

International Colloquium on Ebola Virus Research: Summary Report

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The International Colloquium on Ebola Virus Research was held at the Institute of Tropical Medicine (ITM), Antwerp, Belgium, 4–7 September 1996. The meeting was coorganized by the ITM and the National Institutes of Health (NIH), Bethesda, Maryland, with major support from the Centers for Disease Control and Prevention (CDC), Atlanta, the Commission of the European Communities (CEC), Brussels, and the World Health Organization (WHO), Geneva. A total of 180 persons involved in filovirus research and control from 30 countries participated, including 23 representatives from African countries where Ebola and Marburg virus infections have been detected. Seven themes were addressed: (1) emerging infectious diseases priorities, (2) recent Ebola virus outbreaks, with emphasis on Zaire and Gabon, (3) virology and molecular biology, (4) pathology and pathogenesis, (5) therapy and prevention, (6) natural history, including seroarcheology, and (7) strengthening the filovirus research network. The colloquium commemorated the 20th anniversary of the first isolation, characterization, investigation, and control of Ebola virus, detected in Zaire and the Sudan in 1976 (*Speaker*, S. R. Pattyn, ITM) [1–6].

Filoviruses as Emerging Infectious Diseases (*Chair*, B. Gryseels, ITM; *Rapporteur*, J. LaMontagne, NIH)

There have been 15 filovirus outbreaks recorded in humans since 1967, when the first Marburg virus importation into Germany occurred [7]; the source of infection was monkeys coming from Uganda [8, 9], but monkeys have not been shown to be the natural reservoir of filoviruses. The major foci of human outbreaks have been in sub-Saharan Africa, where evidence of filovirus resides in a triangle covering mainly the middle of the continent (Breman J, figure 1). Human cases of Marburg virus infection occurred in Yugoslavia as an extension of the 1967 importation. Laboratory scientists have been infected, in Germany and Yugoslavia in 1967, in the United Kingdom in

1976 during work with an isolate of Ebola (Sudan subtype) [10], and in Russia in 1990 by an isolate of Marburg virus [11]. In 1989, Ebola virus was imported in macaque monkeys coming from the Philippines to Virginia (Reston), Texas, and Pennsylvania; in 1992, to Sienna, Italy; and, in 1996, to Alice, Texas, illustrating once again the dangers posed by importations of primates for biomedical research [12, 13]. In 1994, a patient from Côte d'Ivoire with Ebola disease was diagnosed in Switzerland and, in late 1996, a patient from Gabon with Ebola virus infection was evacuated by airplane to South Africa.

In response to the threat from filoviruses and other emerging infectious diseases, WHO has recently formed a Division of Emerging and Other Communicable Diseases Surveillance and Control. In addition to its usual activities to strengthen national disease surveillance programs, WHO is developing a global detection system for antimicrobial resistance to drugs and strengthening its rapid epidemic response capability (Heymann D, WHO). The importance of Ebola virus outbreaks and research to developed countries was discussed by a representative of the European Community (Pletschette M, CEC). There are few biosafety level 4 (BSL 4) laboratories in Europe and elsewhere equipped to deal with filoviruses according to the current exacting requirements for such facilities [14]. Importations of emerging diseases will occur more often as travel increases between Africa, Asia, and other continents. Each nation, developed and developing, was urged to develop a plan to deal with filoviruses as part of an overall strategy to confront emerging and reemerging infectious diseases (Colla M, Federal Ministry of Public Health, Brussels; Blinken A, Embassy of the United States of America, Brussels). In the United States, "Infectious Diseases—A Global Health Threat," a report of the National Science and Technology Council, Committee on International Science, Engineering, and Technology, Working Group on Emerging and Re-emerging Infectious Diseases, is an example of such a plan (Khabbaz R, CDC) [15].

