

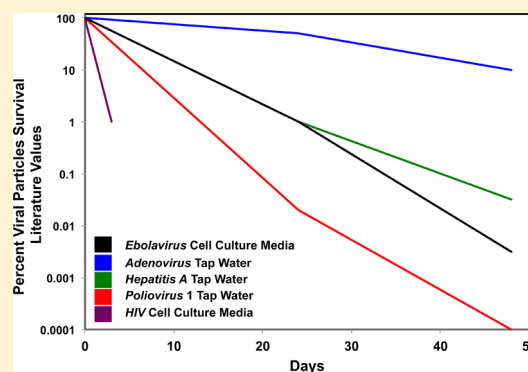
Ebola Virus Persistence in the Environment: State of the Knowledge and Research Needs

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ABSTRACT: In March 2014, an outbreak of Ebola virus (Ebola) arose in western Africa. Since then, there have been more than 10000 cases reported with a mortality rate of approximately 70% in clinically confirmed cases. A significant unanswered question has arisen for the scientific and engineering communities, as well as the general public, surrounding Ebola virus persistence in the environment and the potential for an environmental route of Ebola virus exposure. Here, the authors review the state of knowledge of Ebola virus environmental persistence and highlight future research needs. In general, there are limited data on the environmental persistence or disinfection of Ebola virus available in the open literature. The available evidence suggests that Ebola virus is inactivated at a rate more rapid than or comparable to those of typically monitored enteric viruses. Additionally, while environmental exposure is not the dominant exposure route, available data suggest that it is imprudent to dismiss the potential of environmental transmission without further evidence. A significant research effort, including environmental persistence studies and microbial risk assessment, is necessary to inform the safe handling and disposal of Ebola virus-contaminated waste, especially liquid waste in the wastewater collection and treatment system.



INTRODUCTION

In March 2014, an Ebola virus (Ebola) outbreak began in Western Africa, spreading to the countries of Guinea, Liberia, Sierra Leone, and Nigeria. As of November 28, 2014, the number of Ebola cases had risen to 16933 with 6002 deaths.¹ It is also well-recognized that the number of reported cases is likely underestimated.² The case fatality ratio is estimated to be approximately 70% from persons with a known clinical outcome.³ The estimated basic reproduction number (number of additional persons each infected person will infect) of the current Ebola virus strain ranges from 1.7 to 2.0, depending upon the location.³ The current outbreak is the largest since the discovery of Ebola virus, and the first to spread outside of Africa.⁴ The largest previous outbreak was 425 cases with 224 deaths.⁵ Controlling the outbreak in Africa will take at least several months,⁶ and estimates of the total number of potential cases have ranged from the tens of thousands⁷ to more than 1 million.² In August 2014, the World Health Organization (WHO) declared the Ebola outbreak to be a “public health emergency of international concern”.^{3,8}

The first recorded Ebola outbreak was in 1976 in Zaire (now Democratic Republic of Congo).⁹ Several outbreaks have been recorded in sub-Saharan Africa, including in 1976, 1979, 1994–1997, 1995, 2000, and 2001–2004.^{9,10} The *Ebolavirus* genus is a member of the Filoviridae family, which is comprised of filamentous and enveloped viruses with a single-stranded negative sense RNA genome. Filoviridae have a characteristic

filamentous physiology, with diameters of approximately 80 nm and lengths significantly greater than 1000 nm.^{11,12} The physiology of Filoviridae is unique; there are no other known filamentous mammalian viruses. The *Ebolavirus* genus is comprised of four species, *Zaire*, *Sudan*, *Ivory Coast*, and *Reston*,⁹ with a strain of *Ebolavirus Zaire* causing the current outbreak. The Filoviridae family also contains the *Marburgvirus* and *Cuevavirus* viral genera. Ebola virus disease causes hemorrhagic fever, including high fever, fatigue, diarrhea, vomiting, abdominal pain, and both internal and external hemorrhage.^{3,9} The dose response of Ebola virus is unknown, but the median infectious dose is believed to be small.¹³ Aerosols containing 400 PFU of Ebola virus resulted in the infection and death of all exposed rhesus monkeys,¹⁴ and oral exposure of 10^{5.2} Ebola virus resulted in illness in three and death of two of four rhesus monkeys.¹⁵ In guinea pigs, 1 PFU of the virus contained 400 50% lethal doses,¹⁶ indicating low plaque forming efficiency. Because of high infectivity and mortality, Filoviridae (*Ebolavirus* and *Marburgvirus*) are considered to be Class A bioterrorism agents.^{17,18} Ebola virus is known to be zoonotic⁹ and is believed to continually circulate throughout bat populations,^{19–21} causing sporadic outbreaks in

