Progress in Epidemiology of Ebola Virus Disease

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Abstract

Ebola virus disease (EVD) is a zoonotic disease caused by Ebola virus. It has become one of the most harmful diseases in humans for its high mortality and strong infectivity. Since entering the twenty-first century, the repetition and abruptness of EVD outbreaks in Africa, especially the unprecedented and huge EVD outbreaks in West Africa from 2013 to 2016, has attracted much attention from the global media and public, and made EVD an urgent public health threat worldwide. EVD not only causes serious casualties and leads to heavy economic losses, but also affects the normal operation of the public health system and triggers more social problems. China is one of the earliest countries that assisted EVD epidemic areas in West Africa, and has not only provided financial support and material equipment and dispatched epidemic prevention experts and medical staffs in multiple batches to participate in the prevention and control of EVD epidemic in West Africa, but also made outstanding contributions to researches of EVD etiology, clinical symptoms, pathological changes, pathogenesis, diagnostic and therapeutic drugs, epidemiology, and vaccine development. With the development and acceleration of economic globalization, the more convenient transportation and frequent personnel flow have greatly promoted the cross-regional transmission of EVD, and countries around the world are at risk of EVD importation and transmission; however, there still exist many key scientific issues regarding EVD global prevention and control that are not clear. In order to provide basic information for the development of global prevention and response strategies for the disease, this chapter focuses on EVD transmission dynamics, distribution characteristics, influencing factors, and prevention and control measures to comprehensively and systematically understand the epidemiology and current challenges of EVD, which helps guide the formulation of global public health crisis policies, and provides a scientific basis for the development in the ability to effectively respond to the impact of EVD outbreaks.

Keywords

Ebola virus · Ebola virus disease Transmission · Distribution · Influencing factors · Prevention and control

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5.1 Introduction

Ebola virus disease (EVD) is an infectious disease caused by Ebola virus with symptoms such as acute onset of fever, myalgia, bleeding, rash, and liver and kidney function impairment, which is mainly transmitted by contact with body fluids, excreta, and secretions of patients or infected animals [1–4]. EVD was previously called Ebola hemorrhagic fever, while in the outbreaks in recent years, infected individuals experienced relatively few clinical symptoms of hemorrhagic fever, so this disease is now renamed EVD [3]. The Ebola virus was first discovered in Sudan and Zaire [now the Democratic Republic of the Congo (DRC)] in central Africa in 1976, and endemic areas were mainly distributed in the African region and classified by the World Health Organization (WHO) as one of the most harmful viruses to humans because of their extremely high mortality [4–6]. DRC, as the country with the highest frequency of EVD outbreaks, had four EVD outbreaks during 2014–2018 alone. The most recent two occurred in 2018. In May 2018, the province of Equateur in the DRC experienced the ninth EVD outbreak in the history of the country, which was not declared to end until July. It caused a total of 54 cases and 33 deaths; however, only a few days later, a new EVD outbreak was confirmed in the province of North Kivu in the country, which was still ongoing as of December 27, 2018, with a cumulative incidence of 591 cases and 357 deaths [4, 7, 8].

EVD outbreaks in the African region have become more frequent since entering the twenty-first century. The most alarming and panicky outbreak of EVD occurred in West Africa [9]. In late 2013, a small-scale EVD outbreak in Guinea turned into an unprecedented global public health emergency in a few months, with epidemic areas spreading rapidly to adjacent countries of Sierra Leone and Liberia. The outbreak was declared to end on June 9, 2016, with a total of 28,652 cases and 11,325 deaths reported - with the highest number of morbidity and mortality, the widest impact, and the longest duration in history [4]. Repeated outbreaks of EVD not only bring a lot of casualties, but also lead to heavy economic losses [10]. The World Bank estimates that the West African EVD epidemic has brought economic losses between 2014 and 2015 to $2 billion in West African countries. When the reduction of fiscal revenue, the cost of fighting the epidemic, and the reduction of foreign investment are considered comprehensively, the overall economic loss is about 3.8 billion US dollars [11]. Furthermore, EVD outbreaks in West Africa were different from those in the past; the previous outbreaks often appeared in remote areas or villages with sparse population density and less population movement, so the epidemic could be effectively controlled in a short period. In contrast, EVD outbreaks in West Africa, although they first appeared in rural areas of southeast Guinea, were imported into densely populated urban areas in just a few weeks and transmitted to Liberia and Sierra Leone across borders. Coupled with the scarcity of health resources and backward security systems in West African countries, the lack of EVD prevention and control experience in national health departments, the lack of early identification and diagnostic ability of EVD, as well as the lack of awareness of EVD in the population, large-scale EVD outbreaks in West Africa occurred.

The EVD epidemic in West Africa had spread rapidly and was characterized by a rapid increase in the number of patients, high mortality rate, and deficiency of effective vaccine and therapeutic drug during the epidemic period. The WHO announced this epidemic as a "Public Health Emergency of International Concern" on August 8, 2014 [12], and issued a crisis warning to the world. The three countries in West Africa (Sierra Leone, Liberia, and Guinea) also requested assistance from all countries in the world. The WHO, China, the USA, Médecins Sans Frontières (Doctors Without Borders, MSF), and other countries and organizations subsequently responded to the call to provide materials and equipment and personnel to assist West African countries for carrying out Ebola virus disease epidemic prevention and control. China has close cooperation with African countries in the fields of labor, commerce, education,

tourism, etc., and close personnel contacts, and the foreign aid medical team is resident in African countries, which is one of the earliest countries to respond to assistance. In order to help West African countries better control the EVD epidemic, apart from the aid medical team in Liberia and the established EVD diagnosis and treatment center [13], the Chinese government also dispatched the China Mobile Lab Testing Team to Sierra Leone to carry out the "anti-Ebola and assist Sierra Leone" task, and dispatched experts and medical staffs in multiple batches to participate in the prevention and control of the Ebola epidemic in West Africa, including providing masses of rescue materials, assisting protective equipment vehicles and funds, establishing infrastructures such as EVD observation treatment centers and testing laboratories, and dispatching professionals to help establish a prevention and control system [14, 15]. In addition, like the USA and the UK [1, 16–19], Chinese scientists have also been continuously committed to EVD-related scientific research in recent years. It has contributed a lot to the solution of key scientific problems in EVD prevention and control and made outstanding contributions to the study of EVD etiology, clinical symptoms, molecular evolution, diagnosis and treatment, and vaccine research and development. It mainly includes describing and analyzing the clinical characteristics of the disease and risk factors for poor prognosis [20–23]; providing valuable Ebola virus sequencing data and elaborating the molecular evolution of Ebola virus [24–27]; analyzing the transmission dynamic and intervention efficacy of EVD [28–30]; carrying out the study of early diagnosis and detection reagents of EVD [31–34]; developing new drugs and vaccines [31, 35–38]; and elaborating the prevention and control strategies of the disease [15, 39–41]. China's action to help Africa fight Ebola is a huge step forward for China to truly participate in global public health action.