Understanding the biologic, epidemiologic, and ecologic nature of filoviruses and other pathogens whose essential features remain undiscovered depends on several closely linked events and the interdependence of health workers. The process begins with initial detection of disease in patients and ends with formalization and maintenance of disease control. To assure the most effective and efficient attack on filoviruses, there must be close planning and collaboration

Received 14 April 1997; revised 13 May 1997.

The colloquium was held 4–7 September 1996 in Antwerp, Belgium.

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The Journal of Infectious Diseases 1997;176:1058–63

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0022-1899/97/7604-0028\$02.00

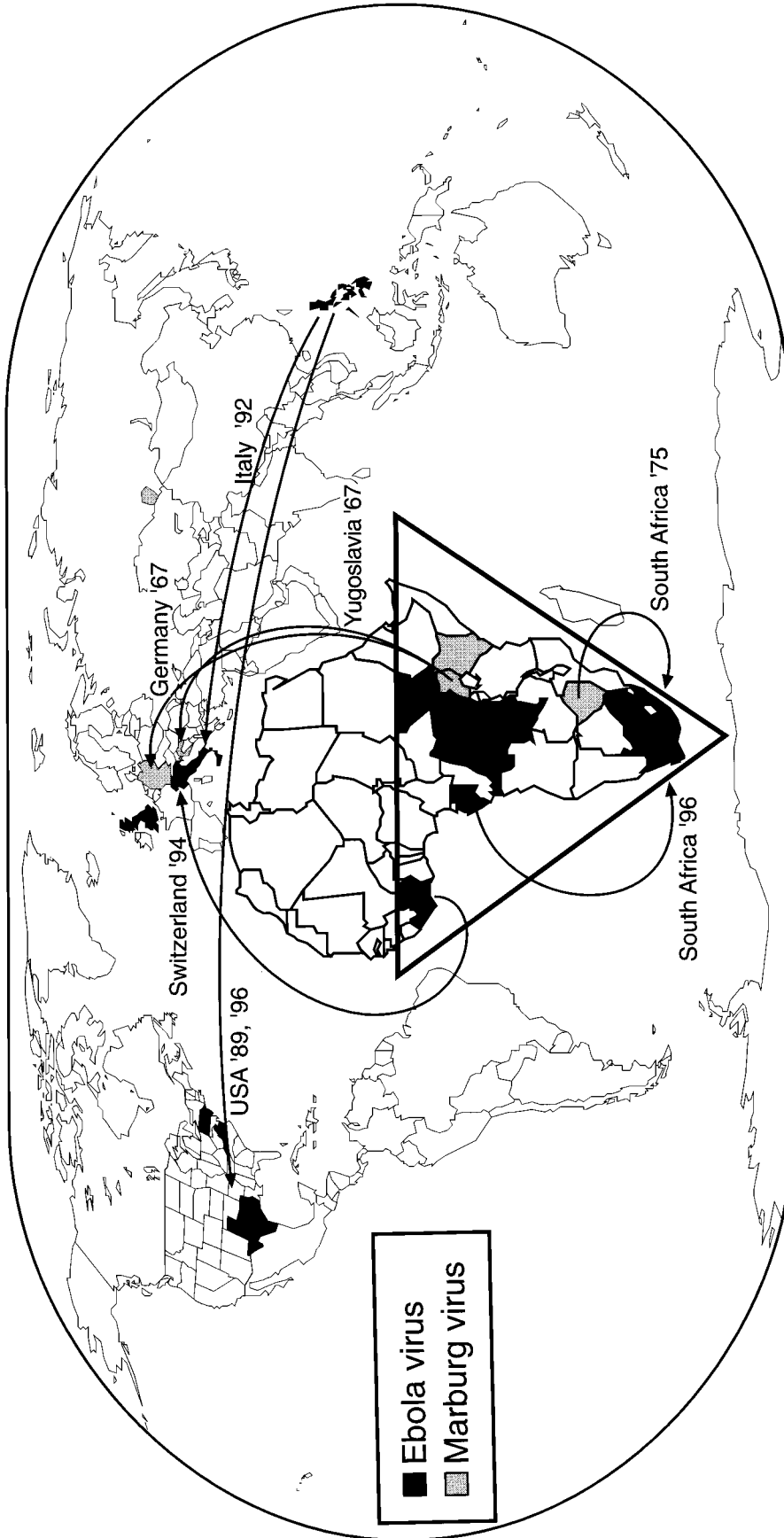


Figure 1. Marburg and Ebola virus outbreaks, 1967–1997: filovirus triangle. Nonhuman primate outbreaks of Ebola (Reston subtype) in the United States (1989, 1996) and Italy (1992) were associated with importations of macaque monkeys from Philippines; human cases of Marburg virus disease in Germany and Yugoslavia (in 1967) were associated with monkeys and other animals imported from Uganda; laboratory-associated human cases occurred in Germany in 1967, United Kingdom in 1976, and Russia in 1990, due to Marburg, Ebola (Sudan subtype), and Marburg viruses, respectively; importation of patient with Ebola (Côte d’Ivoire subtype) into Switzerland occurred in 1994, and from Zimbabwe (Marburg) and Gabon (Zaire subtype) into South Africa in 1975 and 1996, respectively.

between the research scientists in the academic “citadel,” front-line clinicians, and the public health “crusaders” in the field (Murphy F, School of Veterinary Medicine, University of California, Davis).

Recent Outbreaks (*Chair*, C. J. Peters, CDC; *Rapporteur*, O. Tomori, University of Ibadan, Nigeria, and WHO, Harare, Zimbabwe)

The session on recent Ebola virus outbreaks featured the 1995 Kikwit, Zaire, experience as a case study. In Kikwit, the outbreak began 4–5 months before being recognized by health authorities (Muyembe-Tamfun JJ, Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of Congo) [16]. Unsuspecting surgical and medical staff contracted Ebola hemorrhagic fever while operating on a patient diagnosed with an acute abdominal condition; they amplified the disease in the community (Khan A, CDC). Community spread was mainly by close personal contact, particularly during the latter stages of the outbreak, although some patients were infected by contaminated injections. A total of 315 cases occurred, of which 78 (25%) were health personnel; the case fatality rate was 78%. The antigen-capture ELISA, performed on specimens sent from the field to the CDC, was considered the standard for confirming the clinical diagnosis (Ksiazek T, CDC). In several patients, viral antigenemia was present until death (Rollin P, CDC), and systemic filovirus infections with antigen deposition in all organs (including skin) were observed at autopsy using immunohistochemical techniques (Zaki S, CDC). Severe systemic symptoms and signs marked the clinical presentation of 106 hospitalized patients in Kikwit; this outbreak was similar to prior outbreaks, except that hemorrhage was not as prominent, and tinnitus and hearing loss were recorded more often (De Roo A, ITM). Since the Kikwit outbreak, surveillance and diagnosis of Ebola virus in suspected patients (or ruling out the diagnosis) by detection of Ebola antigen in formalin-preserved skin snips, has proven to be an effective diagnostic tool, particularly in terminally ill patients (Lloyd E, CDC). Several thousand animals and 30,000 arthropods were collected by several collaborating institutions during extensive ecologic studies to search for the reservoir of Ebola virus; the results are pending (Ksiazek T).

The recent outbreak of Ebola hemorrhagic fever in Kikwit illustrates the powerful role that the global media plays during dramatic medical events. The challenge for field staff is to understand and accommodate members of the press and to work with them advantageously. The challenge for the media is to develop and respect guidelines for behavior that allow coverage of the event but do not compromise epidemic containment, patient care, or research.