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both human and primate populations. For example, in 2007 an outbreak of *Ebolavirus Zaire* killed approximately 5000 gorillas.¹⁹

Ebola virus is shed by infected individuals through bodily fluids, including saliva, stool, semen, breast milk, tears, and blood.^{22–24} Direct contact is widely believed to be the primary transmission route of Ebola virus, and infection control strategies include early diagnosis, patient isolation, and safe burial.³ In addition, fomites have also been suggested as a potentially important transmission pathway.²⁵ In an epidemiological study of the outbreak in the Democratic Republic of Congo in 1995, five of 19 investigated cases reported no direct contact with an infected individual.²⁶ Additionally, infection in primates has been observed from nondirect contact (aerosols),¹⁴ raising the possibility of an environmental infection route. The U.S. Centers for Disease Control and Prevention (CDC) has recognized public concern regarding the potential for an environmental route of exposure, for example, by issuing specific guidelines for airline crews and cleaning personnel.⁶

In contrast to these well-founded concerns for potential environmental exposure routes, World Health Organization guidelines for dealing with liquid waste from Ebola victims have suggested that direct disposal in the sanitary sewer, without disinfection, is appropriate.

“Waste, such as faeces, urine and vomit, and liquid waste from washing, can be disposed of in the sanitary sewer or pit latrine. No further treatment is necessary.” World Health Organization Guidelines for dealing with bodily waste from Ebola victims²⁷

Somewhat conversely, solid waste from Ebola victims is regulated by the same WHO document and the U.S. Department of Transportation as Class A medical waste (infectious substance), and facilities conducting Ebola virus research (i.e., Biosafety Level 4 facilities) must disinfect all liquid waste on site prior to release to the sewer system.^{27–29} Additionally, the CDC has recently released specific safety guidance for sewage workers.³⁰ In the United States, the CDC and the Environmental Protection Agency have so far upheld the WHO guidance for liquid waste disposal, although some facilities have chosen to disinfect liquid waste prior to disposal, for example, using bleach.³¹ A single infected individual may produce up to 9 L of liquid waste a day.³¹ Previous investigation of infected individuals using molecular methods identified Ebola virus concentrations of 10^5 – 10^7 genome copies/mL of blood plasma in nonfatal infections and 10^6 – 10^8 genome copies/mL of blood plasma in fatal infections.³² The lack of data regarding the safety of nondisinfected disposal of Ebola-contaminated liquid wastes, or the efficacy and application of disinfection approaches, has raised significant concerns about the persistence and disinfection of Ebola virus in the water environment, and the potential for transmission to sewerage workers or animal vectors. Here, the authors review the limited data that are publicly available regarding Ebola virus persistence in the environment and make suggestions for a priority research agenda.

