring the state of our basic knowledge about the filoviruses and the infections they cause to the same state that we have come to expect for all important pathogenic viruses and viral diseases. The following represent some research tacks of high priority; the listing should not be taken as all-inclusive.

1. *Molecular biology of the viruses*: Much progress has been made in characterizing the filoviruses, their genomes, and their proteins. However, we are just beginning to understand the function of the viral proteins, especially from the perspective of their role in the pathogenesis of disease. We know very little, indeed, about how viral gene expression and gene products contribute to the perpetuation of the viruses in nature. Because most molecular biology research is investigator-initiated, if funds and facilities [at biosafety level 4 (BSL 4)] were available, we feel that progress would be rapid in this area.

2. *Pathogenesis of viral infection*: Much progress has been made in regard to descriptive pathogenesis research, but now it is time for manipulative pathogenesis research approaches to be expanded. By manipulating the infectious processes themselves and the host responses that are engendered by the infection (innate inflammatory response, acquired immune response), clues regarding pathogenetic weak links would, it is hoped, be found. With other viral diseases, such clues have often been the keys to developing preventive and therapeutic regimes. In particular, attention must be given to understanding of the potential for filoviruses to employ aerosol transmission, especially in certain settings such as hospitals and experimental animal facilities.

3. *Immunology*: Given the poor neutralizing antibody response evoked by filovirus infections in naturally infected humans and in experimental animals, much more basic immunology research is warranted. Such research must focus on the details of filovirus antigen presentation and processing, means to overcome this hyporesponsiveness, and means to stimulate T-cell-based responses.

4. *Vaccinology*: At present, there is no justification for actual filovirus vaccine development: the number of people at risk is viewed as very small and the cost very large. However, given the long lead time involved and the refractory nature of the viruses in immunoprophylaxis experiments that have been done over the years, it seems prudent to extend basic immunology and molecular biology studies in ways that would accelerate vaccine development should it be necessary. We need to develop the means to be able to move quickly from principles to practice, should this become necessary (i.e., to move from an understanding of protective epitopes to vaccine candidates. It is not enough to wait

until molecular virology and pathogenesis research yields every bit of information that one would want for rational vaccine design—there is never enough information in this regard—but we do need basic information that can be translated into practical vaccine development on short notice. Should filovirus epidemics occur on a larger scale, we would then be in a position to protect the increased numbers of laboratory and field workers and medical care personnel that would be drawn into control programs.

5. *Immunoglobulin therapy*: Russian scientists first showed that very high titered anti-Ebola equine globulins may be valuable in post exposure prophylaxis (Mikhailov *et al.*, 1994; Borisevich *et al.*, 1995; Jahrling *et al.*, 1996b; Markin *et al.*, 1997). Anecdotal evidence has suggested that whole blood from convalescent patients may be protective when transfused into patients with Ebola hemorrhagic fever (Muyembe-Tamfun *et al.*, 1996). On the basis of these clues, work is underway at the Scripps Research Institute to develop highly avid neutralizing human monoclonal antibodies against Ebola virus. This work involves the use of mRNA immunoglobulin gene libraries constructed from bone marrow specimens obtained in Kikwit, Zaire. More of this kind of research must be supported.

6. *Therapeutic drug design and development*: The filoviruses are the only hemorrhagic fever agents for which we have no proven or investigational drug therapy. In the absence of success with vaccine development, there is a desperate need for drugs to protect laboratory workers in case of accident. There is also a desperate need for drugs to treat patients in hospital-based outbreaks in Africa. Indeed, the availability of drugs would also draw patients into hospitals during epidemics, thereby minimizing household and community transmission. The first favorable drug therapy results have finally been obtained (a *S*-adenosylhomocysteine hydrolase inhibitor) (Huggins *et al.*, 1996), but much more needs to be done. Here is a place where the combined resources of the U.S. Army Medical Research Institute of Infectious Diseases and the National Institute of Allergy and Infectious Diseases of the National Institutes of Health should be brought to bear.

## VI. IMPLICATIONS CONCERNING GLOBAL PUBLIC HEALTH

Ebola virus must be dealt with in the context of its character as the etiologic agent of an emerging viral disease (Peters *et al.*, 1991a,b, 1994; 1996a; Murphy, 1993; Murphy and Nathanson, 1994; Sanchez *et al.*, 1995; Monath, 1996). To do this we should examine the emergence of human viral diseases in a historical context. Many of the most important viral diseases of history emerged only following the development of cities containing sufficient inhabitants to support their circulation. Measles is perhaps the best example: classic studies grounded in the work of Panum on the Faroe Islands in 1846 established that about 500,000 people are needed to support continuous transmission of measles virus (smaller populations are intermittently infected from outside) (Panum, 1940; Nathanson and Murphy, 1996). Such large population centers did not occur before the rise of irrigated agriculture in the Middle East around 5000 years ago. This development coincided with increasing domestication of sheep, goats, and cattle, which carry viruses considered to be the progenitors of human mea-

sles virus (sheep and goats: peste-des-petits-ruminants virus; cattle: rinderpest virus).