China closely cooperates with African countries, with nearly 600,000 people coming to Africa each year [42]. Similar to most countries, China is also at risk of importation of virulent infectious diseases such as EVD, Marburg virus disease, and Rift Valley fever. To better master the epidemic regularity of EVD and promote the global prevention and control of EVD, this chapter mainly systematically reviews the research on the transmission regularity, distribution characteristics, and prevention and control of EVD. This will assist us comprehensively and systematically to understand the transmission process, epidemic characteristics, and existing challenges of EVD and provide basic information and scientific basis for the development of EVD prevention and response strategies.

5.2 Etiology and Animal Host

Ebola virus belongs to the order Mononegavirales and Filoviridae, and belongs to the same family as Marburg virus and encode a total of seven structural proteins and one non-structural protein: 3′ -nucleoprotein (NP), polymerase cofactor (VP35), matrix protein (VP40), glycoprotein (GP), transcription activator VP30, matrix protein (VP24), and RNA-dependent RNA polymerase (L) and one non-structural small glycoprotein—sGP [43]. The nucleocapsid with replicase and transcriptase function is composed of genomic RNA encapsidated by the NP and together with polymerase L, VP35, and VP30. VP30 is important for the reinitiation of transcription of subsequent genes, and is a potentially interesting candidate as an antiviral therapy target. Mutations in the sequences encoding the VP40 might lead to inhibition of virus release from the infected cell. Other structural nucleocapsid proteins (VP35 and polymerase L) participate in the synthesis of the viral genome. GP spikes are known to involve in the entry of virions into the host cell and are used in the mechanisms similar to macropinocytosis. It is known that VP24 and VP35 are important virulence factors, because they act as type I interferon (IFN) antagonists. The gene arrangement order is 3′-NP-VP35-VP40-GP/sGP-VP30-VP24-L-5′, which belongs to biosafety level 4 class I pathogens, and the leader and trailer at the 3′- and 5′-ends carry important signals to control tran-

scription, replication, and packaging of the viral genomes into new capsids [43].

At present, six Ebola virus subtypes have been found and named according to their discovery sites, Zaire ebolavirus (EBOV) was discovered in towns in northern Zaire, 1976, Sudan ebolavirus (SUDV) was in southern Sudan, 1976, Reston ebolavirus (RESTV) in cynomolgus monkeys in Leston, Virginia, USA, 1989, Tai Forest ebolavirus (TAFV) in forests in Côte d'Ivoire, 1994, Bundibugyo ebolavirus (BDBV) in western Uganda, 2007, and Bombali ebolavirus (BOMV) in Bombali District in Sierra Leone, 2018, with complete genome sequence differences of less than 30% for the same subtype of Ebola virus. Except for RESTV (which causes only latent infection but not a disease in humans) and BOMV (while BOMV has the potential to infect human cells, there is currently no evidence that the virus causes disease), the other four viral subtypes are pathogenic in humans [2, 4, 44, 45] and vary in virulence, with EBOV being the most virulent, followed by SUDV. After the outbreak in West Africa, scientists found that the sequences of the circulating strains were quite different from those of the previous circulating strains, the mutation rate was getting faster, and there were many accumulated mutation sites. It suggested that the Ebola virus may have been introduced into West Africa from Central Africa long ago and continuously mutated and evolved with the expansion of the epidemic size [24, 27, 46].

Nonhuman primates take an extremely high mortality rate after infection with Ebola virus and are therefore unlikely to be a natural reservoir of Ebola virus. Some scientists believe that rodents and arthropods may be the natural hosts of Ebola virus, and laboratory animals (such as mice, guinea pigs, and rabbits), as well as domestic animals (such as horses, cattle, and pigs), can also be infected with Ebola virus under laboratory conditions [47], and even experimentally infected pigs can transmit Ebola virus to monkeys, but this assumption was quickly denied [48, 49]. Between 1976 and 1998, researchers detected Ebola virus in samples from only six mice and one shrew in Central Africa among approximately 30,000 specimens collected by investigating mammals, birds, reptiles, amphibians, and arthropods in endemic areas [9]. The researchers used the EBOV to infect 24 plant and 19 vertebrate and invertebrate species and found that only bats (including insectivorous and frugivorous bats) supported the replication of EBOV and showed no obvious symptoms, thus speculating that bats may be the natural reservoir of EBOV [50]. The prevalence of Ebola virus was found to be temporally related to bat migration, infection, and death, such as the overlap of migration time and route between DRC EVD outbreak time and location and franquet's epauletted fruit bat and hammerhead fruit bats in 2007 [51]. Moreover, the detection of RNA and antibodies to Ebola virus from the bodies of three African fruit bats suggests that fruit bats are likely to be natural reservoir hosts for Ebola virus, while the rate of Ebola virus infection in bats is very low and Ebola virus can rarely be isolated or detected in bats; therefore, the issue of natural reservoir hosts for Ebola virus has been controversial [16, 52–54].

At present, although great progress has been made in the study of the etiology and host of Ebola virus, it is still a necessity to explore the molecular evolutionary characteristics and infection and transmission mechanism of Ebola virus in more depth to better provide useful information on the origin of Ebola virus and the research and development of drugs and vaccines.