■ EBOLA VIRUS SURVIVAL IN THE ENVIRONMENT

Surfaces. Persistence of Ebola virus on surfaces is a source of significant public concern for secondary transmission of the disease, for example, through deposition from infected individuals prior to quarantine or release in the sanitary sewer. The potential for Ebola virus transmission via fomites has been previously recognized.²⁵ An investigation of Ebola

virus in fomites within an isolation ward found only two of 33 samples to test positive for Ebola virus, leading the authors to conclude that the risk from infection via fomites is low when proper procedures are followed.²² Conversely, in tests of *Ebolavirus Zaire*, >10% and >3% survived on glass and plastic surfaces, respectively, after 14 days at 4 °C. Additionally, 0.1–1% of Ebola virus particles remained viable for up to 50 days at 4 °C.¹³ Investigations of UVC inactivation of Ebola virus on surfaces suggest that a subset of the viral particles (~10%) may be substantially more resistant to inactivation and displayed distinct inactivation kinetics,³³ suggesting a potentially environmentally persistent population. A separate study reported approximately 1% Ebola virus survival in the dark following deposit on a surface after 96 h and that Ebola virus was the most easily inactivated virus on a surface relative to tested alphaviruses and Lassa virus.³⁴ The related Filoviridae Marburg virus can remain infectious for at least 5 days on a surface.³⁵

Aerosols or Droplets. The potential for an infectious exposure route through aerosol or droplet exposure is a highly debated and contentious topic (e.g., refs 36–38). Transmission via aerosols or droplets is considered to be a rare event;^{38,39} however, previous studies have observed the infection of rhesus monkeys with *Ebolavirus Reston* after discontinuation of direct contact and in a separate room, which was presumed to be via aerosol.^{40–42} Additionally, aerosol infection of rhesus monkeys was one of the first identified exposure routes and appears to have an efficacy higher than that of conjunctival exposure.^{14,15} In primates, the observed frequency of illness from aerosol exposure was identical to that of other exposure routes.¹⁴ The rates of biological decay of *Ebolavirus Zaire* and *Ebolavirus Reston* in aerosols have been estimated to be 3.06%/min and 1.55%/min, respectively.¹³ Ebola virus survival in an aerosol was shown to be between 10% and 20% after 1 h and between 1% and 3% after 2 h.¹³ A separate study found 90% inactivation of Ebola virus within a drum reactor in 90 min.⁴³ The potential for transmission of Ebola virus deposited on surfaces or fomites following aerosolization is unknown.

Liquid. Currently, discharging infectious liquid medical waste to the sanitary sewer, including Ebola virus-contaminated medical waste, is allowed. A single study investigating Ebola virus survival in liquid media has been published. This study demonstrated that Ebola virus persisted in guinea pig sera and cell culture media for more than 40 days (Figure 1).¹³ A previous study demonstrated rapid inactivation of Ebola virus at a detergent concentration of 10%, but less than 1 log unit removal of Ebola virus after 24 h at 0.1% detergent.⁴⁴ Ebola virus survival in water, wastewater, or sludge matrices is unpublished in the open literature.

Given the paucity of data available on Ebola virus survival in liquid media, the authors compared published Ebola virus persistence data in liquid media with survival data of other human enteric viruses in tap water or cell culture media (Figure 1). These viruses have a physiology distinct from that of Ebola virus, highlighted in Table 1. In general, persistence would be expected to decrease in water or wastewater compared to that in cell culture media because of increased levels of external stress.⁴⁵ Figure 1 shows the decreased rate of survival of *Poliovirus 1* in drinking water compared to minimal essential media. The actual behavior of Ebola virus in the environment is unknown, and the authors suggest that these data be utilized as a scenario more conservative than current WHO and CDC recommendations for environmental persistence. Additionally, it has been anecdotally reported that Ebola virus is expected to

