Transmission of Ebola Viruses: What We Know and What We Do Not Know

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ABSTRACT **Available evidence demonstrates that direct patient contact and contact with infectious body fluids are the primary modes for Ebola virus transmission, but this is based on a limited number of studies. Key areas requiring further study include (i) the role of aerosol transmission (either via large droplets or small particles in the vicinity of source patients), (ii) the role of environmental contamination and fomite transmission, (iii) the degree to which minimally or mildly ill persons transmit infection, (iv) how long clinically relevant infectiousness persists, (v) the role that "superspreading events" may play in driving transmission dynamics, (vi) whether strain differences or repeated serial passage in outbreak settings can impact virus transmission, and (vii) what role sylvatic or domestic animals could play in outbreak propagation, particularly during major epidemics such as the 2013–2015 West Africa situation. In this review, we address what we know and what we do not know about Ebola virus transmission. We also hypothesize that Ebola viruses have the potential to be respiratory pathogens with primary respiratory spread.**

PAST EBOLA OUTBREAKS

Between the first recognized outbreak of Ebola virus disease (EVD) in 1976 and the onset of the 2013–2015 Ebola epidemic in West Africa, 24 outbreaks of EVD involving approximately 2,400 reported cases had been recognized by the World Health Organization (WHO) (1). One additional outbreak involving 69 cases occurred in the Democratic Republic of the Congo (DRC) between July and October 2014 (2). To date, five species of Ebola viruses have been identified; four from Africa (Zaire, Sudan, Bundibugyo, and Tai Forest) and one from the Philippines (Reston) (1, 3, 4). Most pre-2013 outbreaks were caused by Zaire Ebola virus (EBOV) (14 outbreaks) or Sudan virus (SUDV) (7 outbreaks); Bundibugyo virus (BDBV) caused two outbreaks, and Tai Forest virus (TAFV) was identified in a single case from Côte d'Ivoire (1). Outbreaks caused by Reston virus (RESTV) have occurred in nonhuman primates and pigs, with associated asymptomatic human infections (5).

Only seven outbreaks involved more than 100 reported cases. The maximum number of generations of human-to-human transmission for these outbreaks is unknown but is likely relatively low. One report estimated 15 generations of viral transmission during a 1976 SUDV outbreak (284 cases), which was the most that were identified (6). Investigators recorded four generations of spread during the EBOV outbreak in Kikwit, DRC (315 cases) (7).

Many experts have concluded that the extensive transmission documented in the 2013–2015 West Africa epidemic is due to societal factors (poverty, urban density, population migration patterns, and poor health care and public health infrastructure) rather than unique biological characteristics of the agent (8, 9). Limited data are available, however, regarding virus genomics (affecting phenotype/pathotype), patient viral loads, and certain epidemiological features for this unique EBOV strain. Furthermore, information about Ebola virus transmission in humans remains incomplete, given the relatively small number of outbreak investigations and cases recognized before 2013; as a result, additional questions remain (10). In this review, we explore what we know—and what we do not know—about Ebola virus transmission.

WHAT WE KNOW ABOUT EBOLA VIRUS TRANSMISSION IN HUMANS

Past outbreaks provide opportunities to examine human-to-human transmission of Ebola viruses. Spread within hospitals has been documented repeatedly, and outbreak amplification has occurred in health care settings for both EBOV and SUDV (6, 7, 11). Early outbreak investigations demonstrated the importance of parenteral transmission via nonsterile needles, although this has not been noted more recently (6, 11). In addition, investigators have shown that health care workers are at particularly high risk (6, 7, 11, 12). Use of barrier protection

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TABLE 1 Risk of illness among household contacts by history of direct physical contact with a primary case