5.3 Clinical Features and Prognosis

The incubation period of EVD is 2–21 days, generally 5–12 days. The more viral load, the shorter incubation period and the more severe the disease. The nonspecific symptoms of EVD include fever, malaise, fear of cold, headache, myalgia, anorexia, nausea, vomiting, chest pain, shortness of breath, runny nose, diarrhea, pharyngitis, dysphagia, rash, conjunctival congestion, and edema [1, 55, 56]. However, the nonspecific nature of

these early symptoms prevents EVD from being well separated from other infectious diseases, such as malaria, leptospirosis, influenza, and other respiratory viral infections, as well as yellow fever, dengue, and many other arboviruses or enteroviruses or bacterial infections [16, 57]. During the 2013–2016 outbreak in West Africa, through investigation and research, the WHO Emergency Prevention and Control Group found before the epidemic that the vast majority of cases had symptoms such as fever, fatigue, loss of appetite, vomiting, diarrhea, and headache. Of all patients, 95% presented an incubation period within 21 days, 11.4 days of the mean incubation period, 5.0 ± 4.7 days of the mean time from the onset of symptoms to hospital stay, 6.4 days of the mean hospital stay, and 4.2 ± 6.4 days of the time from the onset of symptoms to death [58, 59]. There are also a few reports of asymptomatic or mildly symptomatic [60, 61] and Akerlund et al. even reported that the initial symptoms of EVD infection in pregnant women were limited to mild abdominal pain and uterine contractions without febrile symptoms [62].

Surviving cases of EVD generally begin to improve in 6–11 days after a few days of fever, but may undergo a rehabilitation period with sequelae such as myelitis, recurrent hepatitis, psychosis, and uveitis, and pregnant women are also at risk of miscarriage [2, 59]. Lotsch et al. found that depression, insomnia, fatigue, anxiety, and posttraumatic stress were common sequelae in patients with EVD, although there were litter data from high-quality studies [63]. EVD dead cases generally present with severe symptoms early in the course of the disease and often die from complications such as multiple organ failure and septic shock between 6 and 16 days [2], and factors or indicators associated with EVD death or poor prognosis have also been reported in recent years. For example, Hunt et al. conducted a study of factors associated with EVD mortality and poor prognosis in the diagnosis and treatment of 150 EVD patients in Sierra Leone and found that early hospitalization of EVD patients contributed to a good prognosis, with Ebola virus RT-PCR Ct values less than 20 (OR 6.7, 95% CI 1.5–30.1), and acute kidney injury (OR 5.8, 95% CI 1.2–29.6) is an independent risk factor for death in infected patients; creatinine levels more than 115 mmol/L and respiratory distress on admission are also factors for poor prognosis [3, 64, 65]; Jiang et al. found that viral load and cytokine and chemokine levels were significantly higher in dead cases than in the early stages of infection [22]; Li et al. found that viral load, age, and diarrhea patients were associated with mortality [20]; Smit et al. reviewed the treatment of 122 children with EVD in West Africa and found that their overall mortality rate was 57%, with factors such as viral load and under 5 years of age associated with mortality in children with EVD [66]; in a study of 2310 adult cases of EVD in Guinea, univariate analysis revealed that patient age, history of visiting or close contact with suspected or confirmed EVD and presence of seven admission symptoms (fever, hiccups, vomiting, diarrhea, cough, sore throat, and unexplained bleeding) were closely related to EVD patients mortality Multivariate analysis showed that patient age was independently associated with EVD mortality (OR 1.06, 95% CI 1.03–1.09), while clinical symptoms at admission were not significantly associated with death, and older age was a factor in the death of EVD patients, which was similar to the conclusions drawn from several studies in Sierra Leone [67]. In addition, Bebell et al. found that pregnancy was also significantly associated with the severity of the disease and the risk of death, but the difference in the course of EVD between pregnant and nonpregnant women is a scientific issue that has not yet been systematically addressed [68].

The nonspecific clinical characteristics of EVD have always been the main bottleneck affecting the accurate diagnosis of EVD. To truly achieve early detection, diagnosis, treatment, and control, scientists worldwide still need to deepen and make breakthroughs in the differences in clinical symptoms between non-EVD patients and EVD patients and in the research of rapid and convenient diagnostic and detection reagents in the early stage of EVD.

5.4 Transmission

Humans and nonhuman primates infected with Ebola virus are the sources of EVD infection, and EVD outbreaks are usually sporadic, and the sources of many outbreaks that have occurred point to wild animals such as gorillas, chimpanzees, and monkeys that die in tropical rainforests in Africa, but also point the source of infection to fruit bats [69]. There are mainly two ways for the transmission of Ebola virus [1]: "animal–human" transmission and "human–human" transmission. "Animal–human" transmission occurs when humans are infected by handling animal carcasses or by direct contact with infected nonhuman primates, and scientists believe that the index patient in each EVD outbreak is infected by contact, handling, or consumption of animals infected by Ebola virus (e.g., chimpanzees, gorillas, fruit bats, monkeys, forest antelopes) [2, 70–73]. The type of human–human transmission is the main route of transmission of outbreaks, which is mainly transmitted by intimate contact between people and infected individuals (including sexual behavior), handling of infected individuals' carcasses, exposure to contaminated environments with body fluids of infected individuals, and exposure of medical staff to patient blood and other body fluids [74]. The primary cases (index cases) of most human outbreaks are hunters, gold seekers, felling workers, and other populations operating in forest areas, while the secondary infected population is the patient's family members or medical staffs who have close contact with them, and the outbreak is basically spread centered on the family of the index case and the hospital where they live [75].

5.4.1 Contact Transmission

Blood, saliva, sweat, secretions, feces, vomitus, semen, and milk from patients with EVD all contain viruses and are infectious [76, 77]. Although Ebola virus has been detected in breast milk, there is no significant evidence to support transmission routes such as breastfeeding, mosquito and insect bites, and aerosol transmission [73, 77]. Contact transmission is the most important mode of transmission for outbreaks of EVD. In EVD outbreaks, the risk of EVD transmission is mainly closely related to three behaviors: close contact during the later stages of the disease, caring for the patient, and preparing the deceased for burial matters [78]. There are many ways of contact, including face-to-face conversation, eating and sleeping, unprotected bedside care, sharing syringes, and cleaning corpses. In the presence of an EVD patient, the virus in the blood or body fluids of an EVD patient can be transmitted through broken skin or mucous membranes to contacts such as family members, physicians, etc., and people can also be infected by contact with objects contaminated with the patient's body fluids. In addition, Ebola virus can also be transmitted from mother to infant in utero, during delivery, or through contact with maternal body fluids including breast milk [4]. Direct contact with infected body fluids, including vomitus, diarrheal material, and blood, has been shown to increase the risk of transmission at least threefold during EVD outbreaks [3, 79]. Therefore, the high-risk group of EVD epidemic is mainly the medical staff who contact and accompany the patients in the family, fail to take correct and effective protective measures to provide treatment for the patients, have contact with the corpses, and participate in the funeral of the patients.

