What is Ebola?

R. A. Stein

SUMMARY

On 23 March 2014, the World Health Organization first announced a new Ebola virus outbreak that started in December 2013 in the eastern part of the Republic of Guinea. Human infections shortly emerged in Liberia, Sierra Leone, and Nigeria. On 30 September 2014, the Centers for Disease Control and Prevention confirmed through laboratory testing the first Ebola virus infection diagnosed in the USA, in a patient who travelled from West Africa to Texas. On 6 October 2014, the first human infection occurring outside of Africa was reported, in a Spanish nurse who treated two priests, both of whom died, and on 23 October 2014, the first human infection was reported in New York City. To date, the 2014 Ebola virus outbreak is the longest, largest, and most persistent one since 1976, when the virus was first identified in humans, and the number of human cases exceeded, as of mid-September 2014, the cumulative number of infections from all the previous outbreaks. The early clinical presentation overlaps with other infectious diseases, opening differential diagnosis difficulties. Understanding the transmission routes and identifying the natural reservoir of the virus are additional challenges in studying Ebola hemorrhagic fever outbreaks. Ebola virus is as much a public health challenge for developing countries as it is for the developed world, and previous outbreaks underscored that the relative contribution of the risk factors may differ among outbreaks. The implementation of effective preparedness plans is contingent on integrating teachings from previous Ebola virus outbreaks with those from the current outbreak and with lessons provided by other infectious diseases, along with developing a multifaceted inter-disciplinary and cross-disciplinary framework that should be established and shaped by biomedical as well as sociopolitical sciences.

Review criteria
Ebola virus, filoviruses, outbreaks, reservoir, transmission routes, risk factors.

Message for the clinic
The 2014 Ebola virus outbreak is the longest, largest, and most persistent one to date, and a considerable challenge for developing as well as developed countries.

Department of Biochemistry and Molecular Pharmacology, New York University School of Medicine, New York, NY, USA

Correspondence to:
Richard A. Stein, Department of Biochemistry and Molecular Pharmacology, New York University School of Medicine, New York, NY, USA
Tel.: +917-684-9438
Fax: +646-754-9632
Email: richardastein@gmail.com; steinr01@nyu.edu

Disclosure
None.

The 2014 Ebola virus outbreak

On 23 March 2014, the World Health Organization (WHO) announced a new Ebola outbreak that started in December 2013 in the Guéckédou district from the southeastern region of the Republic of Guinea (1–5). This was followed by reports of the illness in patients from Liberia, Sierra Leone and Nigeria (3,6,7). By 8 August 2014, when the WHO declared the Ebola outbreak from West Africa a Public Health Emergency of International Concern, there were 1779 reported cases with 961 deaths, and by 14 September 2014, there were 4507 probable and confirmed cases and 2296 deaths in Guinea, Liberia, Nigeria and Sierra Leone (3,5,8,9). The number of infected patients in the current outbreak exceeds, as of mid-September 2014, the combined number of patients from all the previously known outbreaks (10). The estimated case-fatality rate of this outbreak, as of late September 2014, was 70.8% (9). On 30 September 2014, the Centres for Disease Control and Prevention confirmed the first case of Ebola virus infection to be diagnosed in the United States, in a patient who travelled from West Africa to Texas, and on 6 October 2014, the first person to have contacted the infection outside Africa was reported in a nurse from Spain, who treated two Spanish priests, both of whom died of the infection (11–13). On 24 October 2014, the first Ebola virus infection was reported in New York City, in a physician who returned from Africa, where he treated patients with the infection (14). In the USA, as of 24 October 2014, three patients were being treated, five had recovered and one died (14). The 2014 Ebola virus outbreak is the longest, largest and most persistent one on record (5,10). One of the concerns surrounding the 2014 Ebola virus outbreak is the threat of urban spread in Africa; this is in contrast with many previous outbreaks, which occurred in rural areas from Africa near tropical rainforests, making it easier to implement timely public health responses (3,15).

Filoviruses

The *Filoviridae* family contains linear, non-segmented, negative-sense, single-stranded RNA viruses organised into two genera, *Ebolavirus* and *Marburgvirus* (16–18). A new filovirus, the Lloviu virus, which derives its name from the Cueva del Lloviu cave in Spain where it was detected in 2011 in dead insectivorous bats, was proposed to be included in a new genus, *Cuevavirus* (17,19–21). The Lloviu virus virulence for humans and non-human primates has not yet been studied (22). Members of the *Filoviridae* are thought to have diverged approximately 10,000 of years ago from a common genetic ancestor (17).

Ebola viruses have been implicated, since the first known outbreaks in the mid-1970s, in sporadic outbreaks that occurred in Africa and affected humans and non-human primates (23). Genetic and antigenic characterisation led to the description of five Ebola virus serotypes: Sudan (EBOV-S), Zaire (EBOV-Z), Tai Forest (EBOV-TF), which was previously known as Cote d'Ivoire Ebola, Reston (EBOV-R) and Bundibugyo (EBOV-B) (17,18,23). The amino acid identity between Ebola viruses ranges between 55% and 81% (24). One of the seven viral proteins in filoviruses and the most abundant matrix protein, VP40, with important roles in viral assembly and budding, shows approximately 25% amino acid identity between the Ebola and Marburg viruses, supporting distinct mechanisms of cellular entry for the two viruses (24–26). EBOV-TF was implicated in a single non-fatal human infection, EBOV-R was not been implicated in severe human infections to date, but it is highly pathogenic for non-human primates, and EBOV-S, EBOV-Z and EBOV-B were implicated in severe outbreaks (17,18,20,27).

Identifying the natural reservoir of the filoviruses and elucidating the routes of transmission have been particularly challenging (24). Plants, arthropods, small mammals such as bats and rodents and reptiles, have been considered at various points in time as potential reservoirs (19,24,28–32). PCR and antibody data implicated bats as the natural reservoir of EBOV-Z, and studies that show that fruit bats are the reservoir for Marburg virus strengthen the view that bat species may serve as reservoirs for different filoviruses (23,27,28,33,34).

History

Ebola virus was first recognised in 1976 in Central Africa, during two nearly simultaneous outbreaks that were caused by two different subtypes, the Sudan Ebola virus (EBOV-S) and the Zaire Ebola Virus (EBOV-Z) (18,24,35–37). All Ebola virus outbreaks were reported to occur within 10° of the equator (38,39).

The first outbreak started in Nzara and Maridi, two towns from Sudan, where 150 of 284 victims died, with a mortality of 53% (18). The outbreak in Nzara is thought to have originated among workers from a cotton factory. It was reported that insectivorous bats were present in the roof space of the factory (36). The second outbreak occurred in Zaire, near the borders with Sudan and the Central African Republic, and with 318 declared victims and 284 deaths, had an 89% mortality rate (18,40). The first person who developed the disease was a 44-year-old schoolteacher who presented to the Yambuku Mission Hospital on 26 September 1976 with fever and received parenteral chloroquine for suspected malaria (40–42). His fever subsided but subsequently reappeared together with other signs and symptoms, including gastrointestinal bleeding. The patient was admitted on 5 September, died on 8 September and is considered the index case of the outbreak. While travelling during the preceding weeks, he had purchased and eaten antelope meat. Almost all patients who subsequently developed Ebola haemorrhagic fever during this outbreak either received injections at the same hospital or were close contacts of patients who received injections. At the Yambuku General Hospital, it was reported that five syringes and needles were distributed every morning, and were usually rinsed in a pan of warm water between their uses on different patients and sometimes, at the end of the day, they were boiled (40). Eventually, 55 of the 250 neighbouring villages in the area, all within 120 km from Yambuku, reported patients.

After 15 years without known outbreaks, Ebola re-emerged in 1994, and at this time, a new subtype, Côte d'Ivoire Ebola virus was identified (18). In November 1994, three researchers dissected the body of a chimpanzee they found in the Taï National Park, Côte D'Ivoire (43,44). One of them, a 34-year-old woman, became sick 8 days later. She took halofantrine, and as there was no improvement in her condition, with malaria being suspected, she was admitted to a local hospital and treated with intravenous quinine. After several days she was repatriated to Switzerland, where her condition improved and she was discharged. Interestingly, this patient did not develop haemorrhagic signs. Ebola virus was identified from chimpanzee tissues, and it was later shown that the patient was infected with Ebola virus. The human disease was preceded, between October and December 1994, by the observation that about 25% of the 43 members of a wild chimpanzee community disappeared or were found dead in the Taï National Park (43). The high mortality rate among apes

indicated that they are probably not the reservoir for the virus, but probably become infected from a common reservoir that also infects humans. Before the outbreak, the chimpanzee group was reported to have fed on a fig tree. Fruit bats are the most important dispersers of the fig tree's seeds, and some of them are known to fly kilometres in search of a tree with ripe figs (45,46). This became the first example in Africa when a human became infected with Ebola from a naturally infected non-human primate (47).

Between late 1994 and early 1997, northeastern Gabon witnessed several successive Ebola virus outbreaks (18). The first one started in December 1994 and occurred in two waves. Initially, inhabitants from three gold-panning encampments, Mékouka, Andock and Minkébé, became sick and travelled to a hospital in Makokou for treatment. Deaths were also reported within the local populations of gorillas and chimpanzees (48,49). There was a report from a patient who reported having had to kill a chimpanzee that exhibited abnormal behaviour. More cases were reported in January 1995 (18). The second outbreak began in early February 1996 in the Mayibout II village, about 40 km south of Mekouka. It started with 18 children who helped butcher and carry a chimpanzee they found in the forest, and subsequently became ill. Eventually they spread the disease to two neighbouring villages, Mayibout I to the south and Mvadi to the north. Overall, the outbreak caused disease in 31 people, 21 (67.7%) of whom died (18). The third outbreak is thought to have started in July 1996 with the death of a hunter living near the village of Booué (49,50,51). In early August, several chimpanzees were reported to have died in the same area. A second hunter died in the same region in late August, and 12 days later a third hunter became sick, was hospitalized, but left the hospital against medical advice and died in the village of Balimba, where he was treated by a natural healer. In mid-September, the traditional healer and his nephew became sick with similar symptoms, were admitted to Booué Hospital and subsequently to the Makokou General Hospital, and died there soon thereafter. In late October, a physician who performed an endoscopy to an infected patient in Booué developed disease and travelled to Johannesburg for treatment. The South African nurse who cared for him became sick and died (18,49).

The most studied Ebola outbreak was one that occurred between January and June 1995 in Kikwit, 240 miles east of Kinshasa and in the surrounding villages in the Democratic Republic of Congo (DRC; 52,53). In January 1995, a charcoal miner from Kikwit died of Ebola, followed 2–3 weeks later by the death of several family members, who had become infected during the burial rituals. Patients presented haemorrhagic diarrhoea, initially thought to be caused by *Shigella* infections; as a result, Ebola remained undiagnosed for several months (54). The outbreak lasted for 7 months, and involved 316 individuals with suspected infection, 252 of whom died (52). During the Kikwit outbreak, healthcare workers experienced the highest rate of infections, with physicians more likely to become infected (31%) than technicians (11%) and nurses (10%) (55). Epidemiological investigations revealed that direct contact with a sick person and exposure to body fluids such as blood, stool or vomit conferred a heightened risk; being an adult, exposure to patients in the late stages of the infection, or having touched a dead body were additional risk factors (52).

At least 12 additional outbreaks occurred between 2000 and 2013 (16,37,56–67). This period was also characterised by the discovery, during the August 2007–February 2008 outbreak from western Uganda, of a new species, Bundibugyo Ebola virus (EBOV-B), which later was again found in a 2012 outbreak from the DRC (66). The case-fatality rate for infections caused by this subtype is estimated to be about 25% (68). During this outbreak, having visited sick persons or having visited the hospital 3 weeks prior to the onset of symptoms, and participation in funeral rituals, which involved washing and dressing the body of the deceased, emerged as risk factors in those with confirmed and probable infection (69).

Clinical presentation

Filoviruses cause severe haemorrhagic diseases in humans; no licensed vaccines are available to date, and supportive therapies represent the mainstay of treatment (22,24,38). Collecting clinical data from Ebola virus outbreaks has been challenging for several reasons, including the fact that early symptoms are non-specific (7,16). Generally, blood samples are positive by polymerase chain reaction 1 day before symptoms start (7). Ebola viruses are highly pathogenic for humans and for non-human primate species and the acute viral syndrome is known as Ebola haemorrhagic fever (39,70). Particularly outside of epidemics, signs and symptoms are often missed, due to the fact that they overlap with those from other conditions in the areas that are affected, such as malaria, making diagnosis challenging (71). Ebola is often suspected after other interventions, such as antibiotics and antimalarial drugs, do not result in improvement (38).

Early symptoms are similar between survivors and non-survivors, but non-survivors appear to have

100–1000-fold higher levels of viremia (16). Although they are genetically different, the Zaire, Sudan and Ivory Coast subtypes of the Ebola virus cause similar human diseases, with an incubation that can range between 2 and 21 days (16,68,70). The clinical syndromes caused by different Ebola virus subspecies also show some differences; for example, the incubation period appears to be different for the different subtypes (72). The incubation time also depends on the route of transmission; for example, the mean incubation time of EBOV-Z is 6.3 days for transmission by injection and 9.5 days for transmission by contact (68).

The abrupt-onset fever is followed by acute onset non-specific prodromal symptoms including chills, myalgia, malaise, weakness, headache, nausea, vomiting, diarrhoea, chest pain, sore throat and hypotension (16,68,71). Half of the patients usually develop a maculopapular rash on the trunk and shoulders that is usually noticed by days 5–7 of the disease and is often followed by erythema and desquamation (38,68,73). Early in the second week there is a dramatic worsening, with vascular involvement and coagulopathy or improvement (18,24,73). The disease is characterised by multisystemic involvement that includes respiratory, vascular, gastrointestinal, neurological and haematologic manifestations (16,68). The severe manifestations of the disease are thought to be the result of three main factors: rapid viral replication, host immune suppression and vascular dysfunction (16). Death usually occurs because of multi-organ failure and shock; metabolic disturbances occur in late stages, along with diffuse coagulopathy (38,68,73). Case-fatality rates are 60–90% with EBOV-Z and 40–60% with EBOV-S (18,68). The case-fatality rate for infection with EBOV-B was estimated to be 25% based on a single outbreak, and the only person known to become infected with EBOV-TF survived (68). No vaccines or specific antiviral therapeutics are currently available, and supportive measures are the primary method of treatment, with particular attention to hydration, cardiovascular support and supplemental oxygen (5,70).

Basic reproduction number

The basic reproduction number, R0, is used to express the average number of secondary infections that are caused by one primary infection in a completely susceptible population (74,75). For a pathogen to be able to successfully become established in a population, the value of R0 has to be over 1 (76,77). The value of R0, which varies for different infectious diseases and across populations for the same infectious disease, provides a quantitative measure of the efforts that are needed to prevent an outbreak or to eliminate it once it has started in a population (75). Using mathematical modelling, it was estimated that the basic reproduction number was 2.7 (95% CI: 1.9–2.8) for the 1995 Ebola virus outbreak from the DRC and 2.7 (95% CI: 2.5–4.1) for the 2000 outbreak from Uganda (74). This analysis found, for the two epidemics, an estimated R0 for transmission during traditional burial of 1.8 and 0.1, respectively, showing that transmission as a result of funeral practices had a more important contribution during the first outbreak and transmission in the community a more important contribution during the second outbreak. This suggested that in these two outbreaks, community, hospitals and burial ceremonies played different roles in the transmission of the virus in the population (74).

In search of the reservoir

Ebola is a zoonotic disease, and the viruses implicated in human outbreaks are thought to originate in live or dead animals (18). The circulation of the virus among humans is insufficiently understood, possibly explaining the sporadic nature of the outbreaks (7). Despite advances in understanding the molecular biology and the pathogenesis of filoviruses, identifying the reservoir from where the virus spills into humans and non-human primates, and understanding the factors that facilitate this process, remain challenging (19,38). It was proposed that either the reservoir is a rare species, or that transmission within the reservoir itself is not efficient (39).

In most Ebola virus outbreaks, the source of the infection in the index case has not been determined. Examples are the outbreaks from 1976, the 1995 Mekouka and 1996 Bouée outbreaks, the 1995 Kikwit outbreak, and several subsequent outbreaks from Sudan and Uganda (18). In several other human Ebola virus outbreaks, such as certain outbreaks from Gabon and the DRC, human infections occurred concomitantly with an increased mortality among non-human primates, such as gorillas and chimpanzees (42,78). In 1994, an ethnologist became infected in Côte D'Ivoire after performing an autopsy on a chimpanzee that died during an outbreak in the Taï National Park (42,47). In a 1994 outbreak that affected gold-diggers from Minkebé, Gabon, which was initially confused with yellow fever, people killed a sick gorilla for food (42,48). Prior to the 1996 Mayibout outbreak in Gabon, children found and butchered a chimpanzee carcass in the forest, and in several subsequent outbreaks it was documented that prior to developing disease, people handled animal

carcasses that they found (18,47). Since non-human primates are also susceptible to the infection, they are considered to be intermediate hosts (79).

Several studies attempted to identify the natural reservoir of the filoviruses. Even though over 8000 vertebrates and 30,000 invertebrates have been captured and tested for the presence of the virus since the first reported Ebola haemorrhagic fever outbreaks in 1976, the reservoir for the infection remains elusive (80). Small animals, reptiles, arthropods and plants were proposed, at various times and by various authors, as likely reservoirs involved in transmitting the Ebola and Marburg viruses (24,39). Several lines of evidence point towards the possibility that fruit bats could be the natural reservoir of the Ebola virus (81), and it is conceivable that a different reservoir exists for each of the Ebola virus subtypes (39,79). One of these pieces of evidence is the observation that in some outbreaks, such as the 1995 outbreak from Kikwit (the DRC), the 2007 outbreak from Mweka (the DRC), the 2000 outbreak from Gulu (Uganda) and the 2004 outbreak from Yambio (Sudan), the hunting and eating of fruit bats was linked to the human outbreaks (42).

As a result of these observations, several studies proposed to examine evidence of Ebola virus infection in bats and in other species. A 3-month ecological investigation following the 1995 Kikwit outbreak examined 3066 vertebrates, mostly mammals, birds, reptiles and amphibians, but was unsuccessful in isolating the Ebola virus (29). During the dry season of 1979 and 1980, a study of 1664 animals representing 117 species, including over 400 bats and 500 rodents from the Democratic Republic of the Congo and Cameroon near the site of the 1976 Ebola epidemic, failed to identify the natural reservoir of the virus (82). Another study that tested 242 small mammals in three locations within the Central African Republic detected the Zaire Ebola virus in RNA in the organs of seven animals and in the DNA of one animal (83). When 33 varieties of 24 plant species and 19 vertebrate and invertebrate species were experimentally inoculated with the Ebola Zaire virus, it was found that 13 plants wilted or developed lesions on the leaves, but this was attributed to the inoculation process, and infectivity was not recovered from the tissues. However, the same study reported that fruit and insectivorous bats supported replication and circulation of high titres of virus without necessarily becoming ill. Deaths occurred only among bats that had not adapted to the diet fed in the laboratory, in the initial phase of the study. The experimental infection of fruit and insectivorous bats with EBOV-Z led to replication of the virus in these bats, and no deaths occurred among the 12 bats that were

studied before the time that they were sacrificed, 21–28 days into the experiment, demonstrating that viral replication occurred in the animals without causing illness (28).

Between 2003 and 2006, a study that collected 1390 bats representing three fruit bat species in Gabon and the Republic of Congo, from both epidemic and non-epidemic regions, detected EBOV-Z IgG in 40 specimens, with a 2.8% overall prevalence in the entire sample across all the locations (80). In January 2008, a migratory female fruit bat Accra, Ghana, that tested seropositive for EBOV-Z, was fitted with a radio transmitter, and 13 months later when it was detected, it still appeared to be healthy (84).

Between 2002 and 2003, a study conducted three trapping expeditions to areas close to where gorilla and chimpanzee carcasses had been found: one in February 2002, close to Ekata village in Gabon, and two in February and June 2003, to Mbomo village in the Republic of Congo. Among 1030 animals that were captured, there were 679 bats and 16 of these, belonging to three fruit bat species, showed serologic evidence of infection in the form of IgG specific for the Ebola virus. None of the IgG-positive bats were PCR positive, and none of 13 additional bats in which viral nucleotide sequences were amplified by polymerase chain reaction were IgG-positive, indicating that some of the PCR-positive animals had probably become recently infected and did not yet mount a detectable immune response, and pointing towards the possibility of an active infection within the population (33). Another study reported that in 276 bats captured and tested in Bangladesh between 2010 and 2011, antibodies against Ebola viruses were present in five (3.5%) fruit bats, indicating that filoviruses may exist over larger geographical areas than previously thought, and that their range extends to mainland Asia (85).

Although Ebola virus has never been isolated from bats in the wild, the detection of the virus in bats by PCR and serologic evidence indicates that they may be a reservoir for the Ebola virus (86–88). The possibility that bats are a reservoir for the virus is also supported by many studies on the Marburg virus. An ecological investigation in the Python Cave, Uganda, where a Dutch female visitor became infected with the Marburg virus in 2008, and where over 40,000 *Rousettus aegyptiacus* bats are present, revealed that of 1622 bats captured and tested between August 2008 and November 2009, 2.5% were infected with the Marburg virus, based on Q-RT-PCR on samples from the liver and the spleen, and infection was present in the lung, kidney, colon and reproductive tissues, indicating that the virus could be spread by aerosols, urine, faeces and

sexually (89). In 2009, Marburg virus was detected for the first time in the tissues, in addition to viral RNA and antibodies in the sera, in apparently healthy Egyptian fruit bats caught in the Kitaka cave in western Uganda where in 2007 an outbreak occurred among workers mining for lead and gold (90). This represented the first time when filoviral antigens were detected in naturally occurring bats (90).

Demonstrating direct transmission from putative reservoirs, such as bats, is difficult because of the nature of bat bites, which are often invisible and painless, but evidence for this route, even though circumstantial, is powerful (91–94). Bats, also known as 'flying foxes', the only known flying mammals in the world, and among the most ancient of the mammals, inhabit all continents except Antarctica, and represent almost 25% of the recognised mammal species (95–98). Bats live on average 3.5-times longer than a mammal of similar size, promoting the persistence of the virus in the host and increasing the likelihood of transmission (95,99,100). This, along with other characteristics, such as bat migration and their population structure and migration patterns, make them particularly suitable reservoirs for viruses (97). Bats host a significantly higher number of viruses per species as compared into rodents, emerging as unique sources of zoonotic infection (101). They have been implicated in several recent viral outbreaks, including the rabies viruses, the SARS coronavirus, Nipah and Hendra viruses, Rift Valley fever virus, reoviruses and lyssaviruses, and in 2008 it was reported that 85 different viruses have been isolated from or detected in bat tissues (95,97,98,100).

Routes of transmission

The transmission filoviruses to humans is another aspect about their biology that is insufficiently understood (39). Ebola virus transmission appears to be unlikely during the incubation period, and the risk increases with the duration of the illness and with the direct contact during the late stages of the infection (74). Observations on infected patients and experiments on non-human primates point towards several transmission routes, which include blood borne transmission, direct contact with infected patients or body fluids, fomites and possibly aerosolisation (102–110). During the 1976 outbreaks from Sudan and Zaire, reusing contaminated needles played an important role (36,40). In the 1995 Kikwit outbreak, contact with an ill person, exposure to body fluids, touching a deceased person and exposure during the late hospital phases emerged as risk factors (104). An analysis of the 2000–2001 outbreak

from Gulu, Uganda that used culturing and reverse-transcription polymerase chain reaction revealed that the Ebola virus is present in the saliva, semen, stool, breast milk and nasal blood (111). Contact with body fluids emerged as the strongest risk factor, but multivariate analyses suggested that having washed the clothes of a sick person, or having participated in the ritual hand washing during the funeral ceremony, did not appear to be risk factors (103).

While aerosols are not thought to represent the major transmission route during natural outbreaks, and this route has not been demonstrated in humans, it could not be ruled out in some individual cases in which direct contact could not be demonstrated, and there is evidence supporting Ebola virus transmission by this route between non-human primates (22,108,112). This transmission route is facilitated by the stability of filoviruses in aerosols (113). In cynomolgus, rhesus and African green monkeys infected with aerosolised EBOV-Z, the virus was first detected in the blood 3 days after the challenge. While the LD_{50} (median lethal dose) was very low (less than 10 plaque-forming units) and comparable viral titres were detected in the three species, a number of differences were observed. The time to death was different from one species to another but very consistent among individuals from the same species, and the petechial rash was seen on cynomolgus and rhesus monkeys, but not on African green monkeys, after the onset of fever (110). Ebola virus transmission was reported to two of three rhesus monkeys that did not come in direct contact with experimentally inoculated monkeys that were being held in the same room (102). When virus-containing droplets were administered into the respiratory tract of rhesus monkeys via inhalation, as little as 400 plaque-forming units of virus were sufficient to cause fatal disease within 4–5 days after onset of clinical signs, and with the exception of the bleeding from subcutaneous and puncture sites and the nasal discharge, the disease was clinically identical to the one that can be seen after parenteral inoculation of the virus (109). In most instances, the pathology seen after aerosol infection on non-human primates with Marburg or Ebola viruses has been reported to be the same as that seen after parenteral inoculation, the exception being EBOV-S, which caused a viral pneumonia in cynomolgus macaques after inhalation (114).

In 2009, it was reported that domestic swine from the Philippines that developed respiratory problems and an abortion disease symptom in 2008 harboured EBOV-R, the only member of the *Filoviridae* that has not yet been associated with human disease (20). Subsequently, a study that exposed 5-week-old pigs to a porcine isolate of the 2008 virus by drops into

the nostrils and throat and by subcutaneous injection revealed that the virus was able to establish subclinical infection by both routes. Without showing clinical disease, the animals were able to shed the virus via the nasopharynx and, in some instances, via the faecal route (115). In the first experimental interspecies transmission, 4-week-old piglets inoculated oronasally with EBOV-Z were able to transmit the virus to cynomolgus macaques without direct contact. Based on the experimental setting, the virus could have been be transmitted by aerosols, droplets or fomites because of droplets or, possibly, droplets generated while cleaning the rooms, and the evidence suggested that under conditions resembling the ones from the farm, pigs can transmit the infection to non-human primates (81). However, there are no documented examples of respiratory transmission of Ebola virus among humans or non-human primates, and aerosol transmission has occurred only under experimental conditions or when other factors, such as fomites or the cleaning of the rooms, could not be ruled out (81,109). Also, it is important to note that in all human outbreaks studied to date (with the exception of the current one, for which sufficient data are not yet available), the use of standard barrier nursing procedures was able to effectively halt the outbreaks in the hospital settings, and this observation practically rules out aerosol transmission as a major concern for human Ebola virus transmission (68,74,104,116,117). Moreover, there have been concerns that Ebola and Marburg viruses were weaponised in the former Soviet Union (108).

Understanding filovirus-caused infections in humans is additionally complicated by the high seroprevalence to Ebola virus that was found in apparently healthy individuals, indicating either that exposure does not always lead to overt disease, or that Ebola virus variants that are non-pathogenic or have lower pathogenicity and are antigenically similar to the pathogenic strains may exist (66,118,119). Blood collected from 24 asymptomatic individuals who were in contact with symptomatic patients during the 1996 Gabon outbreak found serologic evidence of IgM and IgG responses to Ebola antigens in 11 of them, indicating the possibility of asymptomatic infection in previously exposed individuals (119). Another study that surveyed 4295 serum samples from inhabitants in five ecologically distinct zones in the Central African Republic found that 21.3% were seropositive for Ebola virus antibodies (120). Examination of 1288 serum human samples from individuals living in Germany reported that 6.9% of the sera reacted positively with at least one of three different filoviral antigens, also pointing towards the possibility of subclinical infections (121).

The analysis of 427 serum specimens collected from hunter-gatherer and subsistence farmers from the Central African Republic found that antibodies against filoviruses were more prevalent among hunter-gatherers (37.5%) than among subsistence farmers of similar ages (13.2%) (122). A study conducted in the Central African rainforest in November 1995, at the beginning of the dry season, found a higher antibody prevalence against Ebola virus-Zaire (13.2%) among the pygmy populations from the Sangoumbe, Sakoungbou and Mogboto camps than among a group of non-pygmy villagers from the Gouga village (4%) (123). Pygmy populations practice a seminomadic lifestyle based on hunting and gathering rain forest natural resources, while non-pygmy populations village practice subsistence farming, hunting and limited fishing. These results reveal not only that filoviruses circulate, apparently without any clinical manifestations, in human populations living in forest areas in the Central Africa Republic, but also that certain lifestyles, such as hunting, are associated with higher exposure.

Cyclicity

Infectious diseases have long been remarked for their cyclical occurrence. Seasonal variations have been described since the Hippocratic times (124) and many examples for the cyclic occurrence of infectious diseases are currently known (125). For example, cholera epidemics often occur in one or two annual peaks in the spring and fall, because of the influence of temperature and precipitation on pathogen survival and transmission (125). Seasonality for measles was noticed since the pre-vaccine era and is thought to result from host aggregation during school terms (125,126). Influenza, rubella and rotavirus infections all exhibit peaks in certain seasons of the year, very consistently from 1 year to another (127). This phenomenon was explained by factors that pertain to the pathogen, such as its appearance or disappearance during certain periods of the year, to the environment, such as cyclic temperature or humidity variations, and to host behaviour, such as the crowding of susceptible individuals (127).

A more sporadic pattern of outbreaks was noticed for the Marburg virus, and a more cyclic pattern for the Ebola virus (31), suggesting that ecological factors such as rainfall, or cyclic population changes such as the cyclic turnover of a rodent, bat, or insect, might be involved. Ebola virus is considered to be one of the factors that made western gorillas become critically endangered. By some estimates, the western gorilla population has declined by 60% in the past 20–25 years and Ebola is thought to have killed, over the

past 15 years, one-third of the animals living in protected areas such as national parks (128). Ebola outbreaks in great apes have always been reported at the beginning of the dry seasons – for example, in December 1995 in Mayibout, July 1996 in Booué, July 2001 in Mekambo, December 2001 in Kelle and December 2002 in the second Kelle outbreak (129) and it was, therefore, proposed, that outbreaks are the result of multiple episodic infection of great apes from a yet unknown reservoir, represented by organisms where the virus replicates without causing disease.

An analysis that used spatially continuous satellite data to examine Ebola outbreaks between 1994 and 2002 reported that most outbreaks occurred during the dry conditions at the end of the rainy seasons, indicating the possibility that these conditions favour the transmission of the virus from a cryptic reservoir to humans (130).

In epidemiologic analyses on the Ebola outbreak from the Occidental Kasaï province of the DRC between May and November 2007, which examined ecological conditions and potential animal sources, the authors revealed that the first putative victims purchased bats to eat, from the hunters, and after reconstructing human-to-human transmission events from the beginning of the outbreak, they concluded that seasonal fruit bat migrations could be important in shaping the outbreaks (64). Migrating bats were seen to settle in the area between April and May, nesting in fruit trees and palm trees, and were hunted by local villagers and consumed as sources of protein (64).

Over time, an increase in the Ebola virus outbreaks has been described in Africa, and this is thought to result, at least in part, from the increase of human activities, including deforestation, hunting and mining, which allow increased contacts between humans and wildlife (42,86,118).

Summary

The 2014 Ebola virus outbreak, the largest and the most persistent one to date, represents the first time when the virus has spread outside Africa. Moreover, it is the first time when the outbreak poses a danger to urban, in addition to rural areas, posing difficulties for orchestrating public health responses. Among the most challenging aspects related to the biology of the Ebola virus are identifying the reservoir and understanding the routes of transmission. From observations and studies on previous outbreaks, it appears that in different outbreaks, transmission in different settings contributed to varying degrees to the overall transmission. In the absence of approved therapeutics or vaccines, supportive therapy is currently the most important aspect of disease management. Better understanding the biology of the Ebola virus, transmission from the reservoir to other hosts and to humans, and the dynamics of viral transmission in the population, will play key roles in helping design better prophylactic and therapeutic approaches.

Acknowledgements

References

1 Gatherer D. The 2014 Ebola virus disease outbreak in West Africa. *J Gen Virol* 2014; **95**: 1619–24.

2 Baize S, Pannetier D, Oestereich L et al. Emergence of Zaire Ebola virus disease in Guinea – preliminary report. *N Engl J Med* 2014; **371**: 1418–25.

3 Gostin LO, Lucey D, Phelan A. The Ebola epidemic: a global health emergency. *JAMA* 2014; **312**: 1095–6

4 World Health Organization. Ebola virus disease in Guinea. http://www.who.int/csr/don/2014_03_23_ebola/en/. March 23, 2014 (accessed August 18, 2014).

5 Briand S, Bertherat E, Cox P et al. The international Ebola emergency. *N Engl J Med* 2014; **371**: 1180–3

6 Dixon MG, Schafer IJ. Ebola viral disease outbreak – West Africa, 2014. *MMWR Morb Mortal Wkly Rep* 2014; **63**: 548–51.

7 Fauci AS. Ebola – underscoring the global disparities in health care resources. *N Engl J Med* 2014; **371**: 1084–6

8 World Health Organization. WHO Statement on the Meeting of the International Health Regulations Emergency Committee Regarding the 2014 Ebola Outbreak in West Africa, 2014. http://www.who.int/mediacentre/news/statements/2014/ebola-20140808/en/ (accessed August 18, 2014).

9 WER Team. Ebola virus disease in West Africa – the first 9 months of the epidemic and forward projections. *N Engl J Med* 2014; **371**: 1481–95.

10 Frieden TR, Damon I, Bell BP et al. Ebola 2014 – new challenges, new global response and responsibility. *N Engl J Med* 2014; **371**: 1177–80.

11 Prevention CfDCa. First imported case of Ebola diagnosed in the United States, 2014. http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/united-states-imported-case.html (accessed October 6, 2014).

12 post AP-m, http://www.promedmail.org. PRO/AH/EDR> Ebola virus disease – West Africa (184): USA (TX) first case ex Liberia, 2014. http://www.promedmail.org/direct.php?id=2819341. Archive number: 20140930.2819341 (accessed October 6, 2014).

13 BBC. Ebola outbreak: nurse infected in Spain, 2014. http://www.bbc.com/news/world-europe-29514920 (October 6, 2014).

14 Diamond D. NYC Ebola patient is ninth case in America. Here's what happened to the other eight. Forbes, 2014. October 23, 2014. http://www.forbes.com/sites/dandiamond/2014/10/23/nyc-ebola-patient-is-ninth-case-in-america-heres-what-happened-to-the-other-eight/ (accessed October 24, 2014).

15 Turner C. Ebola virus disease: an emerging threat. *Nursing* 2014; **44**: 68–9.

16 Hartman AL, Towner JS, Nichol ST. Ebola and marburg hemorrhagic fever. *Clin Lab Med* 2010; **30**: 161–77.

17 Carroll SA, Towner JS, Sealy TK, et al. Molecular evolution of viruses of the family Filoviridae based on 97 whole-genome sequences. *J Virol* 2013; **87**: 2608–16.

18 Pourrut X, Kumulungui B, Wittmann T, et al. The natural history of Ebola virus in Africa. *Microbes Infect* 2005; **7**: 1005–14.

19 Negredo A, Palacios G, Vazquez-Moron S, et al. Discovery of an Ebolavirus-like filovirus in Europe. *PLoS Pathog* 2011; **7**: e1002304.

20 Barrette RW, Metwally SA, Rowland JM, et al. Discovery of swine as a host for the Reston ebolavirus. *Science* 2009; **325**: 204–6.

21 Kuhn JH, Becker S, Ebihara H, et al. Proposal for a revised taxonomy of the family Filoviridae: classification, names of taxa and viruses, and virus abbreviations. *Arch Virol* 2010; **155**: 2083–103.

22 Nakayama E, Saijo M. Animal models for Ebola and Marburg virus infections. *Front Microbiol* 2013; **4**: 267.

23 Li YH, Chen SP. Evolutionary history of Ebola virus. *Epidemiol Infect* 2014; **142**: 1138–45.

24 Ascenzi P, Bocedi A, Heptonstall J, et al. Ebolavirus and Marburgvirus: insight the Filoviridae family. *Mol Aspects Med* 2008; **29**: 151–85.

25 Liu Y, Cocka L, Okumura A, et al. Conserved motifs within Ebola and Marburg virus VP40 proteins are important for stability, localization, and subsequent budding of virus-like particles. *J Virol* 2010; **84**: 2294–303.

26 Chan SY, Speck RF, Ma MC, Goldsmith MA. Distinct mechanisms of entry by envelope glycoproteins of Marburg and Ebola (Zaire) viruses. *J Virol* 2000; **74**: 4933–7.

27 Towner JS, Sealy TK, Khristova ML, et al. Newly discovered ebola virus associated with hemorrhagic fever outbreak in Uganda. *PLoS Pathog* 2008; **4**: e1000212.

28 Swanepoel R, Leman PA, Burt FJ, et al. Experimental inoculation of plants and animals with Ebola virus. *Emerg Infect Dis* 1996; **2**: 321–5.

29 Leirs H, Mills JN, Krebs JW, et al. Search for the Ebola virus reservoir in Kikwit, Democratic Republic of the Congo: reflections on a vertebrate collection. *J Infect Dis* 1999; **179**(Suppl. 1): S155–63.

30 Peterson AT, Carroll DS, Mills JN, Johnson KM. Potential mammalian filovirus reservoirs. *Emerg Infect Dis* 2004; **10**: 2073–81.

31 Monath TP. Ecology of Marburg and Ebola viruses: speculations and directions for future research. *J Infect Dis* 1999; **179**(Suppl. 1): S127–38.

32 Olival KJ, Hayman DT. Filoviruses in bats: current knowledge and future directions. *Viruses* 2014; **6**: 1759–88.

33 Leroy EM, Kumulungui B, Pourrut X, et al. Fruit bats as reservoirs of Ebola virus. *Nature* 2005; **438**: 575–6.

34 Towner JS, Pourrut X, Albarino CG, et al. Marburg virus infection detected in a common African bat. *PLoS ONE* 2007; **2**: e764.

35 Johnson KM, Lange JV, Webb PA, Murphy FA. Isolation and partial characterisation of a new virus causing acute haemorrhagic fever in Zaire. *Lancet* 1977; **1**: 569–71.

36 Anonymous. Ebola haemorrhagic fever in Sudan, 1976. Report of a WHO/International Study Team. *Bull World Health Organ* 1978; **56**: 247–70.

37 Leroy EM, Gonzalez JP, Baize S. Ebola and Marburg haemorrhagic fever viruses: major scientific advances, but a relatively minor public health threat for Africa. *Clin Microbiol Infect* 2011; **17**: 964–76.

38 Groseth A, Feldmann H, Strong JE. The ecology of Ebola virus. *Trends Microbiol* 2007; **15**: 408–16.

39 Feldmann H, Wahl-Jensen V, Jones SM, Stroher U. Ebola virus ecology: a continuing mystery. *Trends Microbiol* 2004; **12**: 433–7.

40 Anonymous. Ebola haemorrhagic fever in Zaire, 1976. *Bull World Health Organ* 1978; **56**: 271–93.

41 Tyagi SKS, Singla M. Clinical aspects of Ebola hemorrhagic fever: a review. *Int J Pharma Bio Sci* 2010; **1**: 1–9.

42 Muyembe-Tamfum JJ, Mulangu S, Masumu J, et al. Ebola virus outbreaks in Africa: past and present. *Onderstepoort J Vet Res* 2012; **79**: 451.

43 Formenty P, Boesch C, Wyers M, et al. Ebola virus outbreak among wild chimpanzees living in a rain forest of Cote d'Ivoire. *J Infect Dis* 1999; **179**(Suppl. 1): S120–6.

44 Formenty P, Hatz C, Le Guenno B, et al. Human infection due to Ebola virus, subtype Cote d'Ivoire: clinical and biologic presentation. *J Infect Dis* 1999; **179**(Suppl. 1): S48–53.

45 Shanahan M, So S, Compton SG, Corlett R. Fig-eating by vertebrate frugivores: a global review. *Biol Rev Camb Philos Soc* 2001; **76**: 529–72.

46 Leigh EG. *Tropical Forest Ecology: A View from Barro Colorado Island*. New York: Oxford University Press, 1999: 245.

47 Le Guenno B, Formenty P, Wyers M, et al. Isolation and partial characterisation of a new strain of Ebola virus. *Lancet* 1995; **345**: 1271–4.

48 Amblard J, Obiang P, Edzang S, et al. Identification of the Ebola virus in Gabon in 1994. *Lancet* 1997; **349**: 181–2.

49 Georges AJ, Leroy EM, Renaut AA, et al. Ebola hemorrhagic fever outbreaks in Gabon, 1994-1997: epidemiologic and health control issues. *J Infect Dis* 1999; **179**(Suppl. 1): S65–75.

50 Georges-Courbot MC, Lu CY, Lansoud-Soukate J, et al. Isolation and partial molecular characterisation of a strain of Ebola virus during a recent epidemic of viral haemorrhagic fever in Gabon. *Lancet* 1997; **349**: 181.

51 Anonymous. Ebola haemorrhagic fever. *Wkly Epidemiol Rec* 1996; **71**: 320.

52 Hall RC, Hall RC, Chapman MJ. The 1995 Kikwit Ebola outbreak: lessons hospitals and physicians can apply to future viral epidemics. *Gen Hosp Psychiatry* 2008; **30**: 446–52.

53 Khan AS, Tshioko FK, Heymann DL, et al. The reemergence of Ebola hemorrhagic fever, Democratic Republic of the Congo, 1995. Commission de Lutte contre les Epidemies a Kikwit. *J Infect Dis* 1999; **179**(Suppl. 1): S76–86.

54 De Roo A, Ado B, Rose B, et al. Survey among survivors of the 1995 Ebola epidemic in Kikwit, Democratic Republic of Congo: their feelings and experiences. *Trop Med Int Health* 1998; **3**: 883–5.

55 Tomori O, Bertolli J, Rollin PE, et al. Serologic survey among hospital and health center workers during the Ebola hemorrhagic fever outbreak in Kikwit, Democratic Republic of the Congo, 1995. *J Infect Dis* 1999; **179**(Suppl. 1): S98–101.

56 Muyembe-Tamfum JJ, Kipasa M, Kiyungu C, Colebunders R. Ebola outbreak in Kikwit, Democratic Republic of the Congo: discovery and control measures. *J Infect Dis* 1999; **179**(Suppl. 1): S259–62.

57 Hensley LE, Mulangu S, Asiedu C, et al. Demonstration of cross-protective vaccine immunity against an emerging pathogenic Ebolavirus species. *PLoS Pathog* 2010; **6**: e1000904.

58 Okware SI, Omaswa FG, Zaramba S, et al. An outbreak of Ebola in Uganda. *Trop Med Int Health* 2002; **7**: 1068–75.

59 Anonymous. Outbreak(s) of Ebola haemorrhagic fever, Congo and Gabon, October 2001-July 2002. *Wkly Epidemiol Rec* 2003; **78**: 223–8.

60 Formenty P, Libama F, Epelboin A et al. Outbreak of Ebola hemorrhagic fever in the Republic of the Congo, 2003: a new strategy? *Med Trop* 2003; **63**: 291–5.

61 Boumandouki P, Formenty P, Epelboin A, et al. Clinical management of patients and deceased during the Ebola outbreak from October to December 2003 in Republic of Congo. *Bull Soc Pathol Exot* 1990; **2005**(98): 218–23.

62 Anonymous. Outbreak of Ebola haemorrhagic fever in Yambio, south Sudan, April – June 2004. *Wkly Epidemiol Rec* 2005; **80**: 370–5.

63 Onyango CO, Opoka ML, Ksiazek TG, et al. Laboratory diagnosis of Ebola hemorrhagic fever during an outbreak in Yambio, Sudan, 2004. *J Infect Dis* 2007; **196**(Suppl. 2): S193–8.

64 Leroy EM, Epelboin A, Mondonge V et al. Human Ebola outbreak resulting from direct exposure to fruit bats in Luebo, Democratic Republic of Congo, 2007. *Vector Borne Zoonotic Dis* 2009; **9**: 723–8.

65 MacNeil A, Farnon EC, Morgan OW, et al. Filovirus outbreak detection and surveillance: lessons from Bundibugyo. *J Infect Dis* 2011; **204**(Suppl. 3): S761–7.

66 Albarino CG, Shoemaker T, Khristova ML, et al. Genomic analysis of filoviruses associated with four viral hemorrhagic fever outbreaks in Uganda and the Democratic Republic of the Congo in 2012. *Virology* 2013; **442**: 97–100.

67 MacNeil A, Rollin PE. Ebola and Marburg hemorrhagic fevers: neglected tropical diseases? *PLoS Negl Trop Dis* 2012; **6**: e1546.

68 Feldmann H, Geisbert TW. Ebola haemorrhagic fever. *Lancet* 2011; **377**: 849–62.

69 Wamala JF, Lukwago L, Malimbo M, et al. Ebola hemorrhagic fever associated with novel virus strain, Uganda, 2007-2008. *Emerg Infect Dis* 2010; **16**: 1087–92.

70 Casillas AM, Nyamathi AM, Sosa A, et al. A current review of Ebola virus: pathogenesis, clinical presentation, and diagnostic assessment. *Biol Res Nurs* 2003; **4**: 268–75.

71 Jeffs B. A clinical guide to viral haemorrhagic fevers: Ebola, Marburg and Lassa. *Trop Doct* 2006; **36**: 1–4.

72 Eichner M, Dowell SF, Firese N. Incubation period of ebola hemorrhagic virus subtype zaire. *Osong Public Health Res perspect* 2011; **2**: 3–7.

73 Bente D, Gren J, Strong JE, Feldmann H. Disease modeling for Ebola and Marburg viruses. *Dis Models Mech* 2009; **2**: 12–7.

74 Legrand J, Grais RF, Boelle PY, et al. Understanding the dynamics of Ebola epidemics. *Epidemiol Infect* 2007; **135**: 610–21.

75 Dietz K. The estimation of the basic reproduction number for infectious diseases. *Stat Methods Med Res* 1993; **2**: 23–41.

76 May RM, Gupta S, McLean AR. Infectious disease dynamics: what characterizes a successful invader? *Philos Trans R Soc Lond B Biol Sci* 2001; **356**: 901–10.

77 Heffernan JM, Smith RJ, Wahl LM. Perspectives on the basic reproductive ratio. *J Royal Soc Interface* 2005; **2**: 281–93.

78 Rouquet P, Froment JM, Bermejo M, et al. Wild animal mortality monitoring and human Ebola outbreaks, Gabon and Republic of Congo, 2001-2003. *Emerg Infect Dis* 2005; **11**: 283–90.

79 Georges AJ, Baize S, Leroy EM. Georges-Courbot MC. Ebola virus: what the practitioner needs to know. *Med Trop* 1998; **58**: 177–86.

80 Pourrut X, Delicat A, Rollin PE, et al. Spatial and temporal patterns of Zaire ebolavirus antibody prevalence in the possible reservoir bat species. *J Infect Dis* 2007; **196**(Suppl. 2): S176–83.

81 Weingartl HM, Embury-Hyatt C, Nfon C, et al. Transmission of Ebola virus from pigs to non-human primates. *Sci Rep* 2012; **2**: 811.

82 Breman JG, Johnson KM, van der Groen G, et al. A search for Ebola virus in animals in the Democratic Republic of the Congo and Cameroon: ecologic, virologic, and serologic surveys, 1979-1980. Ebola Virus Study Teams. *J Infect Dis* 1999; **179** (Suppl. 1): S139–47.

83 Morvan JM, Deubel V, Gounon P, et al. Identification of Ebola virus sequences present as RNA or DNA in organs of terrestrial small mammals of the Central African Republic. *Microbes Infect* 1999; **1**: 1193–201.

84 Hayman DT, Emmerich P, Yu M, et al. Long-term survival of an urban fruit bat seropositive for Ebola and Lagos bat viruses. *PLoS ONE* 2010; **5**: e11978.

85 Olival KJ, Islam A, Yu M, et al. Ebola virus antibodies in fruit bats, Bangladesh. *Emerg Infect Dis* 2013; **19**: 270–3.

86 Bausch DG, Schwarz L. Outbreak of ebola virus disease in Guinea: where ecology meets economy. *PLoS Negl Trop Dis* 2014; **8**: e3056.

87 Valmas C, Grosch MN, Schumann M, et al. Marburg virus evades interferon responses by a mechanism distinct from ebola virus. *PLoS Pathog* 2010; **6**: e1000721.

88 Takada A. Filovirus tropism: cellular molecules for viral entry. *Front Microbiol* 2012; **3**: 34.

89 Amman BR, Carroll SA, Reed ZD, et al. Seasonal pulses of Marburg virus circulation in juvenile *Rousettus aegyptiacus* bats coincide with periods of increased risk of human infection. *PLoS Pathog* 2012; **8**: e1002877.

90 Towner JS, Amman BR, Sealy TK, et al. Isolation of genetically diverse Marburg viruses from Egyptian fruit bats. *PLoS Pathog* 2009; **5**: e1000536.

91 Feder HM Jr, Nelson R, Reiher HW. Bat bite? *Lancet* 1997; **350**: 1300.

92 Johnson N, Arechiga-Ceballos N, Aguilar-Setien A. Vampire bat rabies: ecology, epidemiology and control. *Viruses* 2014; **6**: 1911–28.

93 Oczkowski S. The bitten man: reflections on international health. *Open Med* 2007; **1**: e160–3.

94 Edson RS, Bundrick JB, Litin SC. Clinical pearls in infectious diseases. *Mayo Clin Proc* 2011; **86**: 245–8.

95 Smith I, Wang LF. Bats and their virome: an important source of emerging viruses capable of infecting humans. *Curr Opin Virol* 2013; **3**: 84–91.

96 Omatsu T, Watanabe S, Akashi H, Yoshikawa Y. Biological characters of bats in relation to natural reservoir of emerging viruses. *Comp Immunol Microbiol Infect Dis* 2007; **30**: 357–74.

97 Calisher CH, Childs JE, Field HE, et al. Bats: important reservoir hosts of emerging viruses. *Clin Microbiol Rev* 2006; **19**: 531–45.

98 Calisher CH, Holmes KV, Dominguez SR, Schountz T, Cryan P. Bats prove to be rich reservoirs for emerging viruses. *Microbe* 2008; **3**: 521–8.

99 Picard-Meyer E, Robardet E, Arthur L, et al. Bat rabies in France: a 24-year retrospective epidemiological study. *PLoS ONE* 2014; **9**: e98622.

100 Hayward JA, Tachedjian M, Cui J, et al. Identification of diverse full-length endogenous betaretroviruses in megabats and microbats. *Retrovirology* 2013; **10**: 35.

101 Luis AD, Hayman DT, O'Shea TJ, et al. A comparison of bats and rodents as reservoirs of zoonotic viruses: are bats special? *Proc Biol Sci* 2013; **280**: 20122753.

102 Jaax N, Jahrling P, Geisbert T, et al. Transmission of Ebola virus (Zaire strain) to uninfected control monkeys in a biocontainment laboratory. *Lancet* 1995; **346**: 1669–71.

103 Francesconi P, Yoti Z, Declich S, et al. Ebola hemorrhagic fever transmission and risk factors of contacts, Uganda. *Emerg Infect Dis* 2003; **9**: 1430–7.

104 Dowell SF, Mukunu R, Ksiazek TG, et al. Transmission of Ebola hemorrhagic fever: a study of risk factors in family members, Kikwit, Democratic Republic of the Congo, 1995. Commission de Lutte contre les Epidemies a Kikwit. *J Infect Dis* 1999; **179**(Suppl. 1): S87–91.

105 Baron RC, McCormick JB, Zubeir OA. Ebola virus disease in southern Sudan: hospital dissemination and intrafamilial spread. *Bull World Health Organ* 1983; **61**: 997–1003.

106 Roels TH, Bloom AS, Buffington 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): S92–7.

107 Simonsen L, Kane A, Lloyd J, et al. Unsafe injections in the developing world and transmission of bloodborne pathogens: a review. *Bull World Health Organ* 1999; **77**: 789–800.

108 Leffel EK, Reed DS. Marburg and Ebola viruses as aerosol threats. *Biosecur Bioterr* 2004; **2**: 186–91.

109 Johnson E, Jaax N, White J, Jahrling P. Lethal experimental infections of rhesus monkeys by aerosolized Ebola virus. *Int J Exp Pathol* 1995; **76**: 227–36.

110 Reed DS, Lackemeyer MG, Garza NL, et al. Aerosol exposure to Zaire ebolavirus in three nonhuman primate species: differences in disease course and clinical pathology. *Microbes Infect* 2011; **13**: 930–6.

111 Bausch DG, Towner JS, Dowell SF, et al. Assessment of the risk of Ebola virus transmission from bodily fluids and fomites. *J Infect Dis* 2007; **196** (Suppl. 2): S142–7.

112 Twenhafel NA, Mattix ME, Johnson JC, et al. Pathology of experimental aerosol Zaire ebolavirus infection in rhesus macaques. *Vet Pathol* 2013; **50**: 514–29.

113 Reed DS, Mohamadzadeh M. Status and challenges of filovirus vaccines. *Vaccine* 2007; **25**: 1923–34.

114 Herbert AS, Kuehne AI, Barth JF, et al. Venezuelan equine encephalitis virus replicon particle vaccine protects nonhuman primates from intramuscular and aerosol challenge with ebolavirus. *J Virol* 2013; **87**: 4952–64.

115 Marsh GA, Haining J, Robinson R, et al. Ebola Reston virus infection of pigs: clinical significance and transmission potential. *J Infect Dis* 2011; **204** (Suppl. 3): S804–9.

116 Towner JS, Rollin PE, Bausch DG, 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**: 4330–41.

117 Lloyd ES, Zaki SR, Rollin PE, et al. Long-term disease surveillance in Bandundu region, Democratic Republic of the Congo: a model for early detection and prevention of Ebola hemorrhagic fever. *J Infect Dis* 1999; **179**(Suppl. 1): S274–80.

118 Changula K, Kajihara M, Mweene AS, Takada A. Ebola and Marburg virus diseases in Africa: increased risk of outbreaks in previously unaffected areas? *Microbiol Immunol* 2014; **58**: 483–91.

119 Leroy EM, Baize S, Volchkov VE, et al. Human asymptomatic Ebola infection and strong inflammatory response. *Lancet* 2000; **355**: 2210–5.

120 Johnson ED, Gonzalez JP, Georges A. Haemorrhagic fever virus activity in equatorial Africa: distribution and prevalence of filovirus reactive antibody in the Central African Republic. *Trans R Soc Trop Med Hyg* 1993; **87**: 530–5.

121 Becker S, Feldmann H, Will C, Slenczka W. Evidence for occurrence of filovirus antibodies in humans and imported monkeys: do subclinical filovirus infections occur worldwide? *Med Microbiol Immunol* 1992; **181**: 43–55.

122 Johnson ED, Gonzalez JP, Georges A. Filovirus activity among selected ethnic groups inhabiting the tropical forest of equatorial Africa. *Trans R Soc Trop Med Hyg* 1993; **87**: 536–8.

123 Gonzalez JP, Nakoune E, Slenczka W, et al. Ebola and Marburg virus antibody prevalence in selected populations of the Central African Republic. *Microbes Infect* 2000; **2**: 39–44.

124 Fisman DN. Seasonality of infectious diseases. *Annu Rev Public Health* 2007; **28**: 127–43.

125 Altizer S, Dobson A, Hosseini P, et al. Seasonality and the dynamics of infectious diseases. *Ecol Lett* 2006; **9**: 467–84.

126 Anonymous. Measles–United States, 1992. *MMWR Morb Mortal Wkly Rep* 1993; **42**: 378–81.

127 Dowell SF. Seasonal variation in host susceptibility and cycles of certain infectious diseases. *Emerg Infect Dis* 2001; **7**: 369–74.

128 Vogel G. Conservation. Scientists say Ebola has pushed western gorillas to the brink. *Science* 2007; **317**: 1484.

129 Leroy EM, Rouquet P, Formenty P, et al. Multiple Ebola virus transmission events and rapid decline of central African wildlife. *Science* 2004; **303**: 387–90.

130 Pinzon JE, Wilson JM, Tucker CJ, et al. Trigger events: enviroclimatic coupling of Ebola hemorrhagic fever outbreaks. *Am J Trop Med Hyg* 2004; **71**: 664–74.