Study and contact status	Development of EVD		
	No. who became ill (% of total ill)	No. who were not ill (% of total not ill)	Total
Dowell et al. (19)			
Direct contact	28 (100)	67 (46)	95
No direct contact	0 (0)	78 (54)	78
Total	28	145	173
Baron et al. (17)			
Direct contact	27 (93)	59 (57)	86
No direct contact	2[a] (7)	44 (43)	46
Total	29	103	132
Francesconi et al. (18)			
Direct contact	26 (96)	47 (80)	73
No direct contact	1[b] (4)	12 (20)	13
Total	27	59	86
Summation			
Direct contact	81 (96)	173 (56)	254
No direct contact	3 (4)	134 (44)	137
Total	84	307	391

[a] Contact status unknown.
[b] The patient had probable fomite exposure, i.e., used a blanket that the primary case had used before death.

and surgical masks apparently was adequate to halt most nosocomial transmission during past outbreaks of EBOV and SUDV (6, 7, 11, 13–15). In one outbreak of SUDV, however, 14 (64%) of 22 health care workers became infected after barrier precautions were established; this led to reinforcement of infection control practices (16).

Three reports (the first two involving SUDV and the third involving EBOV) examined the risk of illness among household contacts by history of direct physical contact with a primary case (Table 1) (17–19). In the first report, infection did not occur in any of 23 familial contacts who had been in the same room but did not have direct contact with the primary case; the authors therefore postulated that the virus is not easily transmitted by the airborne route (17). In the second study, investigators found that direct contact with body fluids of ill patients conferred the greatest risk; however, sleeping in the same hut and sleeping on the same mat were independent risk factors, suggesting that other modes of transmission played a role (18). In the third study, the authors indicated that while they found no evidence of "small-particle aerosol transmission," based on confidence limits of their findings, they estimated the upper limit of risk in their study for this mode of transmission was no more than 4% (19).

In a fourth report involving an outbreak of EBOV, investigators identified 55 patients out of 316 outbreak-associated cases who had no reported source of exposure during initial investigation. They obtained further information directly from four survivors and via surrogates for 40 deceased patients (20). Of 23 for whom exposure to a suspected EVD case was subsequently identified, 19 had visited the primary case before becoming ill; 14 had merely touched the case (without apparent body fluid exposure), and five reported no physical contact or body fluid exposure. The authors stated that touch alone may result in transmission and postulated that "large droplets, aerosolized particles, or fomites" may have explained the mode of transmission for the five without reported physical contact.

Available data indicate that direct physical contact and exposure to infected body fluids are the primary modes of Ebola virus transmission. In support of this, Ebola virus has been cultured from saliva, breast milk, urine, and semen of infected patients; in addition, viral RNA (identified by reverse transcription-PCR [RT-PCR]) has been found in stool, tears, and sweat and in rectal, conjunctival, vaginal, and skin swabs (21–25). Because copious amounts of virus can be found in skin (by direct visualization and immunohistochemistry staining), and sweat may contain the virus, touching an infected person may result in transmission (26, 27). Infected persons can shed virus for prolonged periods of time after infection (several weeks to months). The virus has been cultured from semen up to 82 days after illness onset (25), but sexual transmission has not been documented (28).

Fomite transmission apparently can occur, but the role of fomites also appears to be relatively small, based on limited epidemiological data. In support of fomite transmission, studies demonstrate that filoviruses can survive on solid surfaces and in liquids for several days to several weeks (29–31). One field study analyzed 31 environmental samples obtained from an Ebola isolation ward during an SUDV outbreak; all were negative by RT-PCR and culture, but two control samples (taken from a blood-stained glove and an intravenous insertion site) were positive for viral RNA by RT-PCR (21). This study did not demonstrate significant environmental contamination; however, sampling took place after routine cleaning of the ward, methods used had not been validated for environmental sampling, and the findings are not generalizable to situations where greater environmental contamination is present. For example, environmental sampling in 2014 at the Ebola treatment center in Kailahun, Sierra Leone, demonstrated multiple positive samples by RT-PCR. Samples were collected early in the morning before daily cleaning and disinfection, and many were taken from surfaces that were not visibly bloody or soiled. Interestingly, the outer surfaces of three out of 16 masks worn by health care workers were RT-PCR positive; they were not visibly bloody or soiled (J. Strong, unpublished data). Identification of viral RNA on masks suggests either the presence of aerosols in the patient-care environment or cross-contamination of the masks (such as when workers doffed personal protective equipment [PPE]). Finding viral RNA in the environment, however, does not necessarily indicate that viable infectious viral particles are present.