5.4.2 Household Transmission

In the 2014–2015 Sierra Leone EVD outbreak, Ajelli et al. found that 74% of transmission events occurred between family members or extended family members, 18% between friends and other community contacts, and 8% within hospitals [80]. Fang et al., by including the data analysis of 634 families in Sierra Leone, showed that the household transmission force (i.e., the probability that an indicator case infects a family member during its infection period) was 0.056–0.062, and it was found that males were less likely to be

infected by family members than females, with an odds ratio (OR) between them of 0.62 (95% CI 0.44–0.88), and juvenile females belonged to the group most susceptible to Ebola virus infection at home, with a household transmission force of 0.11 (95% CI 0.063–0.18) [30]. Another study showed that 32% (95% CI 26–38%) of household contacts who reported direct contact cases had a low risk of disease transmission (OR 1%, 95% CI 0–5%) between household members who did not have direct contact. In addition, the higher the risk of acquiring EVD, the higher the risk of taking care of EVD cases (especially deaths) in the community and participating in behaviors such as traditional funerals [78].

5.4.3 Nosocomial Transmission

Contact transmission within families or communities is often confined to small areas or families, but iatrogenic transmission often causes large-scale and cross-regional outbreaks, such as 85 (26.7%) of the 318 patients in the 1976 Zaire EVD outbreak who were infected with Ebola virus due to the use of contaminated syringes [5]. The basic reproduction number (R0) of EVD epidemic in Uganda in 2000 was 2.7 (95% CI 2.5–4.1), including 2.6 (95% CI 0.3–2.8), 0.01 (95% CI 0.0–3.5), and 0.1 (95% CI 0.0–3.2) for regional transmission, nosocomial transmission, and funeral transmission, respectively, which decreased to 0.3 (95% CI 0.2–0.4) after considering intervention parameters. In addition, studies have compared the two types of outbreaks, "small area-centered" and "hospital-centered," and found that when the epidemic spreads in a small village, the epidemic will eventually end spontaneously due to geographical and patient range of motion restrictions, such as the Zaire EVD epidemic that occurred in 55 villages in the Yambuku area of Zaire (now DRC) in 1976 and 25 villages in the Kikwit area in 1995, with basically less than 10 cases per village, and similar situations have occurred in the outbreak that occurred in the Ekata area of Gabon in 2001. However, when the epidemic occurred in hospitals, due to backward sanitary conditions and medical equipment and lack of awareness of protection among the medical staffs, the epidemic could easily spread among medical staff and cause high mortality, such as the outbreak in Kikwit Hospital in Zaire (DRC) in 1995, which caused more deaths of medical staff [81–83]; studies related to the epidemic in West Africa found that as of May 20, 2015, a total of 869 medical staffs were infected, of which 507 died [84], and during May 23 to October 31, 2014, the incidence of EVD in the medical population was 103 times that in the general population [85].

5.4.4 Sexual Transmission

Ebola virus can persist in the host, and the virus can still be detected in semen and eye fluid for a certain period (e.g., within three months) in cases after recovery [86–88]. A follow-up survey of EVD rehabilitation patients found that the virus can be detected in the semen of rehabilitation patients for a long time: RNA of the virus was detected in the semen of 65% and 26% of surviving patients tested at 4–6 and 7–9 months after the onset of infection, respectively [89]. In addition, Mate et al. conducted an epideiological investigation and molecular epidemiological study, suggesting that Ebola virus can indeed be sexually transmitted. The source of infection was an EVD patient who recovered for 500 days. Through this mode of transmission, 10 new cases occurred in Guinea in March 2016 [86], which also supported the statement that Ebola virus can remain in the body for a long time. Due to the possibility of sexual transmission of Ebola virus, the WHO and the US CDC recommend that all male survivors of EVD do have safe sex until 12 months after EVD onset or after two consecutive virus-negative tests of semen samples [3, 90].

5.4.5 Other Possible Transmission

At present, it has not been confirmed whether Ebola virus can be transmitted by aerosol. However, the scientific community generally

believes that Ebola virus has the possibility and potential for airborne transmission. The International Convention on the Prohibition of Biological Weapons had classified the Ebola virus as a potentially lethal biological warfare agent. In 2004, Leffel et al.'s article published in *Biosecurity and Bioterrorism* and, in the same year, Salvaggio et al.'s article published in *Dermatologic Clinics* were highlighting the risk of Ebola virus as a potential biological warfare agent, especially in the form of aerosol transmission [91–93]. In recent years, scientists have constructed animal models of aerosol infection of Ebola virus including macaques, rhesus monkeys, and African green monkeys in the laboratory, which can infect nonhuman primates through aerosols, and can cause fatal infections at very low doses. The successful establishment of these animal models not only confirms that Ebola virus can infect experimental animals by aerosol under existing experimental conditions, but also lays a foundation for future in-depth studies on the mechanism of this viral infection. In the future, we can use such animal models to carry out studies on Ebola virus infection and transmission under simulated natural environmental conditions [4, 93].

The Ebola virus can also be detected in patients' breast milk and body fluids, which suggests the risk of mother-to-child transmission or vertical transmission [77]. One study revealed that four out of the five breastfed infants with EBOV-positive breast milk were found positive for Ebola virus infection, and all of these Ebola virus-positive infants died [94]. With the current evidence, there is no certainty that mother-to-child transmission or vertical transmission is the main route of EBOV transmission.

The current mainstream hypothesis on the natural history of Ebola virus disease is [44]: Ebola virus is mainly transmitted naturally and circularly among fruit bats, and humans are infected with the disease by contact with animals such as infected bats, chimpanzees, gorillas, forest antelopes, and porcupines, and then close contact between human leads to outbreaks of the disease [2]. However, the circulation mode, infection mechanism, and cross-species transmission mechanism of Ebola virus in nature are still undefined scientific problems, and only by solving these scientific problems can spillover transmission of Ebola virus be prevented from the source.