In Gabon, an early 1994 outbreak was diagnosed first as yellow fever but was later confirmed as Ebola infection (Georges A, Centre International de Recherches Médicales de Franceville, Gabon) [17]. The persistent series of outbreaks in

Gabon since 1994 [18], an outbreak in Côte d’Ivoire in 1994 (Le Guenno B, Institute Pasteur, Paris) [19], and recent importations of Ebola (Reston subtype) into Italy (1992) (Brown D, Public Health Laboratory Service, Colindale, UK) and the United States (1989, 1996) (Jahrling P, United States Army Medical Research Institute for Infectious Diseases [USAMRIID], Frederick, MD [12, 13, 20, 21]) were reviewed. A chimpanzee was the source of the case in the Côte d’Ivoire and the first cases in a later outbreak in Gabon.

The recent monkey importations of Ebola (Reston subtype) have come from one commercial macaque breeder colony in the Philippines, which had an outbreak of fatal disease in monkeys infected locally with Ebola virus [22]. There have been several instructive lessons from these outbreaks; quarantine regulations have been improved and are effective (Tippie M, CDC). Consequently, spread between monkeys was very limited in the 1996 Alice, Texas, importation, and no human infections occurred, as was recorded in Reston. The Texas 1996 and Virginia outbreaks suggested aerosol spread of Ebola virus between monkeys.

Virology and Pathogenesis (*Chair*, M. Kiley, Federal Laboratories for Health, Winnipeg, Canada; *Rapporteur*, A. Sanchez, CDC)

Phylogenetic analyses of the Filoviridae indicate that Marburg and Ebola viruses are of different lineages (Sanchez A). Four genetic sequence patterns of RNA distinguish the Zaire, Sudan, Côte d’Ivoire, and Reston subtypes of Ebola [7, 23, 24]. The sequence patterns are associated with clinical severity: The Zaire subtype of Ebola is most virulent for humans; the Reston subtype caused infection but not disease in 4 humans. A remarkable genetic stability of some Ebola viruses in the wild has been observed. For example, between the Yambuku 1976 isolate and the Kikwit 1995 isolates of the Zaire subtype, only a 1.6% difference was observed in the nucleotide sequences of the glycoprotein gene containing 1172 nucleotides. However, differences of up to 40% in nucleotide sequences in the glycoprotein genes have been observed among different Ebola subtypes.

Systemic Ebola virus infection by parenteral and aerosol inoculation of monkeys has been induced under experimental conditions (Jaax N, USAMRIID; Ryabchikova E, Vektor Laboratories, Novosibirsk, Russia) [25, 26]. Viral invasion of macrophages has been prominent [27, 28]. Platelet function deteriorates in nonhuman primate infections, and multiorgan hemorrhage is characteristic of severe illness (Fisher-Hoch S, Aga Khan University, Karachi, Pakistan) [29]. Mouse (Bray M, USAMRIID) and guinea pig (Ryabchikova E) models are also useful in understanding the pathogenesis of filovirus infections.

During the meeting, there was a “late-breaker” presentation (Lundsgaard T, The Royal Veterinary Agricultural University, Copenhagen) showing a filovirus-like organism in a leafhopper,

Psammotettix alienus (Dahlbom), which is native to Denmark. The insect feeds on leaves of *Festuca gigantea* plants collected in Denmark. This raised speculation that insects could, somehow, be involved in transmission of filoviruses in the wild.

Therapy and Prevention (*Chair*, T. Monath, OraVax, Cambridge, MA; *Rapporteur*, R. Colebunders, ITM)

Is Ebola-specific immunoglobulin effective in treating patients [30]? Evidence from animal studies (Jahrling P; Netesov S, Vektor Laboratories) indicated that Ebola-specific immunoglobulin of equine origin has some activity in suppressing viremia and delaying disease onset and death of nonhuman primates. A limited uncontrolled study using whole blood transfusions from convalescent patients for treating alleged active disease suggested efficacy within the limitations of the study (Muyembe-Tamfun JJ). Research is progressing on the construction of IgG Fab anti-Ebola antibodies (Parren P, Scripps Research Institute, La Jolla, CA). Another promising therapy was an *S*-adenosylhomocysteine hydrolase (SAH) inhibitor given to rodents infected with Ebola virus (Huggins J, USAMRIID). Nonhuman primates and guinea pigs currently are the best animal models in which to study the pathogenesis and pathophysiology of filoviruses and to test drugs and immunologic interventions to cure and prevent infections. Recombinant technology has been applied to anti-Ebola vaccine development, and animal testing is foreseen soon (Schmaljohn A, USAMRIID).