Since the overwhelming majority of EVD cases have resulted from prior contact with a symptomatic Ebola patient, public health authorities have concluded that transmission does not occur from persons who are incubating disease but not yet symptomatic. Enzyme-linked immunosorbent assay (ELISA) testing of close contacts of clinical cases found that contacts can develop asymptomatic infection (demonstrated by IgM and IgG responses) and they harbor very low levels of circulating virus, suggesting that infected persons without symptoms are not very infectious and, therefore, are unlikely to transmit to others (32). This is probably also true for persons in the presymptomatic phase of illness. Investigators have noted, however, that mildly ill patients who are early in the clinical course can transmit infection (19). Similarly, many secondary cases in past outbreaks had ongoing exposure to a primary household case, so it is not possible to determine when transmission actually occurred. Finally, for at least one secondary case, identified during an EBOV outbreak, the

only recognized potential source of exposure was transmission from an asymptomatic person 2 days before that person became ill (11), but such reports are exceedingly rare in the available literature. Even though mildly ill persons can possibly transmit the virus, review of available data suggests that, if this occurs, it is an infrequent event and not of substantial epidemiological importance.

Several additional observations are germane to understanding human-to-human Ebola virus transmission. First, the amount of virus in the blood increases substantially over the course of illness; this likely influences the degree of viral shedding, which, in turn, influences patient infectiousness over time (18, 19, 33). Second, patients in the late stages of disease who are at their most infectious and who are experiencing severe diarrhea, vomiting, bleeding, or coughing may be more likely to shed infectious virus with aerosol particles of various sizes, given the presence of virus in various body fluids. Third, investigators have raised the possibility of "superspreading events." In one report, a single SUDV case apparently transmitted the virus to 10 family members; additional details regarding this case are not available (34). Investigation of an EBOV outbreak found that one case was an apparent source for 38 secondary cases and another was a source for 21 secondary cases; both had gastrointestinal hemorrhaging, and exposures most likely occurred during primary-case burials (7). In Sierra Leone, more than 300 cases were linked to the burial of a patient who died in May 2014 (35). Fourth, the infectious dose for Ebola viruses in humans appears to be extremely low, with 10 or fewer viral particles being sufficient for infection (36). This may be particularly important for health care workers when doffing contaminated PPE, since without meticulous care during doffing, health care workers may come into contact with small numbers of viral particles.

Limited information regarding transmission is available for the current West Africa epidemic. One dramatic feature is the large number of infected health care workers (37). The WHO has postulated that inadequate training in specialty care, shortages of medical staff, shortages of PPE, improper use of PPE, and misdiagnosis of EVD have contributed to this occurrence. The exact transmission mechanism remains unknown for two American nurses who acquired infection while caring for a patient in Dallas, TX, and for a nurse in Madrid, Spain, who cared for a returning missionary (38, 39). For the Dallas patient, aerosol-generating procedures (such as intubation) were performed before his death, but it is not clear if these contributed to transmission to the health care workers.

EBOLA VIRUS TRANSMISSION AT THE HUMAN-ANIMAL INTERFACE

Ebola viruses can infect a number of animal species, most notably nonhuman primates. In 1994, an Ebola outbreak occurred among chimpanzees in the Tai National Park, Côte d'Ivoire (40). An ethologist was infected with TAFV while conducting a necropsy on a wild chimpanzee that had been found dead; she was wearing "household gloves" but no mask or gown (41).