5.5 Overview of EVD Epidemic Distribution

During 1976–2018, a total of 28 human EVD outbreaks have occurred in the African region (Fig. 5.1), including 24 epidemic outbreaks and 4 sporadic outbreaks [see Fig. 5.1, (3), (5), (10), (21)]; no primary cases of EVD have been found outside Africa, except imported or laboratory infection cases [4, 8, 95, 96]. In addition, nine outbreaks of animal EVD caused by RESTV infection occurred outside of Africa, of which seven occurred in non-primates and two in pigs, and three of these outbreaks detected RESTV antibodies in the relevant population (Table 5.1). By 2014, the EVD epidemic was confined to the Central African rainforest and savannah of southeast Africa, mainly five countries: Gabon, Republic of the Congo, the DRC, Uganda, and Sudan, and EVD patients were mainly from relatively closed remote rural areas, with sporadic cases also occurring in Côte d'Ivoire in western Africa and South Africa in southern Africa. The EVD outbreak from 2013 to 2016 in West Africa was the largest and most widespread outbreak in history, mainly in three countries in West Africa: Guinea, Liberia, and Sierra Leone, and most of the EVD patients came from densely populated large cities, such as Conakry, Monrovia, and Freetown. In the second half of 2014, the West African outbreak also spread to seven countries: Nigeria, Senegal, the USA, Spain, Mali, the UK, and Italy (see Fig. 5.1) [16, 49]. The reported number of cases in this outbreak had exceeded the total number of cases in previous outbreaks.

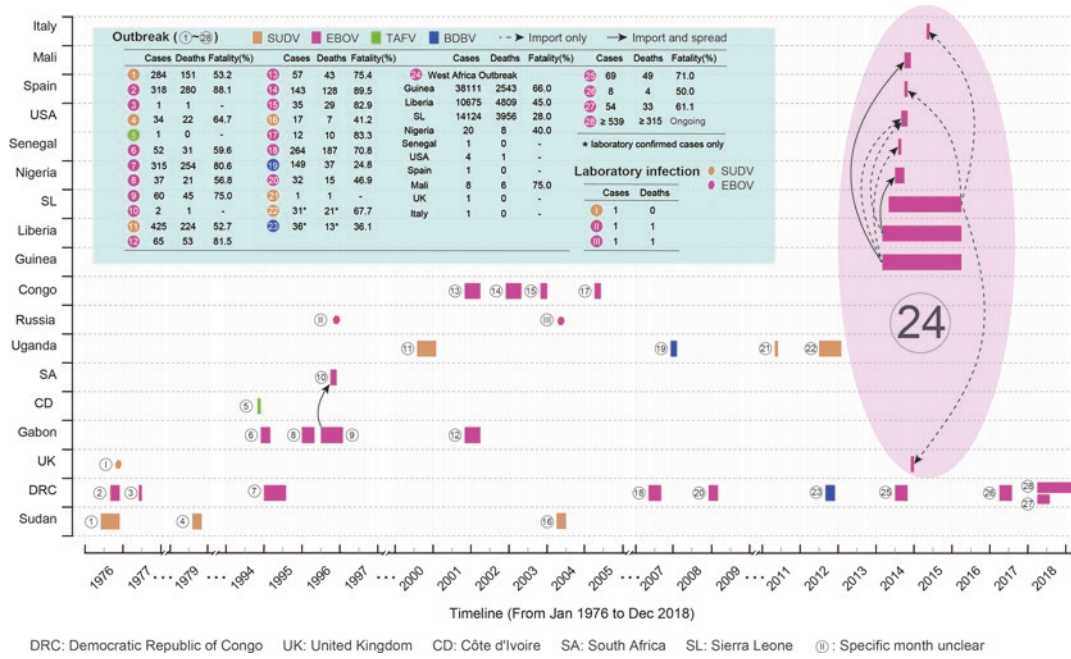

Fig. 5.1 The timeline and situation of human EVD outbreaks from January 1976 to December 2018

Table 5.1 List of RESTV outbreaks occurred outside of Africa

Date(year)	Country	Number of outbreaks of human seropositive antibody	Animal outbreaks
1989–1990	Philippines	3	Crab-eating macaques
1989	United States	0	Monkeys from Philippines
1990	United States	4	Monkeys from Philippines
1992	Italy	0	Monkeys from Philippines
1996	Philippines	0	Monkeys from Philippines
1996	United States	0	Monkeys from Philippines
2008	Philippines	6	Pigs
2011	China	0	Pigs
2015	Philippines	0	Monkeys

5.5.1 Seasonal, Population, and Regional Distribution Characteristics

The epidemic time of EVD covers the whole year, does not show specific seasonal characteristics, and shows no significant correlation between outbreak years [49]. For example, the outbreak in Sudan in 1976 occurred from June to November; the four outbreaks in Gabon mainly occurred around January; the outbreak in Gabon in 1996 occurred from January to April; and the outbreak in the border area between Gabon and Congo occurred from October 2001 to March 2002; the three outbreaks in Uganda mainly occurred around October, while the four outbreaks in Congo and the outbreaks in DRC were distributed in all months of the year [4]. The population is generally susceptible to Ebola virus, which affects people of all ages, from infancy to old age [5, 16], with a lower incidence in children and the elderly and a higher incidence in women and healthcare workers, which may be related to the

frequent exposure of women and healthcare workers accompanying EVD patients.

As shown in Fig. 5.1, as of December 2018, DRC had a total of 10 EVD outbreaks, the most recent one still continuing [4]; Sudan had three EVD outbreaks during 1976–2004, mainly in the southern region; Gabon had four EVD outbreaks, mainly in the northern region; Uganda had four EVD outbreaks, mainly in the northwestern region; and the Republic of the Congo had four EVD outbreaks, mainly in the northwestern region. The emergence of Ebola virus subtypes in Côte d'Ivoire differs from the past, and only one case has been found. Sporadic cases in South Africa came mainly from the importation of the EVD epidemic in Gabon [97]. The 2013–2016 EVD outbreak in West Africa, Guinea, Liberia, and Sierra Leone had EVD cases spread throughout the country, while Nigeria and Mali had local transmission due to the importation of EVD cases, but were limited to transmission in small areas, and Senegal had only imported cases and no local transmission [16].