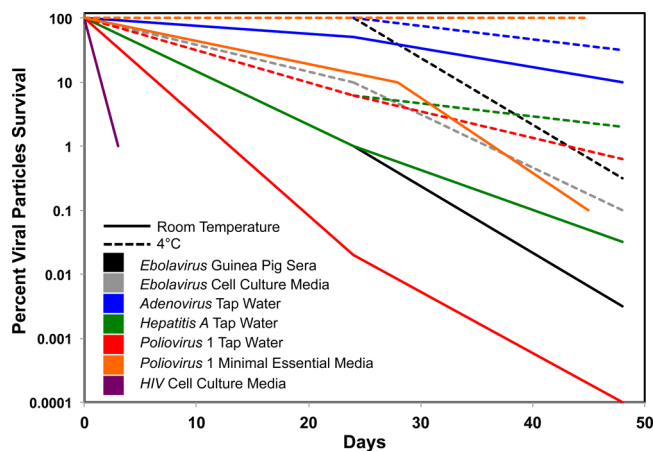


Figure 1. Published Ebola virus survival data in cell culture media and guinea pig sera, compared with published die-off data of model human pathogens in tapwater. *Ebolavirus* data from ref 13. *Adenovirus*, *Hepatitis A*, and *Poliovirus 1* data in water adapted from ref 49. *Poliovirus 1* data in minimal essential media adapted from ref 50. HIV data adapted from ref 51.

Table 1. Physiology of *Ebolavirus*, Model Enteric Viruses in Figure 1, and Proposed Surrogates

| virus | filamentous | genome | genome size (kb) | enveloped? |
|--|-------------|--------|------------------|------------|
| <i>Ebolavirus</i> | yes | ssRNA | 18 | yes |
| model enteric viruses | | | | |
| Adenovirus | no | dsDNA | 35 | no |
| Hepatitis A | no | ssRNA | 7.4 | no |
| Poliovirus 1 | no | ssRNA | 7.4 | no |
| HIV | no | ssRNA | 9.1 | yes |
| proposed surrogates | | | | |
| MS2 (<i>Escherichia coli</i> bacteriophage) | no | ssRNA | 3.5 | no |
| Phi6 (<i>Pseudomonas</i> bacteriophage) | no | dsRNA | 13 | yes |
| Tobacco Mosaic Virus | yes (rod) | ssRNA | 6.4 | no |
| Carrot Mottle Virus | no | ssRNA | 4.2 | yes |

be rapidly inactivated, as it is an enveloped virus.³⁰ However, a study investigating enveloped surrogates of coronaviruses (transmissible gastroenteritis and mouse hepatitis) demonstrated that these enveloped viruses remained infectious in wastewater for weeks, with 1 to 2 log unit removal after 1 week.⁴⁶ Additionally, enveloped *Influenza* viruses have previously been detected in sewage;⁴⁷ however, the potential for infection through water exposure remains unknown. These data suggest the potential for both the environmental persistence and transmission of the enveloped Ebola virus. As a conservative estimate, and without better data, the authors recommend utilizing enteric virus transport and survival as a model to understand and assess Ebola virus in the environment, rather than an assumption of negligible persistence.

RESEARCH NEEDS

It is apparent that the scientific and engineering communities are underprepared to answer questions regarding environmental persistence and transmission for the current Ebola virus

outbreak. Several research areas of immediate need are necessary to respond to the current outbreak and inform the response to future outbreaks.

(1) Elucidate the survival and disinfection of Ebola virus in the environment, especially the water environment.

Ebola virus survival in the water environment is currently unknown in the open literature and precludes informed recommendations for waste disposal, protective measures for sewage workers, and disinfection. In light of this current knowledge gap, it is essential to clearly identify the survival and persistence of Ebola virus in the water environment. This includes identification of Ebola virus persistence in drinking water and wastewater, the disinfection kinetics of Ebola virus, and the survival of Ebola virus in sludges, including during sludge treatment. Additionally, public health data clarifying the role of environmental exposure will inform the appropriate response.

(2) Develop surrogates of Ebola virus for environmental studies that do not require Biological Safety Level 4 (BSL4) access.

Researchers from many fields will likely be interested in evaluating Ebola virus survival and disinfection approaches following the current outbreak; however, BSL4 access, which is necessary for directly working with Ebola virus, is limited and costly. Additionally, access to the Ebola virus itself is limited for laboratory investigations. The development of surrogates that may be handled at lower biosafety levels and have been verified against Ebola virus survival will allow more detailed analysis of the environmental fate of Ebola virus. The authors are not aware of any nonpathogenic virus that captures all of the physiological aspects of *Filoviridae*, suggesting evaluation, and perhaps utilization, of multiple surrogates to capture biological complexity. While nuanced survival characteristics will likely vary between Ebola virus and surrogates, the development of surrogates will serve to allow the general assessment of the behavior of enveloped viruses in environmental systems. Proposed surrogates are highlighted in Table 1. Surrogates were chosen on the basis of characteristics that were physiologically similar to those of Ebola virus.