Between 2001 and 2003, several human outbreaks of EBOV occurred in Gabon and the neighboring Republic of the Congo. These outbreaks began with index cases handling infected wild animal carcasses, including gorillas, chimpanzees, duikers (small antelopes), and possibly monkeys (42, 43). Investigation of a 2007 EBOV outbreak suggested that fruit bats migrating up the Lulua River in the DRC were the source; before illness onset, the index case had bought freshly killed bats for food (44). Since Ebola IgG antibodies and viral RNA have been detected in bats, researchers believe that fruit bats may be a primary natural reservoir for Ebola viruses (45).

Several outbreaks of RESTV occurred between 1989 and 1996 in captive monkeys in the Philippines or imported from the Philippines into the United States or Italy (3, 46, 47). During the first outbreak, four U.S. animal workers seroconverted to positivity for RESTV; none developed symptoms. One likely became infected during necropsy on a monkey. For the remaining three, investigators suggested that direct contact with infected monkeys was the most likely route; conjunctival exposure and inhalation were considered possible routes. A WHO report published in 2009 indicated that an additional five monkey handlers in the Philippines tested positive for IgG antibodies to RESTV; none recalled a significant illness (48).

In 2008, RESTV was isolated from pigs in the Philippines (by RT-PCR and viral isolation). At the time, an increase in mortality was occurring on swine farms; the pigs were infected with porcine reproductive and respiratory syndrome (PRRS) virus, and some had evidence of coinfection with RESTV (5). Six workers in contact with infected pigs had RESTV IgG antibodies, supporting past infection, which suggests that pigs can transmit RESTV to humans. The most likely routes of transmission from pigs to humans include direct contact with body fluids of infected animals and possibly respiratory transmission (48). Coinfection of pigs with PRRS, which causes respiratory disease, may have facilitated respiratory transmission of RESTV (49).

Serologic evidence suggests that dogs can be naturally infected with Ebola viruses. During one outbreak, investigators found an EBOV IgG seroprevalence rate of 31.8% by ELISA testing among dogs from African villages with both infected animal carcasses and human EVD cases (50). Ebola-like illness has not been reported in dogs, EBOV has not been isolated from dogs, and the validity of the ELISA has been questioned; therefore, the significance of this finding, particularly with regard to transmission to humans, remains unknown.

ANIMAL TRANSMISSION STUDIES

Several experimental studies have examined animal-to-animal Ebola virus transmission in various animal species. One study involving EBOV-inoculated rhesus monkeys and controls found that two of three control monkeys caged in the same room developed Ebola disease 10 and 11 days after the inoculated animals had died; the control monkeys were housed approximately 3 m from the inoculated monkeys (51). The authors postulated the control monkeys became infected through aerosol, oral, or conjunctival exposure to virus-laden droplets. The pattern of pulmonary antigen staining on pathology specimens suggested aerosol infection. Alternatively, transmission could have occurred through certain behaviors of caged nonhuman primates (such as spitting and throwing feces) (52) or through routine animal husbandry. In another study, six piglets were infected with EBOV and then housed with four caged macaques (53). The piglets and macaques were separated by a wire barrier 20 cm in front of the monkey cages. All macaques developed infection. Transmission could have resulted from inhalation of aerosols, inoculation of mucous membranes by droplets, or droplets landing in the cages with subsequent fomite transmission; animal workers took care to avoid

cross-contamination of the cages during husbandry practices, but this remains a possibility (53). In a third study, two monkeys infected with EBOV intramuscularly were placed next to two noninfected monkeys—both were in open-barred cages without protective barriers. At 6 days postinoculation, infected monkeys had high titers of virus in blood, but testing of oral, nasal, and rectal swabs did not yield infectious virus. The infected monkeys did not transmit virus to uninfected monkeys (54).

Other studies have examined the role of mucosal or inhalational exposures in transmission. In one, researchers inoculated rhesus monkeys with EBOV orally, conjunctivally, or by intramuscular inoculation (one positive-control monkey) (55). Three of four monkeys exposed by the oral route, four of four monkeys exposed by the conjunctival route, and the positive-control monkey became infected with EBOV and died. In another, six rhesus monkeys were randomly assigned to one of three groups of two animals each: low inhaled dose, high inhaled dose, and noninfectious aerosol (control group). All EBOV-exposed monkeys developed a rapidly fatal illness (56). Investigators have shown that pigs develop a severe respiratory illness following mucosal inoculation with EBOV and can shed large quantities of virus from the oronasal mucosa (57). In these studies, the inoculum was suspended in a uniform carrier and delivered directly to the animals for a certain period of time in a controlled environment; therefore, this information is not necessarily applicable to human-to-human transmission under real-world conditions.

KEY PATHOLOGY FINDINGS IN HUMANS AND NONHUMAN PRIMATES

Clinical pathology studies of EVD in humans are limited because of biosafety concerns and lack of medical infrastructure in areas with Ebola outbreaks. To date, autopsies have been performed for only about 30 cases of filovirus infection; Ebola outbreaks in 1976 (SUDV) and 1995 (EBOV) remain the primary sources of information (27, 58, 59). Pathology data demonstrate that Ebola viruses can infect numerous cell types (primarily macrophages and dendritic, endothelial, and Kupffer cells) and tissues (notably liver, spleen, kidneys, lymph nodes, testes, gastrointestinal mucosa, and skin). Ebola virus is not known to cause pneumonitis in humans, although congestion, focal intra-alveolar edema, diffuse alveolar damage, and hemorrhage can occur in the lungs. Viral inclusions can be found within alveolar macrophages, and free viral particles can be seen within alveolar spaces (59).

Pathology findings in nonhuman primates are consistent with data from human autopsies. Of particular interest are studies involving virus inoculation by aerosol challenge. One study demonstrated that monkeys infected with EBOV by the aerosol route developed a rapidly fatal Ebola-like illness and on necropsy had mild to moderate, patchy interstitial pneumonia with a bronchocentric pattern (56). Severe lymphoid depletion, necrosis, vasculitis, thrombosis, and hemorrhage were noted in tracheobronchial lymph nodes. Large amounts of viral antigen were present in secretions on mucosal surfaces of the airways, oropharynx, and nose. Another EBOV aerosol challenge study involving rhesus macaques demonstrated significant primary infection of lymphoid tissues in the upper and lower respiratory tract, which is generally not noted in primates inoculated by other routes (60). A study involving probable aerosol transmission of EBOV from piglets to cynomolgus macaques showed interstitial pneumonia and focal areas of alveolar hemorrhage and edema in the macaques. Viral antigen was detected in alveolar and septal macrophages, pneumocytes, and pulmonary, bronchiolar, and tracheal epithelial cells (53). In another study, eight guinea pigs were exposed to an aerosol containing a guinea pig-adapted EBOV strain; all developed a lethal interstitial pneumonia that was distinct from pulmonary pathology observed in guinea pigs challenged subcutaneously (61). Another study showed that guinea pigs inoculated intranasally had extensive lung pathology and EBOV antigen in the trachea, suggesting that guinea pigs challenged intranasally were more infectious for naive cage mates than animals inoculated intraperitoneally (62).

GENOMICS OF EBOLA VIRUS

Since initial isolation of Ebola viruses in 1976, the glycoprotein gene has remained stable enough to be useful for diagnostics and phylogenetic analysis (63). Before the West Africa epidemic, GenBank included 48 completed genomic sequences covering the five Ebola viruses (64). Eighty-one additional genomic sequences have been added since the 2013–2015 epidemic in West Africa began (65). Seventy-eight are from Sierra Leone (collected in late May to mid-June 2014) (65) and three are from Guinea (collected in late February to mid-March 2014) (64). Data from these sequences suggest that the current outbreak can be traced to two distinct introductions from Guinea into Sierra Leone, the Guinea outbreak was the result of a single zoonotic event, and this EBOV strain represents a unique clade (64, 65). Two phylodynamic analyses of the Sierra Leone data using several different models suggest that superspreading events (as evidenced by high variance in transmission rates in the best-fitting models) are occurring and driving transmission (66, 67).

THE COMPLEXITIES OF AEROSOLS AND DROPLETS: A CHANGING PARADIGM

Public health officials generally consider disease transmission of infectious agents to fall into three categories—contact transmission (direct and indirect), droplet transmission, and airborne transmission; the definition of each follows (68). Direct contact transmission occurs when pathogens are transferred from one infected person to another person without a contaminated intermediate object or person. Indirect contact transmission involves the transfer of an infectious agent through a contaminated intermediate object or person. Droplet transmission involves respiratory droplets carrying infectious pathogens that travel directly from the respiratory tract of the infectious individual to susceptible mucosal surfaces of the recipient, generally over short distances. Airborne transmission occurs by dissemination of either airborne droplet nuclei or small particles in the respirable size range containing infectious agents that remain infective over time and distance. In further understanding the complexities of disease transmission, two other terms should be considered. The first is aerosol transmission, which involves inhalation of infectious aerosols suspended in the air either near a person or at a distance and can involve aerosol particles of various sizes that either land on mucosal surfaces, such as the nose and mouth, or are inspired deeper into the respiratory tract (69). Both traditional models of droplet transmission and airborne transmission can fit under the broader category of aerosol transmission. Aerosols can be generated from the respiratory tract, via forceful emission of body fluids such as

vomitus or diarrhea, or from aerosol-generating procedures. This leads to the last concept, which is respiratory transmission. Respiratory transmission is limited to generation of aerosols (either droplet or small-particle aerosols) from the respiratory tract (e.g., nasal passages, trachea, or lungs) that then enter the airspace and, to use the traditional model, cause infection by droplet or airborne spread.

Breathing, talking, coughing, and sneezing generate particles suspended in air (aerosols), which range in diameter from less than 1 μm to greater than 100 μm. These particles traditionally have been divided into two categories: droplet and airborne. Droplets have been thought to range from 5 μm to 100 μm in diameter, to travel no more than a few feet, and to be projected onto surfaces, skin, or mucous membranes. Airborne particles (often referred to as "droplet nuclei") have been thought to be less than 5 μm, to remain suspended in the air for a period of time, and to transmit infection by inhalation only at some distance from the source patient. In the traditional infection control paradigm, transmission via particles can only occur in two ways: (i) by large droplets directly impacting skin or mucous membranes and (ii) by inhalation of small airborne particles at a distance from the source. Larger particles also may settle on objects, allowing fomite transmission. This paradigm fails to recognize that infectious aerosols include suspended particles in a wide range of particle sizes (small to large droplets) that are easily inhaled by someone standing near the point of generation. Thus, aerosol inhalation can occur both near and far from an infectious source. Distinguishing transmission from droplet impact on skin or mucous membranes, direct body fluid contact, or inhalation of small-particle aerosols near the source is not possible without more detailed and careful exposure assessment during epidemiological investigations. Larger particles can be deposited in the nasal passages, pharynx, and upper regions of the lungs, while smaller particles are more likely to be deposited in the alveoli.

We now know that aerosols contain particles of various sizes and that the traditional model of droplet versus airborne spread is an oversimplification of a complex process (70, 71). For example, coughing was thought to produce primarily large droplet-sized particles (68). Recent data suggest, however, that most particles produced during coughing are under 1 μm and that cough aerosols are multiphased and capable of extended suspension of inhalable particles both near and far from a source (72, 73). These findings are consistent with a review on particle size, which found that droplets involving respiratory pathogens are a heterogeneous mix of large and small particles (74). A similar paradigm may be applied to generation of aerosols outside the respiratory tract, such as aerosols generated through medical procedures or forceful episodes of vomiting or diarrhea. For example, vomiting associated with enteric norovirus infection can result in emission of infectious aerosols that result in transmission (75). In real-world environments, a number of factors impact the likelihood of aerosol transmission, such as the length of time particles reside in the air, the length of time particles remain infectious once expelled, variations in particle size and density, and issues such as ambient temperature and humidity.

In 2004, Roy and Milton proposed classifying aerosol transmission of respiratory pathogens as obligate, preferential, or opportunistic, based on the ability of the agent to be transmitted through small-particle aerosols (76). According to this system, *Mycobacterium tuberculosis* is transmitted primarily through small-particle aerosols and thus involves obligate aerosol transmission. Pathogens with preferential aerosol transmission include agents such as varicella zoster virus that can be transmitted in multiple ways but are transmitted primarily by small-particle aerosols. Pathogens with opportunistic aerosol transmission include those that are transmitted primarily by other routes but can be transmitted by small-particle aerosols under certain conditions; as noted above, norovirus is an example. Ebola virus may also fit into this category, since infectious aerosols (either large or small droplet), which could result in transmission, may be generated and emitted during the course of disease.

WHAT WE NEED TO LEARN ABOUT EBOLA VIRUSES

The number of past outbreaks and associated epidemiological studies carefully examining transmission patterns is small; therefore, conclusions about transmission are based on relatively limited data sets. Furthermore, inferences from past studies have been based on retrospective reconstructions of chains of transmission, and exposure histories were often obtained from surrogates in situations where cases could not be interviewed; as a result, an inherent recall bias exists for most of the available epidemiological studies. Because of limitations in the current data, the role of aerosol transmission remains unclear. Transmission potentially occurs via virus-laden aerosols generated through forceful emission of body fluids during vomiting, diarrhea, or coughing (particularly if hemorrhaging is involved). In support of this, investigators created Ebola-containing aerosols and, on the basis of decay rates, estimated that EBOV can survive in aerosols for approximately 100 min and RESTV can survive for approximately 160 min (at 50% to 55% relative humidity and 22 $\pm$ 3°C) (31). In addition, available studies have not been able to effectively tease out exposure via fomites from exposure via direct contact or body fluid contact; therefore, the epidemiological significance of fomite transmission also is unknown. Further studies are needed to better define the potential roles for aerosol and fomite transmission in spreading disease.

To date, investigators have assumed that EBOV strains are similar with regard to infectivity, pathogenicity, and virulence. Past experience, however, may not necessarily predict future Ebola virus behavior. Also, we know that the West Africa EBOV strain represents a unique clade (64), and its full phenotypic range has not been elucidated. For example, quantitative RT-PCR testing in 2014 of blood specimens from 41 Sierra Leone patients and 23 patients from a concurrent EBOV outbreak in the DRC, which involved a different EBOV strain, showed significantly lower C_T (cycle threshold) values (indicating higher viral loads) for the Sierra Leone patients (mean C_T, 22.44 $\pm$ 0.90) than for the DRC patients (mean C_T, 30.08 + 1.23; $P < 0.0001$ by an unpaired two-tailed t test). The C_T value is the cycle number at which the fluorescence generated within a reaction crosses a predetermined threshold and is inversely correlated to the amount of target nucleic acid in the sample. The same protocols, reagents, and equipment were used for both sets of specimens, and the time from illness onset to specimen collection did not differ for the two groups. These findings suggest that some patients infected with the West Africa EBOV strain (Makona) have higher viral loads, which could contribute to transmission and result in more superspreading events (G. Kobinger, unpublished data). Furthermore, limited information is available about Ebola virus evolution dur-

ing serial passage over time, since past outbreaks have involved relatively few generations of spread. The West Africa epidemic involves many generations of spread, the impact of which remains unknown.

Several other issues important to transmission and outbreak propagation deserve mention. First, in explosive situations such as the current epidemic, we do not know the potential role of superspreading events. Such events may often be explained by epidemiological or behavioral factors such as association with ritual burials; however, other possible explanations deserve further investigation. It could be, as suggested by phylodynamic modeling, that superspreading events have played an important role in transmission dynamics in West Africa. Second, limited data are available on environmental survival of Ebola viruses. Third, we know that in the vast majority of cases, infected persons do not transmit the virus in the absence of symptoms, likely because of low viral shedding; however, additional data are needed to further refine the onset of infectiousness. Fourth, we know that the virus can be shed for several months following recovery, but the epidemiological significance of this remains unknown. Finally, in such a large epidemic as the one in West Africa, we do not know if transmission is amplified by sylvatic or domestic animal populations at the human-animal interface.

RESPIRATORY TRANSMISSION OF EBOLA VIRUSES: A HYPOTHESIS

It is very likely that at least some degree of Ebola virus transmission currently occurs via infectious aerosols generated from the gastrointestinal tract, the respiratory tract, or medical procedures, although this has been difficult to definitively demonstrate or rule out, since those exposed to infectious aerosols also are most likely to be in close proximity to and in direct contact with an infected case. To date, investigators have not identified respiratory spread (either via large droplets or small-particle aerosols) of Ebola viruses among humans. This could be because such transmission does not occur or because such transmission has not been recognized, since the number of studies that have carefully examined transmission patterns is small.

Despite the lack of supportive epidemiological data, a key additional question to ask is whether primary pulmonary infections and respiratory transmission of Ebola viruses could be a potential scenario for the future. A fair amount of evidence suggests that such transmission could be possible, even without dramatic evolution or genetic changes in Ebola viruses (although viral evolution over time could enhance this possibility). First, Ebola viruses can be isolated from saliva, and viral particles have been identified in pulmonary alveoli on human autopsies, suggesting that infectious aerosols could be emitted from the respiratory tract. Second, Ebola viruses can infect several cell types found in the respiratory tract, including macrophages and epithelial cells (77). Third, cough can be a symptom of EVD, and coughing is known to generate aerosols, although prevalence of reported cough is variable in case series (ranging from "rare" to 49%) (2, 6, 78). Fourth, animal studies indicate that EBOV can be transmitted through aerosols and that respiratory infection with pneumonitis can occur following this route of inoculation. Fifth, experience with RESTV suggests that respiratory transmission of that species can occur between animals and possibly from animals to humans. Finally, people can generate and emit aerosols with particles of various sizes, including fine particles, which could enter the lower respiratory tract and infect susceptible cells; Ebola virus is in the respirable range (800 to 1,000 nm).

If aerosols containing Ebola virus were to enter the lungs of uninfected individuals, it is possible that primary pulmonary infections could occur (as shown in animal studies), which could then result in active viral shedding from the respiratory tract, thus potentially setting up a cycle of ongoing respiratory transmission in humans (79), similar to what occurs during outbreaks of pneumonic plague. Investigators of a nosocomial outbreak of Lassa fever virus, another African hemorrhagic fever virus that is usually spread via contact with rodents (especially rodent urine), postulated that transmission may have occurred through the respiratory route (80, 81). Similarly, investigators of a nosocomial cluster of Crimean-Congo hemorrhagic fever, a vector-borne infection that occurs in Eastern Europe and Africa, identified probable aerosol transmission due to aerosol-generating medical procedures (82). Experts in bioterrorism have long been concerned that hemorrhagic fever viruses, particularly filoviruses (Ebola virus and Marburg virus), could be used as potential agents of bioterrorism, with an aerosol being the most likely form of dissemination (83, 84). This concern has been grounded in uncertainty concerning the potential for aerosol transmission of such viruses. A strain of Marburg virus was weaponized by the Soviet Union, highlighting this risk (58).

Leading public health agencies have stated that airborne transmission (i.e., transmission via small-particle aerosols traveling over time and distance) of Ebola viruses is unlikely to occur in the future because this would require specific genotypic changes in the virus (85, 86). We agree this is an improbable (although not impossible) scenario; however, with phenotypic changes in the virus, aerosol transmission (and possibly respiratory transmission if primary pulmonary infections were to occur) involving droplets of various sizes from cases in relatively close proximity to uninfected persons remains plausible. The West Africa Ebola epidemic surprised even the most astute infectious disease experts in the global public health community; we should not assume that Ebola viruses are not capable of surprising us again at some point in the future.

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