From this history lesson, we might speculate that future emergent viruses will come from new ecological niches, sites where new selective pressures favor the emergence of new variant viruses. The new megacities of Africa and other tropical zones of the world may provide such new niches for the emergence of new variant viruses and other infectious agents. Modern air transportation could deliver a new pathogen to any other megacity in the world in hours. Further, we might speculate that the most dangerous new, emerging viral disease would be one that is spread by the airborne route (Mims, 1991). This notion follows on the concept of "The Ultimate Pathogen" (Kilbourne, 1983, 1996). Diseases transmitted by the respiratory route, such as influenza, have proved to be very difficult to control, partly because of their rapid spread. This notion as it pertains to filovirus diseases has certainly been brought to the attention of the public by the press/media; even so, it must be dealt with by appropriate research. We know that Ebola virus has the capacity to invade the lung and to replicate very productively there. We suspect that invasion of lung comes late in course of infection in humans and evokes too little cough to generate an effective aerosol. However, we do not know whether this character of the infection might change in the future, with mutations favoring aerosol transmission being fixed through Darwinian selective forces.

Why should the average scientist or citizen be concerned about filoviruses? Is there a significant risk to Africa that compares with the everyday problems of malaria, yellow fever, pneumonia, diarrhea, and other more common causes of infectious disease mortality? Should there be a real concern in North America or Europe?

The danger from filoviruses is difficult to evaluate because of our limited knowledge base, and therefore these questions are difficult to answer objectively. There is a need to understand these viruses and the diseases they cause just because the risk they represent is unknown and the risk of future episodes is so unpredictable. This judgment takes nothing away from our need to understand the more common infectious agents and diseases of the tropics as well. We must not lose sight of the need to develop a knowledge base for all dangerous pathogens, particularly where the benefit from a small investment in epidemiological and laboratory research is likely to be so great. For example, we need to find the natural reservoir of the filoviruses and learn how their prevalence in the environment is regulated. We need to find out how transmission of these viruses to humans is regulated. In Africa, the emergence of Ebola virus could dramatically increase if the unknown reservoir increased in numbers, if it changed its behavior, or if ecological factors brought additional reservoir hosts into play. We need to know enough to anticipate such changes and to intervene rapidly should they occur.

Ecological changes that can contribute to disease emergence are common happenings in these times of rapid, uncontrolled exploitation of the tropical forests of the world and rapid, uncontrolled development of the cities of the tropics. Perhaps most important is the reality that across sub-Saharan Africa population centers lack the social organization that is needed for disease prevention and control. Present conditions of hygiene and sanitary management

and the paucity of medical care and disease surveillance will continue, and they will continue to present risks of new infectious disease emergence. As western-style hospitals become more affordable for Africans, nosocomial Ebola amplification will increase. In this context, it is elementary to predict that outbreaks of filovirus disease will continue to occur in Africa, in all likelihood at an increasing frequency and in larger and larger epidemics.

Again, why should we in the developed world be concerned? Even if we say that we live in a global community and that there is a possibility that air travel could bring Ebola virus to our doorstep, quickly, what is the worst that might happen? If the worst that might happen is an occasional importation resulting in a small cluster of cases, possibly involving medical staff, should we be concerned? If such episodes are unpredictable in time and place, should we not just wait and react after the fact? Of course, the answer to such questions lies in past experiences: the same questions were asked when acquired immunodeficiency syndrome (AIDS) first appeared in Los Angeles and New York, and the wait-and-see answer did not serve our society well at all. One of the poorly understood findings from the Kikwit epidemic was that some Ebola patients were much more dangerous than others. Two individual patients were the cause of more than 50 contact cases (Khan *et al.*, 1996). We do not think that the concerned public would be satisfied if its public health leaders decided on a wait-and-see approach for dealing with Ebola or the other diseases with similar epidemic potential.

The over-arching global impact of emerging infectious diseases was begged by the U.S. Institute of Medicine study, published as *Emerging Microbial Threats* (Lederberg *et al.*, 1992), and answered by the Centers for Disease Control and Prevention Report, *Addressing Emerging Infectious Disease Threats, A Prevention Strategy for the United States* (CDC, 1994). The World Health Organization has answered similarly. The answer is based on the development of a global integrated enterprise, an early warning system, with new capacity for (a) disease surveillance, (b) diagnostics, (c) an integral research base, (d) a communications system, (e) a technology transfer system, (f) a global prevention/intervention and emergency response infrastructure, (g) a global training program, and (h) a stable funding base.

This enterprise need not be thought of as so expansive, so expensive, as to be unrealistic. For example, in regard to the filovirus diseases, surveillance need not be expensive and emergency response need only provide hospital hygiene and training and supplies for strict barrier-nursing practices and simple laboratory procedures to make diagnosis easier (Peters *et al.*, 1991b; CDC, 1995d; Lloyd *et al.*, 1996). In particular, this enterprise must be built on a more substantial research base, and this in turn requires adequate trained staff and laboratory facilities for work on the BSL 4 pathogens (CDC/NIH, 1993). Safe, productive research demands a core of trained, career scientists with knowledge of the pathogens and procedures to work with them, and these persons are not created in a short didactic course or readily carried over directly from other fields; indeed, there is underutilized BSL 4 research space in the United States. The nature and extent of present disease risks are such that present facilities around the world cannot support an appropriate scope and scale of urgently needed research work. Greater high containment laboratory capacity is urgently

needed, along with funding to allow experts from academic institutions to collaborate with colleagues in government agencies in the needed work. This need must be met in all concerned developed countries, on behalf of the people of all less developed countries.

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