(3) Performing a risk assessment of exposure scenarios.

Many of the response recommendations for the current Ebola virus outbreak, including waste handling and the 21 day quarantine period,⁴⁸ appear to have not been informed by careful risk assessment. The authors recommend careful risk assessment and cost–benefit analysis for the management decisions recommended in response to the outbreak, including liquid and solid waste disposal.

(4) Develop a dose–response model for Ebola virus.

Currently, there is no dose–response model for Ebola virus. The lack of a dose–response model hinders risk assessment and informed management decisions. The authors highlight the necessity of a dose–response model for multiple exposure pathways, including aerosol exposure.

In conclusion, the authors suggest that environmental transmission of Ebola virus should be more carefully considered, and significant questions regarding the proper handling of Ebola-contaminated liquid waste remain. In the absence of better data, the authors suggest using persistence data of well-studied enteric viruses in the water environment as a more conservative estimate than current WHO and CDC recommendations. Additionally, the authors have suggested research topics to address priority unknowns to inform the response during the current outbreak and any future outbreaks.

More broadly, the authors utilize this opportunity to highlight the need for more careful evaluation of disposal practices for infectious liquid medical waste.

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Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) CDC 2014 Ebola Outbreak in West Africa (<http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/index.html>) (accessed November 14, 2014).
- (2) Meltzer, M. I.; Atkins, C. Y.; Santibanez, S.; Knust, B.; Petersen, B. W.; Ervin, E. D.; Nichol, S. T.; Damon, I. K.; Washington, M. L. Estimating the future number of cases in the Ebola epidemic—Liberia and Sierra Leone, 2014–2015. *MMWR Surveillance Summaries* **2014**, *63* (Suppl.3), 1–14.
- (3) WHO Ebola Response Team. Ebola Virus Disease in West Africa: The First 9 Months of the Epidemic and Forward Projections. *N. Engl. J. Med.* **2014**, *371* (16), 1481–1495.
- (4) Frieden, T. R.; Damon, I.; Bell, B. P.; Kenyon, T.; Nichol, S. Ebola 2014: New Challenges, New Global Response and Responsibility. *N. Engl. J. Med.* **2014**, *371* (13), 1177–1180.
- (5) Outbreak of Ebola hemorrhagic fever Uganda, August 2000–January 2001. *MMWR. Morbidity and mortality weekly report; Centers for Disease Control and Prevention: Atlanta*, 2001; Vol. 50, Issue 5, p 73.
- (6) CDC Interim Guidance about Ebola Infection for Airline Crews, Cleaning Personnel, and Cargo Personnel. (<http://www.cdc.gov/quarantine/air/managing-sick-travelers/ebola-guidance-airlines.html>) (accessed October 27, 2014).
- (7) Kupferschmidt, K. Estimating the Ebola epidemic. *Science* **2014**, *345* (6201), 1108.
- (8) WHO statement on the meeting of the International Health Regulations Emergency Committee regarding the 2014 ebola outbreak in West Africa. (<http://www.who.int/mediacentre/news/statements/2014/ebola-20140808/en/>) (accessed October 27, 2014).
- (9) Pourrut, X.; Kumulungui, B.; Wittmann, T.; Moussavou, G.; Délicat, A.; Yaba, P.; Nkoghe, D.; Gonzalez, J.-P.; Leroy, E. M. The natural history of Ebola virus in Africa. *Microbes Infect.* **2005**, *7* (7–8), 1005–1014.
- (10) Peters, C. J.; Peters, J. W. An Introduction to Ebola: The Virus and the Disease. *J. Infect. Dis.* **1999**, *179* (Suppl. 1), ix–xvi.
- (11) Feldmann, H.; Geisbert, T. W. Ebola haemorrhagic fever. *Lancet* **2011**, *377* (9768), 849–862.
- (12) Beer, B.; Kurth, R.; Bukreyev, A. Characteristics of Filoviridae: Marburg and Ebola Viruses. *Naturwissenschaften* **1999**, *86* (1), 8–17.
- (13) Piercy, T. J.; Smither, S. J.; Steward, J. A.; Eastaugh, L.; Lever, M. S. The survival of filoviruses in liquids, on solid substrates and in a dynamic aerosol. *J. Appl. Microbiol.* **2010**, *109* (5), 1531–1539.
- (14) Johnson, E.; Jaax, N.; White, J.; Jahrling, P. Lethal experimental infections of rhesus monkeys by aerosolized Ebola virus. *Int. J. Exp. Pathol.* **1995**, *76* (4), 227.
- (15) Jaax, N. K.; Davis, K. J.; Geisbert, T. J.; Vogel, P.; Jaax, G. P.; Topper, M.; Jahrling, P. B. Lethal experimental infection of rhesus monkeys with Ebola-Zaire (Mayinga) virus by the oral and conjunctival route of exposure. *Arch. Pathol. Lab. Med.* **1996**, *120* (2), 140–155.
- (16) Bukreyev, A.; Yang, L.; Zaki, S. R.; Shieh, W.-J.; Rollin, P. E.; Murphy, B. R.; Collins, P. L.; Sanchez, A. A Single Intranasal Inoculation with a Paramyxovirus-Vectored Vaccine Protects Guinea Pigs against a Lethal-Dose Ebola Virus Challenge. *J. Virol.* **2006**, *80* (5), 2267–2279.
- (17) Leffel, E. K.; Reed, D. S. Marburg and Ebola viruses as aerosol threats. *Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science* **2004**, *2* (3), 186–191.
- (18) Borio, L.; Inglesby, T.; Peters, C.; Schmaljohn, A. L.; Hughes, J. M.; Jahrling, P. B.; Ksiazek, T.; Johnson, K. M.; Meyerhoff, A.; O'Toole, T. Hemorrhagic fever viruses as biological weapons: Medical and public health management. *JAMA, J. Am. Med. Assoc.* **2002**, *287* (18), 2391–2405.
- (19) Bermejo, M.; Rodríguez-Teijeiro, J. D.; Illera, G.; Barroso, A.; Vilà, C.; Walsh, P. D. Ebola Outbreak Killed 5000 Gorillas. *Science* **2006**, *314* (5805), 1564.
- (20) Leroy, E. M.; Epelboin, A.; Mondonge, V.; Pourrut, X.; Gonzalez, J.-P.; Muyembe-Tamfum, J.-J.; Formenty, P. Human Ebola outbreak resulting from direct exposure to fruit bats in Luebo, Democratic Republic of Congo, 2007. *Vector-Borne and Zoonotic Diseases* **2009**, *9* (6), 723–728.
- (21) Biek, R.; Walsh, P. D.; Leroy, E. M.; Real, L. A. Recent common ancestry of Ebola Zaire virus found in a bat reservoir. *PLoS Pathog.* **2006**, *2* (10), e90.