In the EVD outbreak in West Africa, imported cases of EVD occurred in the USA, the UK, Spain, and Italy, but did not cause local transmission [16]. The incidence of infection in laboratory workers was reported three times in the UK and Russia (see Fig 5.1) [8, 44, 95]. As shown in Table 5.1, EVD outbreaks in macaques have also been reported in the UA, Italy, and the Philippines, in which infected macaques in both the USA and Italy were from the Philippines; the virus was first isolated from pigs in 2008 in the Philippines and pigs in 2011 in China; Ebola virus antibodies have been detected in the sera of people who have been exposed to macaques and pigs in the USA and the Philippines, but none of them developed clinical symptoms of EVD [98–102]. Apart from laboratory infections, no locally infected cases of EVD have been identified outside Africa, but antibodies to Ebola virus can be detected in residents of some African regions without EVD outbreak [83, 103].

5.5.2 Distribution Characteristics of Virus Subtypes

Among the 28 EVD outbreaks, there were 19 EBOV outbreaks, six SUDV outbreaks, two BDBV outbreaks, and one TAFV outbreak (see Fig. 5.1), EBOV outbreaks were most common in western Central Africa and West Africa, and SUDV and BDBV outbreaks were most common in eastern Central Africa [16]. EBOV was mainly distributed in Liberia, Guinea, Sierra Leone, Nigeria, and Mali of West Africa, and also in Gabon, DRC, and Congo; SUDV was mainly distributed in Sudan and Uganda; BDBV was distributed in Uganda and Congo; TAFV was isolated from only one patient in Côte d'Ivoire, and RESTV was mainly distributed in the Philippines, the USA, Italy, and China. Each human EVD outbreak has its specific viral subtype, and outbreaks caused by EBOV are the most frequent, except for Uganda and DRC, where outbreaks of different viral subtypes have occurred; other countries in Africa are limited to outbreaks of only one viral subtype. In terms of virulence and mortality, the case fatality rate of BDBV infection is about 40% and that of SUDV infection is about 50%, while the case fatality rate of EBOV infection is the highest, approximately 70–90%, and the case fatality rate of TAFV infection is still unclear, with only one case reported historically, while RESTV causes disease and death only in nonhuman primates [2].

It is a fact that outbreaks and transnational, cross-continental transmission of EVD had repeatedly occurred in Africa. Understanding the distribution pattern of natural foci of EVD and mastering the occurrence and dynamic development trend of outbreaks are helpful for targeted disease entry surveillance and follow-up investigation, which is of great significance to prevent the imported transmission of EVD.

5.6 Influencing Factors

The mechanism by which Ebola virus is transmitted from host animals to humans is not clear, but large-scale outbreaks of EVD are inseparable from the influence of factors such as the natural environment, human behavior and cultural customs, and socioeconomic and political factors [49]. Fang et al. evaluated the efficacy of Sierra Leone's national strategic prevention and control program (including isolation of patients, increasing beds, and performing safe funerals) in controlling EVD, and systematically explored the nonlinear relationship between meteorological, socioeconomic factors and EVD transmission. The study found that interventions such as the National Strategic Prevention and Control Program were confirmed to effectively reduce the risk of EVD transmission by 43–65%, and the results of univariate analysis showed that the distribution of primary roads, secondary roads and railways, population density, and building area had a significant positive correlation with the spread of EVD; three factors, including the distance to the nearest hospital, the distance to the nearest Ebola treatment center (ETC), and the forest coverage area, had a significant negative correlation with the spread of EVD; through further multivariate analysis, the optimal model showed that the spread of EVD was closely related to the distribution of primary roads, secondary roads, and hospitals. The study also showed that these influencing factors, such as religion, temperature, humidity, population density, farmland distribution, and healthcare facility distribution, had a significant nonlinear relationship with EVD epidemics [30]. The results of a study on the risk factors associated with the Sudanese EVD outbreak in Uganda in 2000 showed that 20 of 83 contacts in this area were affected, and through epidemiological investigation and univariate analysis of these 83 contacts, it was found that direct contact with patients was more dangerous than indirect contact with patients, and the more routes of contact, the higher risk of infection [79]. Other similar studies had also found that the prevalence and spread of EVD were associated with indicators such as agricultural production, income, road density, hospital bed density, number of unsafe funerals, and days from onset to hospitalization [104, 105].

5.6.1 Natural Environmental Factors

Before 2014, EVD occurred mainly in five Central African countries near the equator, and forests and mines in endemic areas belonged to high-risk sites for this disease source. A study conducted a serological survey of rural residents in Gabon, randomly selected 4349 adults and 362 children, and conducted a correlation study for factors such as EBOV serum antibodies and type of residence in the study population, and logistic regression analysis revealed that the positive rate of IgG antibodies in the serum of people near forest areas (32.4%) was higher than that of the general population (15.3%) [103]. In addition, the typical tropical rainforest climate in central Africa has created lush and dense forests and vast grasslands, making the animals and plants in this area have good natural growth sites. Central African regions are divided into two dry seasons and two rainy seasons throughout the year, and some studies suggest that multiple outbreaks in this region begin at the end of the rainy season or transition to the dry season, and researchers believe that at the end of the rainy season, the sudden dry ecological environment may be a trigger point for the emergence of outbreaks [97, 106]. By studying the location of each outbreak and the corresponding ecological environment, the results of niche model analysis showed that EVD outbreak may be associated with factors such as altitude, temperature, and humidity [107–109].

5.6.2 Factors of Human Behavior and Cultural Practices

The evidence for the correlation between EVD outbreak and human behavior is not yet complete, and some factors are even contradictory between regions [16], but both etiological surveys and epidemiological data have shown that outbreaks of EVD are closely related to forest operation activities of the population, and activities such as going to deep forest, cutting trees, reclaiming agricultural land, hunting and trafficking, and mining gold mines have greatly increased the chance of infection in humans in contact with virus-carrying animal hosts or infected animals [49, 110]. African residents have the habit of hunting animals, trafficking animals, and eating raw animals, such as bats and monkeys, and often have direct or indirect contact with wild animals, increasing their exposure to the Ebola virus; in addition, some local generations of customs, such as injecting monkey blood into humans to treat diseases and removing the clothing and jewelry of the deceased before funerals, have the potential to cause the local spread and prevalence of diseases [49]. Besides, the lack of awareness of prevention and traditional customs of people in some parts of Africa have also promoted the spread of EVD, such as the lack of scientific understanding of EVD, the belief that it is a disease caused by evil or devil, misunderstanding, fear, and exclusion of social and public health services associated with it; for example, residents of the Sadialu village in Sierra Leone who still choose to live with patients, refuse to go to the hospital, believe that the hospital is the place of death sentencing, and some even avoid local healthcare institutions [111].

5.6.3 Socioeconomic and Political Factors

Poverty (e.g., continuous expansion of the range of activities such as hunting, felling, and mining for survival), unsound systems in hospitals (e.g., lack of medical gloves, disposable injection needles), lack of awareness of protection by healthcare workers (lack of awareness of EVD), inefficiency of local government in epidemic prevention and control, and socioeconomic and political factors such as lack of resources, civil conflicts, and civil wars have a decisive impact on the spread and prevalence of EVD [49]. Larger outbreaks often last for months, which is closely related to the backward local medical and health conditions and technical means. Since there is no good diagnostic technique, they usually misdiagnose the epidemic as the infection of other tropical diseases at the early stage, thus misjudging the transmission and risk of the disease, resulting in the spread of the epidemic. For example, the Zaire EVD outbreak in Gabon in 1994 was considered yellow fever at the early stage. Some people also believe that the outbreak area of EVD has been in a state of economic backwardness and imperfect public health system for a long time due to many years of folk conflicts and failure policies, resulting in that the epidemic situation cannot be effectively controlled and even aggravating the spread of EVD. For example, Liberia and Sierra Leone have been devastated by civil war among the three countries with the most severe epidemic in West Africa from 2013 to 2016, while Guinea is a very poor country, and these socioeconomic and political reasons have greatly reduced the effectiveness of government departments in epidemic prevention and control [106].

Small-scale local outbreaks of EVD are inseparable from the results of a combination of natural environment and human production behavior, but large-scale outbreaks often expose the socioeconomic and political factors existing in the country in response to public health crises, which also suggests that countries should pay attention to strengthening the construction of national infectious disease epidemic response mechanisms and emergency response prevention and control systems.

5.7 Prevention and Control

The strong infectivity and incubation period up to 3 weeks of EVD make the disease easy to spread across borders, regions, and even cause local epi-

demics, posing a serious threat to human health and socioeconomic sustainable development. Therefore, preventing and controlling outbreaks and epidemics of EVD has become a global public health problem that must be faced by countries around the world [49, 112] and has also prompted continuous strengthening of relevant research efforts in this field internationally.

5.7.1 Drugs and Vaccines

There is no specific treatment for EVD worldwide, and there is no specific drug approved for marketing. Although some antiviral drugs have obtained good therapeutic effects in animal experiments, their safety and effectiveness in clinical trials still need to be confirmed by scientific research. At present, the treatment is based on symptomatic and supportive treatment to minimize viral replication and slow down the disease process and gain time for eliminating infections through natural and adaptive immunity, and the main measures include general treatment, antiviral therapy, monoclonal antibody therapy, and symptomatic treatment against cytokine storms and multiple organ failure [1, 2, 16, 19, 59]. The outbreak of EVD in West Africa has rapidly driven clinical research on multiple EVD drugs with good results; for example, the USA urgently approved the entry of two drugs, ZMapp and TKM-Ebola, into clinical trials, and ZMapp was also used in two American patients and one Spanish patient, two of whom in the USA improved [113]; a case report from January 2017 showed that the first newborn with EVD successfully survived a combination of the three drugs [114]. Chinese investigators used the favipiravir (T-705) for the first time in Sierra Leone for the treatment of EVD and achieved good efficacy [35]. Chen and other Chinese researchers have developed the world's first ebola virus vaccine with recombinant adenovirus type 5 Ebola virus vector to enter the clinic and completed a phase 2 clinical trial, which has made a breakthrough [38]; the rVSV-ZEBOV recombinant vesicular stomatitis virus vector vaccine developed by US researchers is currently the most effective vaccine confirmed, which has carried out clinical trials in Guinean epidemic areas, and the efficacy rate of the vaccine is as high as 100% (95% CI 74.7–100) [115]. The immune response induced by this type of vaccine has the advantages of rapid response, high level, and long duration, and has a good prospect, which has been demonstrated to have a good protective effect in EVD outbreaks in West Africa and EVD outbreaks in the Congo in the past two years after ring vaccination intervention [116–118]. However, it has also been pointed out that vaccine-induced immunity is still not rapid enough to prevent EVD in humans, even when the vaccine is administered as soon as possible after exposure [119].

5.7.2 Control and Intervention

To control the spread of EVD, prevention and control measures are mainly used to control the source of infection and cut off the route of transmission and protect the population, including emergency isolation, national education, judicial intervention, and monitoring and tracking [15]. It has been shown that the spread of EVD can be effectively prevented by early diagnosis, patient isolation and care, infection control, safe and dignified burial of those who die of EVD, strict tracking of contacts, and the use of targeted vaccines [58, 96, 115, 117, 119]. Key elements of EVD prevention and control have also been published by US CDC experts, involving monitoring, isolation, investigation, tracking, education, reporting, training, etc. [73, 120]. When the EVD epidemic occurs, early detection and confirmation of cases; implementation of strict isolation measures for patients and infected individuals; blockade of epidemic areas and strict control of population movement in epidemic areas; close observation of contacts and timely follow-up investigation and handling; necessary ideological communication and publicity work to strengthen effective personal protection from high-risk exposures, especially self-protection of staff such as hospitals and laboratories;

maintenance of daily case reports; prohibition of holding clustered activities such as traditional funerals; and strengthening effective hospital and community infection control and other measures, including improving personal protective equipment, self-protection skills training, strict disinfection measures, and strengthening laboratory biosafety, can play an important role in controlling the spread of EVD [49, 121, 122]. Muoghalu et al. showed that the transmission rate of EVD in the community declined to a rather low level as EVD cases were removed from the community and entered the community EVD care center. In addition, with the implementation of comprehensive measures for prevention and control, the transmission caused by iatrogenic infection has also been further reduced [104].

The prevention and control of EVD have always been the focus of global attention, and the emergence of EVD has challenged the fragile health and epidemic prevention system in African underdeveloped countries and also reminded other countries to remain vigilant in responding to the threat of infectious diseases. With the acceleration of globalization, cooperation and exchanges in the fields of trade, labor, education, tourism, etc. are becoming more and more frequent among countries around the world, and convenient transportation and frequent personnel movement greatly increase the risk of cross-regional spread of EVD, and countries outside Africa are facing the potential threat of EVD importation. Therefore, international, national, and regional cooperation should be strengthened to establish an effective international quarantine, monitoring, and sharing system to effectively block the export of EVD from natural foci and local outbreak [123, 124].

5.8 Prospects and Challenges

Ebola virus is a type of zoonotic virus, and although related studies on Ebola virus emerge endlessly, some scientific questions remain unsolved, including (1) What is the natural host? (2) Can it be transmitted through aerosols? (3) How does the rate of virus mutation change? (4) How to assess the risk dynamics of its cross-species, cross-regional transmission? (5) What are the exact causes of Ebola virus spillover of wild animals infecting humans and causing outbreaks? (6) How does the change in the physical geographical ecological environment, ecological behavior of host animals, and human production behavior exacerbate the spread of Ebola virus in nature? Answering these questions still needs further in-depth study. To effectively prevent and control the spread of EVD worldwide, it is necessary not only to develop appropriate and effective diagnosis and treatment specifications and anti-EVD drugs and vaccines, but also to further carry out EVD-related social ecology, epidemiology, and molecular biology research, which can help us to more deeply understand the epidemiological characteristics of the epidemic that has occurred, to predict and prevent the epidemic that will occur, including determining the risk area, affected population, and risk factors, and to explore its transmission mechanism. Although the focus of attention is more on how to quickly and effectively eliminate the epidemic in the short term, attention and research on the above scientific issues will be conducive to guiding effective prevention and control.

Since entering the twenty-first century, the EVD epidemic in Africa has become more and more frequent and involves more and more extensive regions. It suggests that in recent years, the contact between humans and wild animals has been increasing, the cross-regional spread of EVD epidemic has occurred continuously, the impact of EVD far exceeds the number of reported EVD cases and deaths, and the economic burden caused by large-scale outbreaks of EVD epidemic and the negative impact on other public healthcare services in the affected area countries have also become a tragic lesson [125]. Therefore, the global public health and safety situation is still not optimistic in the future, and

improving the ability to effectively prevent and control and cope with public health crises should be a key area that needs to be focused on by each country. EVD outbreaks are closely related to the limited and fragile public health system in West Africa, especially related to the old public health infrastructure of countries in affected areas and the lack of public knowledge about Ebola virus and its transmission [49]. In the future, the international community should seek reform in emergency response capacity, international financing, budget allocation and human resource construction, and timely transfer of the focus of work to the recovery and reconstruction of countries in epidemic areas. Besides, the key is to help countries in epidemic areas establish a stronger public health system and actively carry out assistance and cooperation in the fields of disease prevention and control, personnel training, and drug research and development.

For China, global public health events are frequent, and putting forward the gateway of infectious disease prevention and control is the overall trend. Although China sets a good example for global EVD prevention and control and promotes the global understanding of the key scientific problems of EVD with valuable experience in assisting local governments to fight the Ebola epidemic in Africa, together with the existing EVD research results, due to the wide region, large population movement, and high population density, there are still many challenges in EVD prevention and control in China, and many scientific problems still need to make breakthroughs [14, 15, 126, 127]. It includes technical reserves in EVD detection, treatment, and prevention; the gap of the construction of emergency detection forces such as "virus detection technology, biosafety protection, laboratory capacity and mobile capacity construction" compared with Europe and the USA; the shortcomings in biosafety protection and team management; further breakthroughs in vaccines and drugs in clinical trials; the participation of international cooperation, the establishment of foreign research platforms; the trial site carrying out epidemiological prospective studies; and the scientific establishment of emergency response organization command system and mechanism. In addition, the WHO has issued some guidelines related to EVD prevention and control involving virus detection, storage and transportation of experimental samples, clinical nursing, prevention of medical staff, and prevention and control of inbound and outbound infectious diseases. China should share rights and interests and resource allocation with the international community, strengthen the capacity level of international rescue teams, and reflect the image and style of large countries. Furthermore, on the basis of existing results, it should improve the relevant guidelines for EVD prevention and control, diagnosis, and treatment adapted to China's national conditions and with guiding significance and further strengthen the emergency response capacity of public health incidents in China to effectively respond to its cross-border transmission.

5.9 Conflict of Interest

All authors declare no conflict of interest.

References

1. Khalafallah MT, Aboshady OA, Moawed SA, Ramadan MS. Ebola virus disease: essential clinical knowledge. Avicenna J Med. 2017;7(3):96–102.
2. Feldmann H, Geisbert TW. Ebola haemorrhagic fever. Lancet. 2011;377(9768):849–62.
3. Caswell RJ, Manavi K. Emerging sexually transmitted viral infections: 1. Review of Ebola virus disease. Int J STD AIDS. 2017;28(13):1352–9.
4. WHO. Ebola virus disease. https://www.who.int/zh/news-room/fact-sheets/detail/ebola-virus-disease.
5. WHO. Ebola haemorrhagic fever in Zaire, 1976. Bull World Health Organ. 1978;56(2):271–93.
6. WHO. Ebola haemorrhagic fever in Sudan, 1976. Bull World Health Organ. 1978;56(2):247–70.
7. WHO. Ebola situation reports: Democratic Republic of the Congo. [cited Dec 31, 2018]. https://www.who.int/ebola/situation-reports/drc-2018/en/.

8. CDC. Years of Ebola virus disease outbreaks. [cited Dec 31, 2018]. https://www.cdc.gov/vhf/ebola/history/chronology.html.
9. