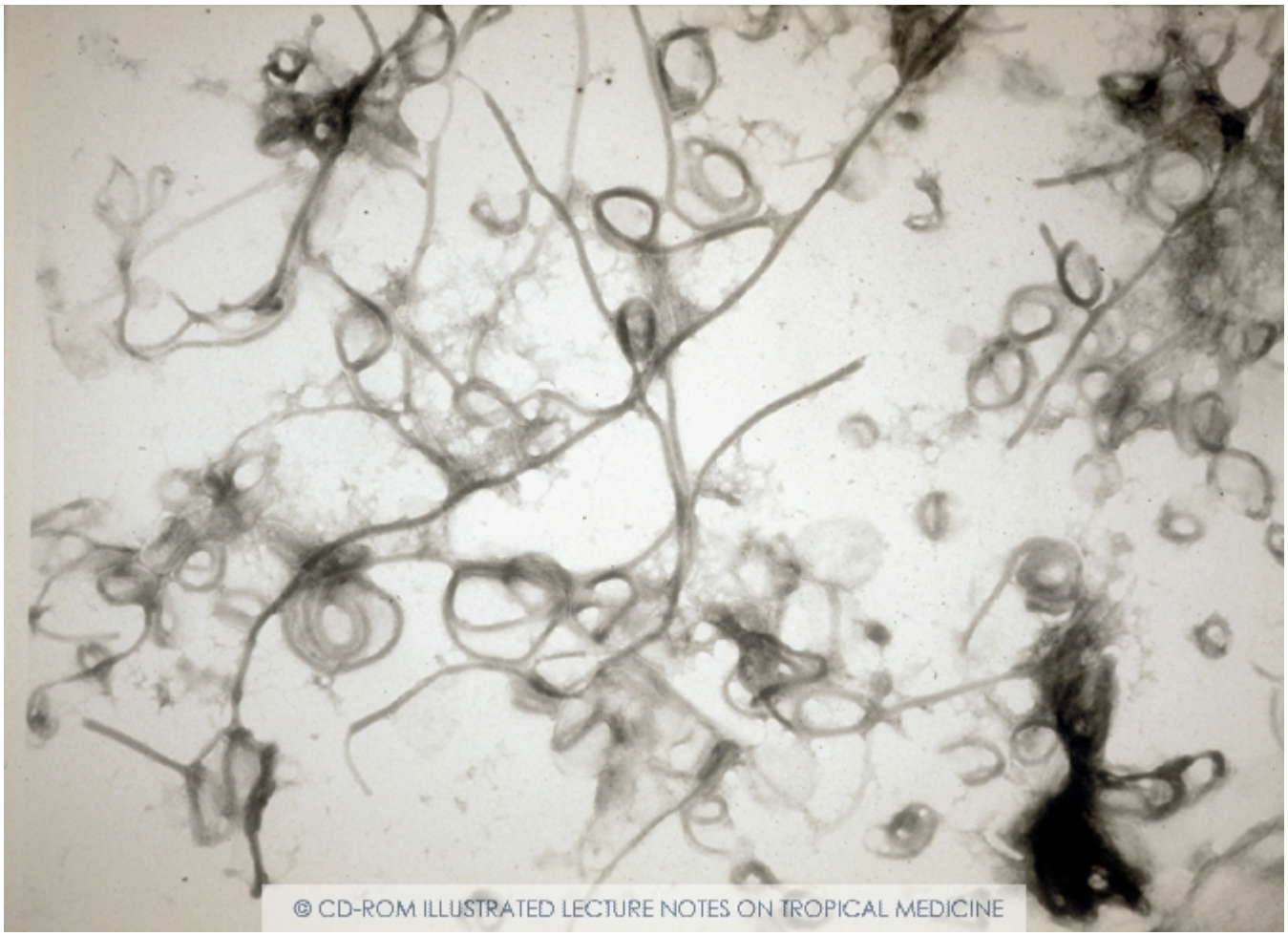
General

Ebola virus is a member of the Filoviridae family (Mononegavirales order). It is a enveloped filamentous particle with a non-segmented, negative-sense RNA genome. The viral spike on the viral envelope is formed by the sole trimeric transmembrane glycoprotein and mediates viral entry; this spike is a target for the host immune response and for vaccine development. EBOV or Ebola virus refers to the Zaire ebolavirus in the genus ebolavirus. The other known species within the genus are Bundibugyo ebolavirus (Bundibugyo virus), Reston ebolavirus (Reston virus),Sudan ebolavirus (Sudan virus), Taï Forest ebolavirus (Taï Forest virus), and Bombali virus. Only Bundibugyo, Sudan, and Ebola viruses have been associated with disease outbreaks in humans. Ebola virus disease (EVD) refers to a disease caused by four of five viruses of the genus Ebolavirus: BDBV, SUDV, TAFV and EBOV.



Ebola virus, Electron microscopy, copyright ITM, with special thanks to Guido Van der Groen

Ebola-Zaire (EBOV) and Ebola-Sudan (SUDV)

In 1976 there was a sudden large-scale epidemic of 2 different Ebola viruses in Maridi (South Sudan) and in Yambuku, on the Ebola river in North Congo. The mortality rate in Yambuku was very high (280 deaths out of 318 cases = 88%) and slightly lower in Sudan (53%). In 1977 there was one fatal case in Tandala, North Congo. New major outbreaks occurred in 1979 in Nzara (South Sudan), in 1995 in Kikwit, Congo and in 2003 in Kelle, Congo Brazzaville. The virus, which emerged in Kikwit, very closely resembled that in Yambuku (less than 1.6% difference in RNA). This is a sign of a genome, which is not under selection pressure, suggesting a stable ecological niche between epidemics. The Sudanese virus isolates of 1976 and 1979 were also almost identical.

From 1994 till 2012, several small epidemics occurred with the number of infections never exceeding 500, with the case fatality rate varying between 41 and 100% (100% was 4 times due to a single case) (see table below). During an outbreak in October 1996, an infected doctor was flown over to South Africa and there caused a fatal secondary case in a nurse. This illustrates how easily pathogenic organisms can be spread in this age of long-distance transport. Early in 2003, a large-scale epidemic occurred in Mbomo and Kelle, a very remote and rural area of Congo Brazzaville, just south of Odzala National Park. It started by a large-scale die-off among the lowland gorillas in the park. The disease flared up again in the same area, in November the same year, but was contained before New Year 2004.

Ebola outbreak in West-Africa

On 14 March 2014, rumours of a ‘mysterious disease’ were reported by the Ministry of Health in Guinea. Several health staff taking care of the sick had died and mortality was very high. Suspicion of Lassa viral haemorrhagic fever rose, but what jumped out were the hiccups, a typical symptom associated with Ebola. 28 March 2014, the World Health Organization was notified of an outbreak of a communicable disease characterized by fever, severe diarrhoea, vomiting, and a high fatality rate in Guinea. Virologic investigation identified *Zaire ebolavirus* (EBOV) as the causative agent. Full-length genome sequencing and phylogenetic analysis showed that EBOV from Guinea forms a separate clade in relationship to the known EBOV strains from the Democratic Republic of Congo and Gabon.

the suspected first case of the outbreak was a 2-year-old child who died in Meliandou in Gu Gundou prefecture on December 6, 2013. A health care worker from Guom Gu with suspected disease, seems to have triggered the spread of the virus to Macenta, Nzcenta, and Kissidougou in February 2014. The initial case fatality rate was 86% (12/14 patients). What followed was an unprecedented outbreak going from bad to worse. Ebola had been stealthily spreading undetected for more than three months. It is not unusual for Ebola to go undiagnosed for a substantial period of time; the past eight Ebola outbreaks each took two months on average to be discovered and investigated. Ebola's symptoms are easily confused with other diseases, such as cholera and malaria, and experts trained to recognise it are rare. However, past outbreaks took place mostly in remote villages in central and eastern Africa, where they were more easily contained. In a twist of geographic fate, Ebola erupted at the junction of Guinea, Liberia and Sierra Leone, where people regularly move across the porous borders. Fear and suspicion of the unknown virus, unsafe burial practices, mistrust in politicians, the hiding of cases, and a weak public health system, which lacked the resources to recognise and efficiently respond to Ebola, all contributed to the virus surging through the region. For months, the epidemic spread faster than the international community's response. The Ebola virus was introduced into Nigeria on 20 July 2014 when an infected Liberian man arrived by airplane into Lagos, Africa's most populous city. The man, who died in hospital 5 days later, set off a chain of transmission that infected a total of 19 people, of whom 7 died.

On August 8 2014, the WHO declared the epidemic to be an emergency of international concern. In Mali 8 people were infected of whom 6 died and 1 case was detected in Senegal. On 6 October 2014, the World Health Organization (WHO) was informed of the first confirmed autochthonous case of Ebola virus disease in Spain. This case represents the first human-to-human transmission of EVD outside Africa. The case is a female healthcare worker with no travel history to West Africa but who participated in the medical care of an EVD case in a Spanish citizen, who had been infected in Sierra Leone and evacuated to Madrid, Spain on 22 September 2014 and who died on 25 September 2014. She was in contact with the repatriated EVD case twice; on 24 and 25 September 2014. On both occasions she is reported to have worn appropriate personal protection equipment (PPE). Following the Spanish national protocol for EVD cases, the healthcare worker was considered a low risk contact and monitored accordingly. The female case developed a fever on 29 September 2014 and was admitted into isolation on 6 October 2014 where she tested positive for Ebola.

In total 28.652 Ebola cases are recorded during the 2013-2016 epidemic with a death toll of 11.325. Ebola has destroyed lives and families, left deep scars, and ripped at the social and economic fabric of Guinea, Liberia and Sierra Leone. The virus cut a vast swathe through the three countries, in a cross-border geographical spread never seen before. Fear and panic set in, the sick and their families were desperate, and national health workers and MSF teams were overwhelmed and exhausted. Medical workers are not trained to deal with at least 50 percent of their patients dying from a disease for which no treatments exist. Nevertheless, the world at first ignored the calls for help and then belatedly decided to act. Meanwhile, months were wasted and lives were lost. No one knows the true number of deaths the epidemic will have ultimately caused. Across the three countries, local healthcare workers were tragically dying by the dozens. In Ebola outbreaks, health facilities without proper infection control often act as multiplying chambers for the virus, become dangerous places for both health workers and patients. This outbreak was no different, but it happened on a massive scale. The resulting collapse of health services means that untreated malaria, complicated deliveries and car crashes will have multiplied the direct Ebola deaths many times over. Why was the world so slow to wake up to its severity and respond? Was it due to fear, lack of political will, lack of expertise, or a perfect storm of all three?

See also: https://www.who.int/features/ebola/storymap/en/

Country	Total Cases (Suspected, Probable, and Confirmed)	Laboratory-Confirmed Cases	Total Deaths
Guinea [2]	3814	3358	2544
Sierra Leone [3]	14124	8706	3956
Liberia [4]	10678	3163	4810
Total	**28616**	**15227**	**11310**

Ebola cases in 3 countries with widespread transmission during the 2013-2016 epidemic. Source: CDC

Country	Total Cases (Suspected, Probable, and Confirmed)	Laboratory-Confirmed Cases	Total Deaths
Nigeria	20	19	8
Senegal	1	1	0
Spain	1	1	0
United States	4	4	1
Mali	8	7	6
United Kingdom	1	1	0
Italy	1	1	0
Total	**36**	**34**	**15**

Countries with lower case load during the 2013-2016 epidemic. Source: CDC

Ebola outside Africa

During the West-African outbreak, 17 persons with EVD disease have been cared for outside Africa of which three persons have contracted Ebola outside Africa. In the United States eleven cases of EVD have been reported: nine of them contracted the disease outside the US and travelled into the country, either as regular airline passengers or as medical evacuees; of those nine, two died. Two nurses have contracted Ebola in the United States, both treating an Ebola patient; both have recovered. Of the eleven cases, four have been diagnosed within the US: the two above mentioned nurses and two travellers that became ill in the US.
Only 6 cases of Ebola have been diagnosed in Europe, all in connection with the Ebola outbreak in West Africa: one in Italy, one in Spain and three in the United Kingdom and one locally acquired in a health care worker in caring for an evacuated Ebola patient in Spain.

In August 2018, the Democratic Republic of Congo MOH tested 4 individuals positive for the Ebola virus in North Kivu. In this war-ravaged province in which there is mistrust of the

government and mistrust of the Ebola response, the outbreak became the second largest ever recorded with a total of 3406 cases (3262 confirmed and 144 probable) and 2243 deaths, corresponding with a mortality rate of 65.9% which is significantly higher than in the West-African epidemic (39.5%).

Ebola Ivory Coast (Thaï Forest virus, TAFV)

In 1994 many chimpanzees died following an Ebola epidemic in the Tai nature reserve in Côte d'Ivoire on the border with Liberia. Here one person was infected during an autopsy on a chimpanzee that had died. She was evacuated to Switzerland where she was treated. The causative agent turned out to be a new genetic subtype of Ebola virus. In late 1995 another (unconfirmed) case occurred in the same area (Plibo) in a Liberian refugee.

Reston virus (RESTV)

In 1989 an epidemic of another Ebola virus occurred in a primate centre in the USA in Reston, a town near Washington D.C. A number of people were infected but without any illness both in Reston (4) and in the Philippines (12) where the monkeys came from. Unlike the case of Ebola-Zaire, there were arguments here for aerogenic transmission. Research was complicated by the fact that another haemorrhagic fever virus epidemic was taking place at the same time among the monkeys (Simian Haemorrhagic Fever Virus). Late 2008 a Philippino farm worker was found infected by the Ebola-Reston virus that was discovered in pigs at 2 farms north of Manila. It was the 1st time Ebola-Reston was found outside monkeys. The infected man had not shown any symptoms and was healthy. Later 5 more persons were found to have been infected, all were asymptomatic. RESTV sequences have been found in Chines pigs, raising fear about food safety.

Bundibudyo virus (BDBV)

Bundibudyo was the region in Uganda were the 2007 Ebola epidemic was centred. The epidemic in Uganda was caused by a fifth viral species. The genome differs by about 32% of its nucleotides, compared with the other Ebola strains. This may complicate efforts to produce a universal vaccine. Fifty-six cases of Bundibugyo Ebola virus infection were

laboratory confirmed during the first epidemic. Signs and symptoms were largely nonspecific. The proportion of deaths among those infected was about 40%. A new outbreak occurred in August and September 2012, centred on Isiro and Viadana, Haut-Uele district.

Bombali virus (BOMV)

In 2018 a new ebolavirus – Bombali virus was detected in free-tailed bats in Sierra Leone: little free-tailed (*Chaerephon pumilus*) and Angolan free-tailed (*Mops condylurus*) bats. The bats were found resting inside houses but it is not known whether human exposure has occurred or if BOMV is pathogenic in humans.

Summary of known human Ebola Disease cases

1972	1 non-fatal case (retrospective diagnosis)	Tandala, DRC (not confirmed)
1976	318 cases, 280 deaths	Yambuku, DRC (discovery of the virus)
1976	284 cases, 151 deaths	Nzara, Maridi, Tembura and Juba, Sudan
1977	1 fatal case	Tandala, DRC
1979	34 cases with 22 deaths	Nzara and Yambio, Sudan
1980	1 suspected case	Kenya (not confirmed)
1994	44 cases, 28 deaths	Minkouka, Gabon
1994	1 non-fatal case	Tai Park, Côte d'Ivoire
1995	315 cases, 255 deaths	Kikwit, DRC
1996	1 non-fatal case	Plibo, Liberia (not confirmed)
1996	37 cases with 21 deaths	Mayibout and Makokou, Gabon
1996	60 cases with 45 deaths	Booué, Gabon. One exported case in South Africa with one fatal secondary case.
2000	425 cases with 224 deaths	Gulu, Masindi, Mbarara (Uganda)
2002	43 deaths in Congo, 53 deaths in Gabon	Gabon – DRC

2002	No reliable numbers available	Mbomo, DRC
2003	About 140 cases with about 120 deaths (February-March). Flare-up in November-December, with 35 cases (29 deaths).	Mbomo, DRC
2004	25 cases with 6 deaths	Mbomo and Mbandza, Congo Brazzaville
2005	About 10 cases	Etoumbi, DRC
2007	About 187 cases About > 90 cases New epidemic in Congo, lasting till early 2009. Number of cases unclear In March 2009, accidental needle stick injury in Hamburg (virologist) Isolated case (May 2011) in Uganda Number of cases unclear 66 cases, 35 deaths	Kampungu, Mweka, Luebo, DRC (Western Kasai) Western Uganda November 2009, outbreak in Mweka, DRC Germany, the first time that vesicular stomatitis virus-based vaccine is used in a human (post-exposure) July 2012, outbreak in Kibaale, Uganda and quasi simultaneous in August 2012 outbreak is Isiro and Viadana, Haut-Uele, Congo
2008		
2008-2009 2009 2011 2012		
2013-2016	28.652 cases with 11.325 deaths	Guinea, Sierra Leone, Liberia, Nigeria, Malia, Senegal, USA, Spain
2014	66 cases, 49 deaths	Équateur province, DRC
2017	8 cases, 4 deaths	Likati, DRC
2018	54 cases, 33 deaths	Bikoko, Mbandaka, DRC
2018-2020	3470 cases, 2287 deaths	North-Kivu and Ituri province, DRC

Epidemiologic and ecologic features



Ebola epidemic in Kikwit, Congo 1995. Small animal trapping and study, as a part of the search for the reservoir of this virus. Notice the protective gear of the researchers. Copyright ITM

Today, neither EBOV nor other filoviruses are endemic anywhere, but the discovery of persistent virus in humans after infection during the 2013-2016 epidemic, indicates that the virus can temporarily circulate in persons. The natural reservoir of these viruses remains unconfirmed, nevertheless bats are the prime suspects. To date the analysis of the numerous arthropods and living vertebrates has not produced a single positive viral isolate, although Ebola virus was demonstrated in several carcasses in the Central African rainforest, esp. primates. Analysis of 98 animal carcasses in Gabon – Congo (study period 2001-2003) showed on 10 Ebola-positive gorillas out of a 50 gorilla carcasses, 3 positive chimpanzees out of 15, and 1 positive duiker (Cephalophus) out of 14. The monkey species, which have been studied thus far, all die from the infection and therefore cannot form the natural reservoir.

Filoviruses are considered regionally epizootic. Contact with infected monkeys plays a role in the beginning of an epidemic but how these animals are initially infected is not known. The epidemic, which started in November 2003 in Mbomo, Congo Brazzaville, was rumoured to have started after villagers found a dead wild pig in the forest and ate its meat. This would be the first case that such an animal would be implicated. Certain fructivorous and insectivorous bats can be experimentally infected and certain species are seropositive in nature. In 3 bat species (*Epomops franqueti, Hypsignathus monstrosus* and *Myonycteris torquata*) Ebola RNA sequences have been detected. These animals usually develop an asymptomatic infection.To date, ebolaviruses surprisingly were never isolated in a bat, which might be explained by low viral loads or inhibitors in bat tissue. Epidemics may start after spillover events from bats to humans and other mammals that serve as end-, intermediate- or amplifying hosts. These animals are often shot and eaten as “bush meat”. The Zaire strain of Ebola virus can also replicate in pigs. Infected animals develop severe lung disease. They shed large numbers of virions in the respiratory tract. Shedding continues for up to 2 weeks after infection. Infected animals can transmit the infection to non-infected pigs and possibly to humans.

Pathophysiology

Transmission takes place through direct contact with infected body fluids (including sexual contact) and nosocomial through infected needles and contact with infected blood. Sexual transmission is described up to 6 months after survival. Aerogenic transmission of Ebola has been demonstrated in the laboratory in Rhesus monkeys, thought this is never described in humans. Viral particles land on mucous membranes or occasionally enter percutaneously. Filoviruses replicate in the cytoplasm of their target cells, which are initially dendritic cells and macrophages and potently shut down early innate immune responses by blocking interferon production. Later, dendritic cells migrate to lymphoid tissues and the virus is released in the circulation with spread to the liver, spleen and other tissues. Disease is caused by the cytopathogenic effects of the virus itself leading to cell lysis, but also by an exaggerated host immune response inducing a cytokine storm causing a septic shock. Several cytokines (IL-1ß, IL-6 and TNF) and chemokines cause T-cell activation, which is rendered ineffective in severe or fatal cases due to T-cell exhaustion followed by an impaired adaptive immune response. Endothelial-cell dysfunction is caused by inflammatory mediators triggering vascular permeability and fluid extravasation. Tissue factor is produced by infected macrophages and lead to fibrin deposition in the spleen, lymphoid tissues, glomeruli and

renal proximal tubules. Diffuse intravascular coagulation arises due to consumption of clotting factors, endothelial dysfunction and platelet dysfunction with coagulopathy and bleeding as a consequence. Multiple organ failure (MOF) with tissue hypoperfusion develops due to microvascular anomalies and hypovolemia due to gastro-intestinal fluid losses. Bacterial translocation can be a consequence of the disrupted gut mucosa triggering bacteraemia and bacterial septic shock.
Fatal cases are associated with defective immune responses and high viremia. Survivors have early and vigorous cellular as well as humoral immune responses. The immunological course early in the infection determines how quickly the Ebola virus replicates and whether the host will die or recover. Surviving an infection is linked to an early appearance of IgM and IgG, followed by the activation of cytotoxic cells.

Clinical aspects



Patient with Ebola haemorrhagic fever with bleeding at injection sites. Photo Dr Van den Enden, Copyright ITM



Ebola, 2003, Kelle, Congo. Patient presenting with bleeding gums, a sign of haemorrhagic diathesis. Photo Dr Erwin Van den Enden, Copyright ITM

The clinical disease is not called Ebola haemorrhagic fever anymore but Ebola virus disease (EVD) which downplays bleeding as a clinical hallmark and stresses the great variability in symptoms. After an incubation period of 2 to 21 days (average 7 days) infection often leads to multiple organ failure, with death occurring on average 6 to 9 days after the onset of symptoms. But asymptomatic infection with Ebola can occur. People infected with Ebola virus initially present with nonspecific febrile illness with malaise, fatigue and myalgia. In a second stage, gastro-intestinal symptoms with anorexia, nausea, abdominal pain, vomiting and diarrhoea develop. Patient can lose up to 10 liters per day and severe electrolyte

disturbances rise: hypokalaemia, hyponatremia, hypomagnesemia, … Dysphagia, headache, conjunctival injections, maculopapular rash and joint pain are other common symptoms. Hiccups can be caused by uncontrolled diaphragm contractions due to viral invasion of the CNS that controls the diaphragm. Hiccups were a clue that led researchers to suspect that the West-African epidemic was not caused by Lassa virus but possibly by Ebola virus. One should not focus too much on bleeding as a presenting symptom as this is a late symptom and cases will be missed. Even in end-stage disease patients, bleeding abnormalities occur in less than half of them. Bleeding from gums, petechiae, persistent oozing from venepuncture sites, subconjunctival haemorrhage, haematemesis and bloody diarrhoea can be present. Hepatitis arises due to lysis of hepatocytes and liver hypoperfusion. Once kidney failure sets in, the fluid management becomes very difficult with the risk of fluid overload, pulmonary oedema and difficult to manage hypo-/hyperkalemia. The occurrence of renal failure almost universally leads to death if renal replacement therapy is not available.
Neurological complications have a multifactorial aetiology: hypoglycaemia, viral meningo-encephalitis, intracranial haemorrhage, hepatic encephalopathy, delirium, …

Healthcare providers should not minimize the psychological impact of receiving the diagnosis ‘Ebola’ on a patient, knowing that the mortality rate in some epidemics surpasses 60 percent. Anxiety and depression are common symptoms and psychological support to help patients cope with their fears is part of good patient care.

The post-Ebola syndrome refers to musculoskeletal pain, headache, encephalitis and ocular problems (uveitis) that were frequently noted in thousands of EVD survivors of the 2013-2016 EBOV epidemic. The mental health effects on survivors, their family and community are considerable.

Diagnosis

Diagnosis during an epidemic is based on clinical suspicion, with serum PCR as confirmation. The viral RNA can be detected via a quantitative reverse transcriptase PCR on a blood sample(qRT-PCR). Results are expressed in Cycle Threshold (C_t) levels: low (< 20) C_t levels indicate detection of the virus after a low number of cycles required for the fluorescent signal to cross the detection threshold, hence ahigh viral load translating in a poor prognosis. Diagnostic studies during the 2013-2016 outbreak have mainly relied on molecular diagnostic

platforms. In general these tests are highly sensitive and specific. Various assays are currently available & FDA approved including an Xpert-based machine (Xpert Ebola Assay), which is a fully automated and closed device now rolled out for tuberculosis diagnosis. If this assay is installed within an Ebola treatment unit, time between sample collection and result was 2.5-3 hours in a study by MSF. The assays itself runs over around 90 minutes and cartridges specific for the EBOV Zaire strain were developed to target highly conserved sequences in the nucleocapsid protein (NP) and glycoprotein (GP) genes. Results can come out positive or negative and a cycle threshold (C_t) for both gene targets is given. These molecular assays may be negative early in the disease course, warranting follow-up testing in patient with recent onset symptoms. If the initial PCR test is negative and the patient has symptoms that started less than 48 hours previously, a second sample must be taken at 72 hours of illness (after another 24-48 hours). Simple bedside antigen-based tests have become available, but their sensitivity and thus negative predictive value is lower than PCR. These tests can thus be used for quick confirmation, but not to exclude the infection. Virus can be cultured in a few BSL-4 laboratories (e.g. on Vero cells).
Serological testing has no place in the diagnosis of an acute ill patient, but can be used for epidemiological research. It is worth knowing that each of the various geographical isolates have their own antigenic structure and therefore problems can arise with serological testing.

Other laboratory findings are elevated transaminases linked with hepatitis and creatinine kinase due to myositis. Consumption of clotting factors due to DIC leads to disturbed coagulation tests (PTT, D-dimers, fibrinogen). Thrombocytopenia is present in most patients and initially there is lymphocytopenia and later neutrophilia. Histologically there is focal necrosis in various organs (testes, kidneys, liver, etc.). Lower baseline viral load, creatinine and aminotransferase levels correlate with improved survival.

Patients can be safely discharged from Ebola treatment units when two sequential tests come back negative ($C_t > 40$) in a patient that has clinically improved.

Treatment

Early diagnosis and prompt initiation of care increase survival ratios. Paediatric patients and elderly are at higher risk of dying (however in the 2018-2020 epidemic in Eastern DRC extremes of age were not associated with poorer outcomes) as well as patients with a high

viral load.
During epidemics, good patient care may lower the mortality. Care for EVD patients is based on three pillars: supportive care to restore normal physiology, management of discomfort or distress and presumptive treatment of concurrent infections. In all epidemics so far, treatment was mostly done in very basic field conditions and treatment in an intensive care unit was rarely possible. Throughout the experiences gained in the recent epidemics, Ebola treatment Centres (ETC) in the field have more and more evolved towards provision of individualized care, with advances in laboratory and technical support. Staffing ratios of 1 or more more clinicians for four patients, and assessments (evaluation of each patient) performed at least three times per 24 hours are recommended. A comprehensive guidance "Optimized Supportive Care for Ebola Virus Disease" has been published by the World Health Organisation (https://www.who.int/publications-detail-redirect/optimized-supportive-care-for-ebola-virus-disease).

Gastro-intestinal symptoms can be controlled with metoclopramide (Primperan®) or domperidone (Motilium®) against vomiting and loperamide against diarrhoea. Omprazole is given as stress ulcer prophylaxis. Prevention intravascular volume depletion and avoidance of organ hypoperfusion is critical. Fluid losses from vomiting, diarrhoea and vascular leakage may require more than five liters per day of crystalloid solution intravenously if the patient is unable to compensate the losses with oral rehydration. In the last epidemics, the use of point-of-care ultrasound has been a useful addition to estimate fluid status and has the potential to increase diagnostic capacity and individually tailored patient care. On-site biochemical testing was often available, permitting correction of electrolyte abnormalities (hyponatremia, hypo-/hyperkalaemia, hypomagnesemia and hypocalcaemia) and hypoglycaemia. Oral nutrition should be encouraged, ideally guided by a nutritionist. If necessary nasogastric tube can considered. High calorie liquid food is easier to swallow than solid food, since many patients suffer from severe throat pain. Antipyretic agents as paracetamol are given to manage pain and to decrease fever. Stronger pain killers (tramadol, morphine) might be needed, but NSAID's should be avoided to minimize the risk of renal failure and to decrease the risk of bleeding. Chlorpromazine and even haloperidol might be considered in case of agitation and confusion. Seizures are treated with diazepam.

Since coinfections are often difficult to diagnose in low resource settings with blood cultures rarely available, presumptive treatment with broad-spectrum antibiotics, in the form of a third generation cephalosporin, are usuallypart of the initial standard treatment. It is not unusual for EVD patients to develop new-onset fever that may be associated with leukocytosis in the second or third week of the hospital course, often despite initial improvement in the presenting symptoms and the viral load. In this setting, the development of ETU-acquired secondary infections while on broad spectrum antibiotics, and the development of resistant gram-negative bacteremia or Clostridium difficile infection, should be considered. Antibiotic management, including drug choice as well as doses, should be adjusted accordingly. In malaria endemic regions, anti-malarial treatment is sometimes added for all admitted patients, but robust data justifying this approach are lacking. A natural experiment due to a 12-day stock rupture of artesunate-lumefantrine in a Liberian MSF Ebola treatment center, noticed a lower risk of death in patients prescribed artesunate-amodiaquine compared with patients that received artesunate-lumefantrine. Amodiaquine is a compound with anti-Ebola activity in vitro. It is however not excluded that artemether-lumefantrine is associated with an increased risk of death due to torsades de points with fatal arrythmias in patients with a long QTc interval especially when combined with hypokalemia/hypomagnesemia and/or ciprofloxacine and/or metoclopramide. Another explanation is that artesunate-amodiaquine use was associated with unmeasured patient characteristics that altered the risk of death (e.g. effective malaria infection, higher viral loads, age, patients admitted during busier periods, patients in need of parenteral treatment with worse prognosis, …).

In the rare events when parenteral nutrition, renal-replacement therapy and mechanical ventilation where available, these treatments probably had a lifesaving impact.

Survivors of EVD need comprehensive follow-up care, including rheumatological, auditory, and ocular function with special attention to visual acuity deficits or raised intraocular pressure. Appropriate psychological and social support should be offered after mental health screening examinations.

Aside from good supportive care, several investigation treatments with anti-EBOV activity exist. The main categories are antibodies (plasma from convalescent patients, whole blood or monoclonal antibodies) and antivirals. A study with convalescent plasma in Guinea did not

show sufficient mortality benefit, neither did a study with whole blood transfusion. A phase II study with the antiviral favipiravir only decreased case fatality rate in patients with a low viral load and seemed to increment mortality in patients with higher viral loads. At the end of the 2013-2016 outbreak in West-Africa, a randomized clinical trial (PREVAIL II) with ZMapp – a cocktail of three potent monoclonal antibodies – showed a fatality rate of 37% (13 of 35 patients) in those receiving the standard of care and a fatality rate of 22% (8 of 36 patients) in those receiving standard of care together with ZMapp. Although this result seems beneficial, the decline in the epidemic reduced participant enrolment hence results did not reach the statistical threshold for efficacy.

During the 2018-2020 outbreak in DRC, the Pamoja Tulinde Maisha (PALM "Together Save Lives" in Swahili) trial compared ZMapp with three newer agents: mAb114 (Ridgeback Biotherapeutics) which is a single monoclonal antibody derived from the memory B cells from a survivor of the Kikwit EVD epidemic, REGN-EB3 (Regeneron Pharmaceuticals) combining three triple monoclonal antibodies obtained by immunizing mice and the antiviral remdesivir (Gilead), a prodrug nucleotide analogue. mAb114 and REGN-EB3 have the advantage over ZMapp that they are given as a single dose whereas ZMapp is given in 3 doses, spaced 3 days apart. Remdesevir is given in daily doses for at least 10 days. An interim analysis showed superiority of Mab114 and REGN-EB3 to ZMapp and remdesivir with respect to mortality. In the mAb114 and REGN-EB3 group mortality was 35% and 33% as compared with 50% in the ZMapp and 53% in the remdesivir group. Shorter duration of symptoms before admission with earlier treatment initiation improved survival, which had not been the case in previous epidemics. Surprisingly, mortality with ZMapp in the PALM trial was 50% compared to 22% in the above mentioned PREVAIL II trial. The reasons remain unclear and subgroup analysis is ongoing to shed more light on potential differences among treatment groups. After the interim analysis patients were randomized to receive mAb114 or REGN-EB3, dropping the ZMapp and remdesivir study arm. Final results of this trial are still pending.
Hundreds of patients in the recent outbreak in Eastern DRC that were not included in the PALM trial still received above mentioned investigations drugs under the Monitored Emergency Use of Unregistered and Investigational Interventions (MEURI) framework. Despite the lack of randomization, an analysis of patients receiving drugs under MEURI showed remarkably similar results for the same therapeutics that were provided in the PALM trial.

It is important to notice that patients developing EVD despite previous vaccination for EBOV

had much better outcomes.

Future research might focus on combination therapy considering the possible synergistic effect of remdesivir – that has a delayed onset of action as compared with a antibodies – combined with antibodies. A next generation human antibodies (i.e. MBP134, FVM04 and CA45) have shown protection against EBOV, SUDV and BDBV, whereas ZMapp, REGN-EB3 and Mab114 only protect against EBOV. Nevertheless, these products will first have to prove their non-inferiority in a well-designed future trial.

Table 1. Clinical Trials of Vaccines and Antiviral Therapies for Ebola Virus Infection in Humans.*

Treatment and Study Design (Country)	Filovirus Species (Strain)	Dose	Regimen	No. of Patients and Outcome	Study
Vaccine					
rVSV-ZEBOV; open-label, cluster, randomized trial of ring vaccination (Guinea)	Ebola (Makona)	2×10^7 PFU	Single injection (IM)	5837 vaccinated; estimated efficacy, 100% (95% CI, 79.3–100.0)	Henao-Restrepo et al.[51]
rVSV-ZEBOV; randomized, placebo-controlled phase 2–3 trial (Liberia)	Ebola (Makona)	2×10^7 PFU	Single injection (IM)	500 vaccinated (phase 3 eliminated because of decline of Ebola in Liberia)	Kennedy et al.[52]
rVSV-ZEBOV; open-label, cluster, randomized trial of ring vaccination (DRC)	Ebola (Kivu)	2×10^7 PFU	Single injection (IM)	93,965 vaccinated; efficacy, 97.5% (95% CI, 95.8–98.5)	World Health Organization[53]
ChAd3-EBO-Z; randomized, placebo-controlled phase 2–3 trial (Liberia)	Ebola (Makona)	2×10^{11} particle units	Single injection (IM)	500 vaccinated (phase 3 eliminated because of decline of Ebola in Liberia)	Kennedy et al.[52]
Antiviral Therapy					
Convalescent plasma; nonrandomized comparative study	Ebola (Makona)	Unknown	Two consecutive IV transfusions of 200–250 ml each	84 enrolled; no significant survival benefit	van Griensven et al.[44]
Convalescent blood; nonrandomized comparative study	Ebola (Makona)	Unknown	One IV transfusion of 450 ml given over a period of 1–4 hr	43 enrolled; no significant survival benefit	Sahr et al.[45]
ZMapp; phase 2–3 trial (Liberia, Sierra Leone, Guinea, United States)	Ebola (Makona)	50 mg/kg	One dose every 3 days (IV) for a total of three doses	36 enrolled, 28 survived (77.8% survival rate)	PREVAIL II Writing Group[46]
ZMapp; PALM trial (DRC)	Ebola (Kivu)	50 mg/kg	One dose every 3 days (IV) for a total of three doses	323 enrolled, 160 survived (49.5% survival rate)	Mulangu et al.[54]
MAb114; PALM trial (DRC)	Ebola (Kivu)	50 mg/kg	One dose (IV)	174 enrolled, 113 survived (64.9% survival rate)	Mulangu et al.[54]
REGN-EB3; PALM trial (DRC)	Ebola (Kivu)	150 mg/kg	One dose (IV)	155 enrolled, 103 survived (66.5% survival rate)	Mulangu et al.[54]
Remdesivir (GS-5734); double-blind, placebo-controlled, natural history trial (Liberia)	Ebola (Makona)	100 mg	Once daily for 5 days (IV)	Ongoing, with planned enrollment of 60 survivors to assess viral shedding in semen	Siegel et al.[55]
Remdesivir (GS-5734); PALM trial (DRC)	Ebola (Kivu)	200 mg loading dose; 100 mg thereafter	Once daily for 9–13 days (IV)	175 enrolled, 82 survived (46.9% survival rate)	Mulangu et al.[54]
Favipiravir (T-705); single-group trial with historical controls (Guinea)	Ebola (Makona)	6000 mg loading dose; 2400 mg thereafter	Two 1200-mg doses daily on days 1–9 (oral)	126 enrolled; no significant survival benefit	Sissoko et al.[48]
TKM-130803; single-group, phase 2 trial with historical controls (Sierra Leone)	Ebola (Makona)	0.3 mg/kg	Once daily for up to 7 days (IV)	12 enrolled; no significant survival benefit	Dunning et al.[47]

* CI denotes confidence interval, IM intramuscular, IV intravenous, PALM Pamoja Tulinde Maisha, and PFU plaque-forming units.

Source: NEJM, 2020; 382:1832-42, Feldmann et al.

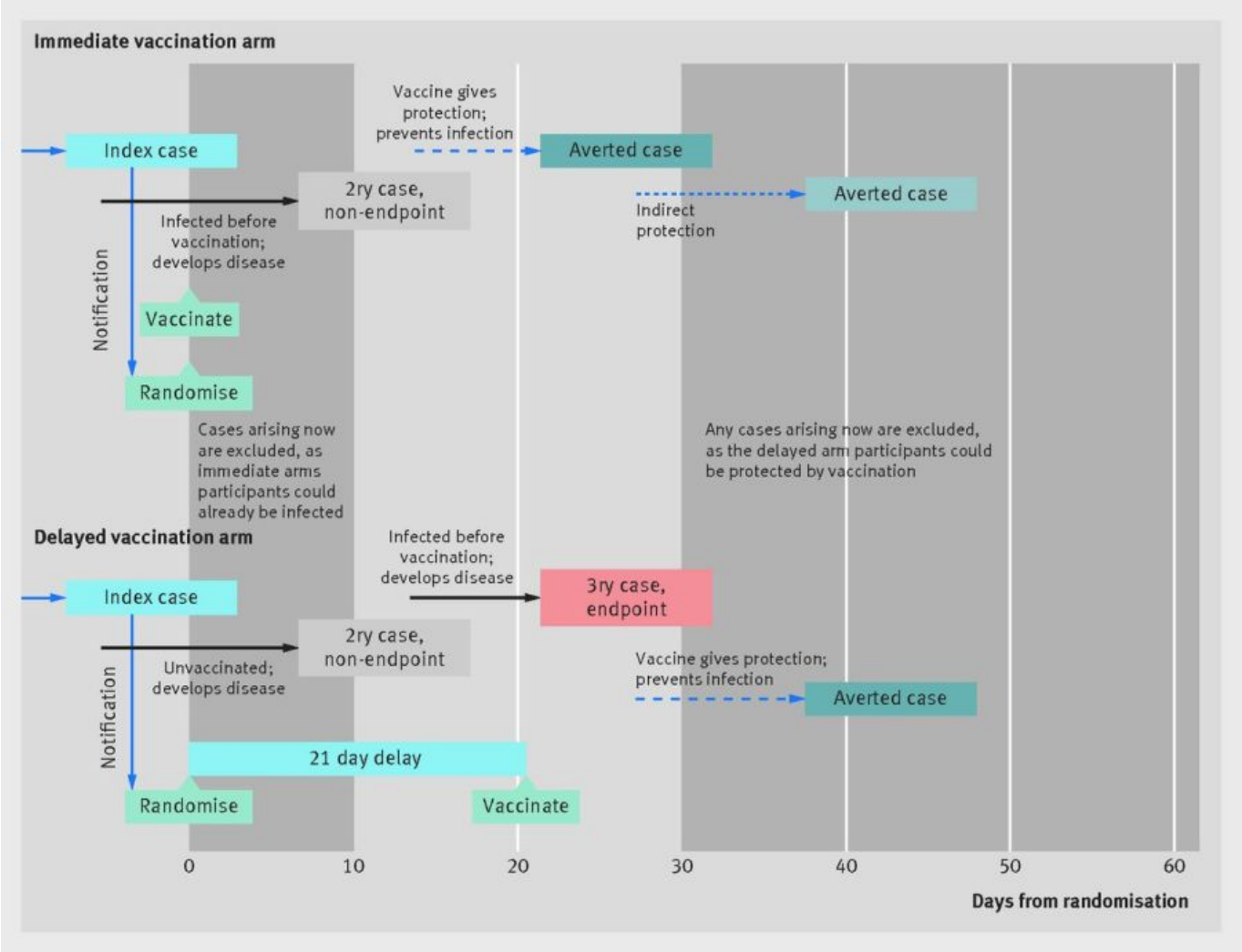
Prevention

Prior to the 2013-2015 Ebola outbreak, no effective vaccine was commercially available nevertheless, such a vaccine was explored with several purposes, such as after lab-accidents, during epidemics, and probably in the stockpile for biowarfare defence.

Clinical development of several vaccines has advanced substantially during the 2013-2015 EBOV outbreak. Successful vaccination relies on the development of an immune response against the viral glycoprotein (GP), which is critically involved in cell attachment, fusion and cell entry. While assumed that the protection against EVD predominantly relies on the development of anti-GP antibodies, the role of the cellular immune response in vaccine protection remains to be defined. There is indeed not yet a well-defined immune marker (biomarker) that correlates with protection against EVD after vaccination. Currently, the level of IgG antibodies against the EBOV GP is the most commonly used measure of immunogenicity in vaccine trials.

The vesicular stomatitis virus (VSV)-based vaccine (rVSV-ZEBOV), a live (replication-competent) vaccine expressing the *Zaire ebolavirus* glycoprotein has shown limited reactogenicity (“toxicity”) and good immunogenicity in phase I/II studies, although arthritis was documented in some. This vaccine was subsequently evaluated in a phase III trial in Guinea using a ring vaccination strategy, as previously successfully employed during the eradication of small pox. In principle, ring vaccination represents a strategy of targeted vaccination (in contrast with vaccination in the general population), targeting risk individuals at high risk of exposure to and development of EVD. After the identification of a new EVD case (‘index’) case, his/her contacts, and the contacts of these contacts are eligible for vaccination. The trial compared clusters with immediate vaccination with clusters with delayed vaccination 21 days later. As cases occurring early after vaccination might have been infected before the vaccination, and since it takes some time for the vaccine to induce immunity, only cases occurring ten days or more after vaccination were taken into account. No EVD cases were seen after this ten day period in the immediate vaccination group, whereas cases continued to accrue with delayed vaccination. This yielded a vaccine efficacy of 100% although with a wide confidence interval (74.7%–100%). The vaccine effectiveness, taking into account all individuals that could/should have been vaccinated was 76.3% (95% CI –15.5% to 95.1%).

The rVSV-ZEBOV has also been used in front-line workers in Guinea and Sierra Leone. During the latest epidemic in eastern DRC, more than 265,000 people have received it as part of a ring vaccination strategy (with ring vaccination not only offered to 1st, but also to 2nd and 3th generation contacts) with an efficacy of 97.5% for vaccines with an onset of illness more than 10 days after vaccination, and 88.1% for all those with EVD regardless of the timing of illness onset. The rVSV-ZEBOV vaccine is now approved for use by WHO during epidemics and is approved for use in Europe and the United States, mainly to protect international healthcare workers that will work in Ebola treatment units.



Source: http://www.bmj.com/content/351/bmj.h3740

Since November 2019, a second vaccine Ad26.ZEBOV/MVA-BN was used to complement the

ongoing ring vaccination with rVSV-ZEBOV. This vaccine is given in two doses, 2 months apart: the first dose consists of a recombinant human adenovirus 26 encoding the *Zaire ebolavirus* glycoprotein, while the second dose is a modified vaccinia Ankara virus (MVA) containing glycoproteins of *Zaire* and *Sudan ebolavirus* and *Marburg Musoke* virus as well as the nucleoprotein of the *Tai Forest ebolavirus*. The 2 dose prime-boost regimen is expected to give longer protection and therefor the vaccine is given to at-risk populations neighbouring an Ebola epidemic regions where there is no active transmission yet and to health care workers. It is not part of the ring vaccination strategy when rapid immunity is needed since the single-shot rVSV-ZEBOV appears to induce a quicker immune response. Future work on vaccine efficacy, stability, storage, transport and administration as well as supply adequacy are needed.

Future clinical research

Clinical research initiatives started only late during this EVD outbreak. Only in August 2014, WHO declared the outbreak as a “public health emergency of international concern” and funding became available from the main funding organizations from September on, in part driven by EVD infections of health care workers from international healthcare workers with the threat of EVD spreading to the US and Europe. WHO developed an inventory of vaccines and therapeutics in the pipeline. However as many had often not undergone clinical evaluation, there was a lot of discussion whether it was ethical to use/evaluate these interventions during the 2014-2015 outbreak. In September 2014, the WHO Ethics Working Group released a statement recommending that “investigational drugs or vaccines that have shown promising results in the laboratory or in animal models be urgently tested in humans by scientifically sound, rigorous methods”. Since then, many clinical studies have been launched in a relatively short time span, complemented with studies in non-human primates. A process that would otherwise take several years now had to be done in months.

Ebola outbreak management

Recognition

The very first step is to recognize possible clinical cases, which is why case definitions must be determined and widely distributed. The current case definition used by WHO for Ebola

Virus Disease is: a patient with any ONE of the following:

- Sudden onset of fever (≥38□C) AND contact with confirmed or probable Ebola case or dead or sick animal; OR
- Sudden onset of fever (≥38□C) AND ≥3 symptoms (Headache, vomiting, diarrhoea, anorexia/loss of appetite, lethargy, stomach pain, myalgia, dysphagia, breathing difficulties, or hiccups); OR
- Contact AND ≥3 symptoms; OR
- Unexplained bleeding or miscarriage; OR
- Sudden unexplained death.

This generic suspect case definition may be adapted to local circumstances (clinical presentation, mode of transmission). During outbreaks, expanding the suspect case definition to include patients with mild symptoms increases sensitivity, but increases the case load in triage centres. The performance of the case definition during outbreaks should be assessed.

Steps should be taken to identify and type the virus (send a blood sample safely to a well-equipped laboratory). In a laboratory which is protected and equipped to work with dangerous pathogens (biosafety level 4), an attempt will be made to detect viral antigen, antibodies and viral RNA (reverse transcriptase PCR) and carry out an analysis of the genome in order to establish which Ebola subtype is involved.

Central organization

If it is established that it really is Ebola, the government will be notified. Central control, registration and coordination is essential for combating an epidemic. WHO and CDC will be notified. Groups specifically responsible for a certain part of the campaign will be set up: clinical care, surveillance in the community, logistics, collecting the dead and safe burials, investigating rumours, informing the population, epidemiological study, research, reception center, etc. These days it is also useful to appoint someone who can handle the press correctly. Every day information will be exchanged between the various teams and the latest developments will be reported to the WHO in Geneva.

Vaccination

In the most recent and future epidemics, vaccination of health care workers, ring vaccination and vaccination of populations at risk play a much bigger role than in earlier epidemics (cfr. above). More info can be found in the Strategic Advisory Group of Experts (SAGE) on Immunization document by WHO: https://www.who.int/immunization/policy/position_papers/interim_ebola_recommendations_may_2019.pdf?ua=1

Isolating patients

The patients' movements should be limited. They should be isolated (no direct physical contact with patients, blood, excreta etc.). Any newpatientmust be directed to a triage zone. Here, based on the history (contact with Ebola patients, fever, symptoms), patients must be divided into Ebola suspects and non-Ebola patients according to the predefined case definitions. Ebola suspects must be kept in isolation, awaiting results of their PCR. If fever came up less than 3 days ago and the PCR result is negative, the PCR test will be repeated after 2 days before a patient is considered definitely negative. Often contact with Ebola will not be reported due to superstition, fear of stigmatization or if there was sexual contact with a person who subsequently developed Ebola infection. The absence of a lab facilities on-site can be a practical problem for the clinicians working in the field.

Barrier nursing

During an outbreak, there is a crucial need to protect health care workers. The small inoculum and the high mortality rates despite (investigational) treatments impose a zero-tolerance practice. Personal protection (masks, goggles, aprons, boots, disinfection supplies) for medical staff and for people who care for the sick person in case of refusal to admission to an ETC (often family) is necessary.

Demonstration of how to use the protective equipment and proper explanation are imperative. Donning and doffing is done through standard operational procedures and under direct supervision of a team member. Personal protective equipment has many

inconveniences, but none greater than heat stress, limiting the time that can be spent for caring patients under tropical conditions.

Reusable equipment should be disinfected rigorously with, for example, bleach (hypochlorite solution). Objects that cannot be sterilized must be burnt under supervision. People who are suspected of being infected with the Ebola virus should be cared for by people who understand and use personal protection. Basic needs (drink, food, pain-relief, hygiene, etc.) have to be met. Vomitus, sputum, faeces and urine must be collected in a plastic bucket and mixed with strong bleach before disposal.

Centers with a poor medical infrastructure and with a high risk of nosocomial transmission must be closed down temporarily. This applies both to large hospitals and small one-person clinics with only a few needles and syringes. Strict guidelines have to be issued to centers which continue functioning, particularly with regards to triaging of suspect cases, disinfection, the use of needles and syringes, vaccinations and surgical procedures. In many places non-qualified private individuals have only a few (non-sterile) needles and syringes, which they use for all injections.Ebola, field treatment

Cfr. section on treatment above.

Surveillance and contact tracing

The goals of Ebola virus disease (EVD) surveillance are to promptly detect new, suspected EVD cases and deaths so as to trigger an appropriate response. Communities and local authorities should always report all deaths. In past epidemics a system of alerts was put in place. An alert is a condition that meets a very broad (sensitive) definition that aims to identify all signals that could potentially be an EVD case or death. Alerts can be generated by the community, at health facilities, or picked-up in the media. Often checkpoints are put up at so called points of entry and exit. Alerts are reported to those in charge of surveillance through various means, e.g. a telephone hotline. If an alert is validated and a new case identified, it is primordial to establish the chain of transmission.

People who have recently had contact with Ebola patients but do not display symptoms have

to be placed under supervision (surveillance) for 3 weeks, the maximum incubation period. A contact is defined as: any person who has been exposed to a suspected, probable, or confirmed case of EVD in at least one of the following ways:

- has slept in the same household as a case
- has had direct physical contact with the case (alive or dead) during the illness
- has had direct physical contact with the (deceased) case at a funeral or during burial preparation rituals
- has touched the blood or body fluids (including urine, faeces, vomit, tears, or sweat) of a case during their illness
- has touched the clothes or linens of a case
- a baby who has been breastfed by the case

Note: This should include health workers (including those involved in cleaning, waste management, laboratory technicians, nursing, etc.)

If symptoms arise, immediate investigations should be carried out. Each diagnosed patient has on average 10 to 15 contacts which are to be monitored daily for 21 days. In large epidemics, contact tracing and follow-up can be a vastly resource-intensive activity.

Convalescent patients

Ebola virus might persist up to several months in selected immunologically privileged body sites of survivors. Sexual transmission is possible up to 6 months or longer after clinical recovery. Male survivors and their partners should be counselled on safe sex practices for 6 months or until their seminal fluid is free of viral RNA.

Convalescent serum can be stored if necessary, even though studies with convalescent serum so far failed to prove survival benefit This serum has the possibility to produce monoclonal antibodies as was the case in mAb114 development.

Information

Nothing may be as important as community engagement and public perception. A part from education about the disease and control measures, populations should be encouraged to quickly alert authorities about febrile cases and unexplained deaths. Transmission of the disease will only stop when the community is no longer caring for the sick in unprotected settings and burying the dead in an unsafe manner. Trust is not always a given in an epidemic, which was clearly shown in the 2018-2020 DRC outbreak, with several structures of the outbreak response attacked. A general large-scale information campaign with adequate and practical information for the population should be started. If this results in many questions and tips, a permanent center can be set up where information about possible new cases can be examined. In view of the extreme virulence, the incomplete knowledge about these pathogens and memories of the impact of the earlier plague and yellow fever epidemics, these pathogens can capture the imagination of the general public. Superstition and belief in witchcraft can lead to misunderstandings and violence. In an environment of mistrust towards the national or local government and towards international organizations, experimental countermeasures as vaccination and experimental drugs can fuel rumours of unsavoury experimentation. Crystal clear communication about the ongoing interventions with transparent answers to questions are a prerequisite for the interventions to be successful.

Burials

The deceased should not be washed, and the bodies have to be isolated and buried as quickly as possible and reasonable. This sometimes causes problems with the family and acquaintances of the deceased because of the disruption of traditional rituals. The government has a role to play here in law enforcement avoiding the disrespect of cultural values as much as possible.

Social impact

Caring for orphans in the community should be organized if this does not take place through the traditional system of the extended family. The latter sometimes does not work because of

fear, prejudice and practical problems.

Logistics

Logistics play a very important role and include, among other things, infection control, equipment and materials, administrative support, accommodation, money and wages, communication, transport, fuel, safety and stock management. Good management is essential and has to be entrusted to reliable people. The NGO Médecins Sans Frontières and Alima (Alliance for International Medical Action), an international non-profit medical organization have a lot of experience in handling the logistics of such operations. Specific "Ebola kits" of different sizes have been prepared and are kept in stock, ready to be used in emergency situations.

Personnel

Experts in various areas cannot, in most cases, make themselves available quickly for a long time and a rotation system should be organized. It is best if (international) staff do not change too frequently in order to achieve a minimal continuity locally. Realistic guidelines for cases in which medical personnel are infected accidentally must be drawn up. According to the scale of the outbreak, the need for formation of national and/or international staff should be assessed.

Epidemiology

Epidemiological research should attempt to identify transmission routes and secondary cases. Risk factors for infection should be identified: unsafe burials, screening systems in health facilities, fear/mistrust in the community. An attempt will also be made to trace the first case in order to understand how the chain of infection started. However this person may well have died. Several people, such as customers, work colleagues, neighbours, family and friends may be able to provide useful information. A reminder of the terminology: the index case is the patient in whom the disease first indicated the existence of an outbreak. The index patient always remains the same person irrespective of whether earlier cases are discovered later. The very first case is called the primary case, not the index case. Later

secondary, tertiary, etc. cases can follow. The first case might change over time with incoming retrospective data, whilst the index case of an epidemic will never change in the future.

Reservoir

Because an animal reservoir is assumed to exist where the virus “hides” between epidemics, extensive attempts have been and are being made to identify this. An “ecological” team should be exclusively involved in this and will study different animals in the vicinity. An investigation should also be carried out into whether the virus is “exported” from the isolation units in the hospital to the environment. In addition to the fieldwork itself, there then follows the tedious analysis of the various potential hosts, both for the presence of the virus and their taxonomic identification.

Laboratory

Rapid sample analysis (blood samples of patients, samples of other body liquids, etc.) and rapid transmission of the results is recommended. Logistical problems can hinder this. Investment in research and cooperation will pay dividends.

Looking for isolated cases

The maximum known incubation period is 21 days. After the end of the epidemic (no more cases for a minimum of 6 weeks), surveillance can be carried out locally. It is possible that isolated cases and limited outbreaks occur. In order to obtain a better understanding of this disease, long-term surveillance is necessary. Regular flare-ups of the disease in the aftermath of the devastating epidemic in Guinea, Sierra Leone and Liberia, were seen due to sexual transmission, even several months after the declaration of the end of the epidemic.

Future prevention

We do not know how all epidemics started, but several followed the consumption of infected apes. The risk of nosocomial transmission is clear. Owing to modern rapid means of

transportations, Ebola fever can emerge anywhere in the world. Naturally this does not only apply to Ebola, but to the whole spectrum of communicable diseases.