- (22) Bausch, D. G.; Towner, J. S.; Dowell, S. F.; Kaducu, F.; Lukwiya, M.; Sanchez, A.; Nichol, S. T.; Ksiazek, T. G.; Rollin, P. E. Assessment of the Risk of Ebola Virus Transmission from Bodily Fluids and Fomites. *J. Infect. Dis.* **2007**, *196*, S142–S147.
- (23) Formenty, P.; Leroy, E. M.; Epelboin, A.; Libama, F.; Lenzi, M.; Sudeck, H.; Yaba, P.; Allarangar, Y.; Boumandouki, P.; Nkounkou, V. B.; et al. Detection of Ebola Virus in Oral Fluid Specimens during Outbreaks of Ebola Virus Hemorrhagic Fever in the Republic of Congo. *Clin. Infect. Dis.* **2006**, *42* (11), 1521–1526.
- (24) Rodriguez, L. L.; De Roo, A.; Guimard, Y.; Trappier, S. G.; Sanchez, A.; Bressler, D.; Williams, A. J.; Rowe, A. K.; Bertolli, J.; Khan, A. S.; et al. Persistence and Genetic Stability of Ebola Virus during the Outbreak in Kikwit, Democratic Republic of the Congo, 1995. *J. Infect. Dis.* **1999**, *179* (Suppl.), S170–S176.
- (25) Francesconi, P.; Yoti, Z.; Declich, S.; Onok, P. A.; Fabiani, M.; Olango, J.; Andraghetti, R.; Rollin, P. E.; Opira, C.; Greco, D. Ebola hemorrhagic fever transmission and risk factors of contacts, Uganda. *Emerging Infect. Dis.* **2003**, *9* (11), 1430.
- (26) Roels, T. H.; Bloom, A. S.; Buffington, J.; Muhungu, G. L.; Mac Kenzie, W. R.; Khan, A. S.; Ndambi, R.; Noah, D. L.; Rolka, H. R.; Peters, C. J.; et al. Ebola Hemorrhagic Fever, Kikwit, Democratic Republic of the Congo, 1995: Risk Factors for Patients without a Reported Exposure. *J. Infect. Dis.* **1999**, *179* (Suppl. 1), S92–S97.
- (27) World Health Organization. Interim Infection Prevention and Control Guidance for Care of Patients with Suspected or Confirmed Filovirus Haemorrhagic Fever in Health-Care Settings, with Focus on Ebola; World Health Organization: Geneva, 2014.
- (28) Chosewood, L. C.; Wilson, D. E. *Biosafety in microbiological and biomedical laboratories*; Diane Publishing: Darby, PA, 2007.
- (29) U.S. Department of Transportation. Safety Advisory: Packaging and Handling Ebola Virus Contaminated Infectious Waste for Transportation to Disposal Sites. 2014.
- (30) Centers for Disease Control and Prevention. Interim Guidance for Managers and Workers Handling Untreated Sewage from Individuals with Ebola in the United States; Centers for Disease Control and Prevention: Atlanta, 2014.
- (31) Lowe, J. L.; Gibbs, S. G.; Schwedhelm, S.; Nguyen, J.; Smith, P. W. Nebraska Biocontainment Unit perspective on disposal of Ebola medical waste. *Am. J. Infect. Control* **2014**, *42*, 1256–1257.
- (32) Towner, J. S.; Rollin, P. E.; Bausch, D. G.; Sanchez, A.; Crary, S. M.; Vincent, M.; Lee, W. F.; Spiropoulou, C. F.; Ksiazek, T. G.; Lukwiya, M.; et al. Rapid Diagnosis of Ebola Hemorrhagic Fever by Reverse Transcription-PCR in an Outbreak Setting and Assessment of Patient Viral Load as a Predictor of Outcome. *J. Virol.* **2004**, *78* (8), 4330–4341.

- (33) Sagripanti, J.-L.; Lytle, C. D. Sensitivity to ultraviolet radiation of Lassa, vaccinia, and Ebola viruses dried on surfaces. *Arch. Virol.* **2011**, *156* (3), 489–494.
- (34) Sagripanti, J.-L.; Rom, A. M.; Holland, L. E. Persistence in darkness of virulent alphaviruses, Ebola virus, and Lassa virus deposited on solid surfaces. *Arch. Virol.* **2010**, *155* (12), 2035–2039.
- (35) Belanov, E.; Muntianov, V.; Kriuk, V.; Sokolov, A.; Bormotov, N.; P'iankov, O.; Sergeev, A. Survival of Marburg virus infectivity on contaminated surfaces and in aerosols. *Vopr. Virusol.* **1995**, *41* (1), 32–34.
- (36) MacIntyre, C. R.; Chughtai, A. A.; Seale, H.; Richards, G. A.; Davidson, P. M. Surgical mask or no mask for health workers not a defensible position for Ebola. *International Journal of Nursing Studies* **2014**, *51* (12), 1694–1695.
- (37) Martin-Moreno, J. M.; Llinás, G.; Martínez-Hernández, J. Respiratory protection for healthcare workers treating Ebola virus disease (EVD): Are facemasks sufficient to meet occupational health and safety obligations? *International Journal of Nursing Studies* **2014**, *51* (11), 1421–1426.
- (38) Martin-Moreno, J. M.; Llinás, G.; Hernández, J. M. Is respiratory protection appropriate in the Ebola response? *Lancet* **2014**, *384* (9946), 856.
- (39) Alimonti, J.; Leung, A.; Jones, S.; Gren, J.; Qiu, X.; Fernando, L.; Balcewich, B.; Wong, G.; Stroher, U.; Grolla, A. Evaluation of transmission risks associated with in vivo replication of several high containment pathogens in a biosafety level 4 laboratory. *Sci. Rep.* **2014**, *4*, 5824.
- (40) Jaax, N.; Jahrling, P.; Geisbert, T.; Geisbert, J.; Steele, K.; McKee, K.; Nagley, D.; Johnson, E.; Jaax, G.; Peters, C. Transmission of Ebola virus (Zaire strain) to uninfected control monkeys in a biocontainment laboratory. *Lancet* **1995**, *346* (8991), 1669–1671.
- (41) Dalgard, D.; Hardy, R.; Pearson, S.; Pucak, G.; Quander, R.; Zack, P.; Peters, C.; Jahrling, P. Combined simian hemorrhagic fever and Ebola virus infection in cynomolgus monkeys. *Lab. Anim. Sci.* **1992**, *42* (2), 152–157.
- (42) Ebola virus infection in imported primates—United States. *Canada Diseases Weekly Report* **1990**, *16*, 17–18.
- (43) Smither, S. J.; Piercy, T. J.; Eastaugh, L.; Steward, J. A.; Lever, M. S. An alternative method of measuring aerosol survival using spiders' webs and its use for the filoviruses. *J. Virol. Methods* **2011**, *177* (1), 123–127.
- (44) Chepurinov, A. A.; Bakulina, L. F.; Dadaeva, A. A.; Ustinova, E. N.; Chepurnova, T. S.; Baker, J. R., Jr. Inactivation of Ebola virus with a surfactant nanoemulsion. *Acta Trop.* **2003**, *87* (3), 315–320.
- (45) Sobsey, M. D.; Meschke, J. S. Virus survival in the environment with special attention to survival in sewage droplets and other environmental media of fecal or respiratory origin. Report for the World Health Organization; World Health Organization: Geneva, 2003.
- (46) Casanova, L.; Rutala, W. A.; Weber, D. J.; Sobsey, M. D. Survival of surrogate coronaviruses in water. *Water Res.* **2009**, *43* (7), 1893–1898.
- (47) Heijnen, L.; Medema, G. Surveillance of influenza A and the pandemic influenza A (H1N1) 2009 in sewage and surface water in the Netherlands. *J. Water Health* **2011**, *9* (3), 434–442.
- (48) Haas, C. N. On the Quarantine Period for Ebola Virus. *PLoS Current Outbreaks* **2014**, DOI: 10.1371/currents.outbreaks.2ab4b76-ba7263ff0f084766e43abbd89.
- (49) Enriquez, C. E.; Hurst, C. J.; Gerba, C. P. Survival of the enteric adenoviruses 40 and 41 in tap, sea, and waste water. *Water Res.* **1995**, *29* (11), 2548–2553.
- (50) Krikelis, V. Survival of Poliovirus Type I (Mahoney) and Coxsackie Virus B5 (Faulkner) at Different Temperatures under Laboratory Conditions. *EAA Hnikot Inetitottot Haztep Archives de l'Institut Pasteur hellénique* **1983**, 42.
- (51) Casson, L. W.; Sorber, C. A.; Palmer, R. H.; Enrico, A.; Gupta, P. HIV survivability in wastewater. *Water Environ. Res.* **1992**, 213–215.