Natural History, Including Seroarcheology (*Chair*, R. Shope, University of Texas, Galveston; *Rapporteur*, B. Le Guenno)

T. Monath presented a conceptual filovirus zoonotic model of the transmission cycle considering virologic, serologic, epidemiologic, and ecologic evidence that, to date, suggests inclusion of chimpanzees and bats in the cycle. The most perplexing aspect of the Filoviridae story is the enigma of where these viruses reside during interepidemic periods.

Active surveillance for Ebola hemorrhagic fever conducted in northern Zaire by WHO during 1980–1985 identified 21 likely cases. At that time, a convalescent patient was required to have an IFA titer of $\geq 1:64$. The case fatality rate was 43%, and there was a 15% prevalence of antibody among contacts. That these cases did not result in large outbreaks suggests that person-to-person transmission of Ebola virus is limited unless amplified nosocomially (Heymann D). Widespread serologic studies of humans in western and central Africa during the 1980s and early 1990s have indicated the presence (by IFA) of antibodies to Ebola and Marburg viruses in humans and animals in several African countries (Gonzales JP, Institut Français de Recherche Scientifique pour le Développement en Coopération, Paris, and Yale University, New Haven, CT) [31–38]. Frequencies of Ebola virus antibodies appear much higher

in humans in western Africa than in eastern Africa, whereas the prevalence of Marburg virus, although lower than for Ebola virus, is higher in eastern Africa than in the west. A major problem with previous serologic surveys has been the reputed low specificity and lack of sensitivity of the IFA test used [39]. Recently, the IFA test has been replaced by an IgG ELISA (Ksiazek T).

The most important recent breakthrough in finding the natural reservoir of filoviruses has been the discovery that certain African species of fruit and insectivorous bats, after being experimentally infected, can support replication of Ebola virus (Zaire subtype) [40]. The animals remain asymptomatic during infection, even when high titers occur. This finding, if confirmed, can lead to more focused ecologic studies.

Strengthening the Filovirus Research Network and Recommendations (*Chairs*, J. J. Muyembe-Tamfun and D. Heymann; *Rapporteur*, H. Artsob, Laboratory Centre for Disease Control, Ottawa, Canada)

There were several colloquium recommendations, and all depend upon close international collaboration and coordination. This is because there are relatively few laboratories that have the trained staff, facilities, and experience to deal with Filoviridae and other class 4 pathogens [14]. All BSL 4 laboratories are in developed countries, except for one in South Africa. These laboratories, and others interested in filoviruses, need to work very closely with scientists and public health officials in Africa, the Philippines, and elsewhere in the developing world. Is it possible that filoviruses exist in the Americas, other areas of Asia, and elsewhere? Time will tell.

Early discovery of suspected patients and prompt diagnosis are necessary to assure that control measures and investigations begin promptly and are tailored to the current episode. One area in the Tai Forest of western Côte d'Ivoire, where a European primatologist was infected by a chimpanzee, appears to be an excellent site for a prospective study of the natural history of filoviruses (Formenty PBH, WHO, Abidjan, Côte d'Ivoire). The government of Côte d'Ivoire and WHO are supporting a study site in the Tai Forest where international collaborative research on Ebola ecology can be conducted. Another area of special interest and potential for a field site is eastern Gabon, where three outbreaks of Ebola have occurred since 1994 [18, 41].

To strengthen the diagnostic capability of collaborating laboratories, there needs to be a consensus on which standard diagnostic tests to use. Sensitivity, specificity, and predictive value (positive and negative) must be shown to be optimal before a diagnostic antibody test will be accepted as the standard. Standardized reagents for antigen and antibody detection by designated laboratories will need to be prepared and shared through a collaborating network. Quality control of the reagents and of the work done by the users of the reagents is essential.

Improved management of patients with Ebola virus disease can occur with current technology. Patient care should focus on the maintenance of blood pressure, avoidance of pulmonary edema, and prevention and treatment of pneumonia and other bacterial complications. SAH inhibitors and other promising related compounds need toxicologic and pharmacokinetic studies and controlled testing in nonhuman primates and in humans in a clinical setting. Disseminated intravascular coagulation (DIC) associated with filoviruses merits use of fresh-frozen plasma (FFP) and platelet concentrates; however, there is no evidence that this combination would prevent DIC. The use of heparin with FFP and platelets has not been evaluated for the treatment of filovirus-induced DIC. Immunotherapy and immunoprevention require more laboratory study before field testing can be done in humans.

While monkeys and guinea pigs have been used for studying the pathophysiology of filoviruses, major efforts should be directed toward further developing an animal model. Field studies could be restricted to focusing on species incriminated by the animal inoculation studies or epidemiologic evidence.

Training of scientists and public health workers in the developing world, particularly western and central Africa, on the manifestations and control of filovirus outbreaks is of utmost priority (McCormick J, Aga Khan University). Infections of laboratory workers with filoviruses remain a peril [10, 11]. Because of the constant danger of importation of patients with filovirus infections, developed and developing countries need to strengthen surveillance and establish protocols for managing suspected patients and contacts of patients with hemorrhagic fevers and for quarantining selected animals coming from areas with endemic filoviruses (Lloyd E, Tipple M). International support for these activities is needed, and a coordinating meeting should be held soon to examine the resources needed and their possible origin. WHO should have a major role in establishing an international filovirus scientific commission and be responsible for coordinating support for the activities detailed above.

One of the most pressing issues for a scientific group to consider is the type of laboratory and field containment required to work with the different aspects of filovirus surveillance, diagnosis, patient management, and research (Johnson K, Bozeman, MT). If the virus is inactivated [42, 43], can diagnostic work, with possibly infected material from the field, proceed in facilities less than BSL 4? Even if Ebola virus remains intact, is it possible to create a safe and inexpensive environment for work with filoviruses, including management of patients, as has been the experience with Lassa fever in Africa? More filovirus outbreaks will occur in Africa and elsewhere. More scientists are needed urgently to work in the field and laboratory, and resources are needed to support and protect them. A reassessment of how we perform surveillance and research in the field and at the bench with filoviruses is in order.

A supplement to the *Journal of Infectious Diseases* will contain papers from this colloquium and other contributions to filovirus research.

Acknowledgments

We thank Karl M. Johnson (Bozeman, MT) for encouragement and review of the summary; James M. Meegan (National Institutes of Health [NIH], Bethesda, MD) for excellent collaboration and for arranging a bibliography of filoviruses for colloquium participants; and Nancy Coulter (NIH) and Ciska Maeckelbergh and staff at the Institute of Tropical Medicine (Antwerp, Belgium) for excellent administrative support. We thank the following institutes and companies for sponsoring the colloquium: In Belgium: Algemeen Bestuur voor Ontwikkelingssamenwerking (Belgian Administration for Development Cooperation), Commission of the European Communities, Federal Ministry of Public Health, Flemish Government (Department of Foreign Affairs and Department of Education), Institute of Tropical Medicine, Nationaal Fonds voor Wetenschappelijk (National Fund for Scientific Research), and Organon Teknika, Merck-Belgolabo, Epacar, Canon, and Rank Xerox; in Geneva: World Health Organization; in the United States: Centers for Disease Control and Prevention (Atlanta), NIH (Fogarty International Center, National Center for Research Resources and National Institute of Allergy and Infectious Diseases), Office of International and Refugee Health, Department of Health and Human Services (Rockville, MD), and US Army Medical Research Institute for Infectious Diseases (Frederick, MD).

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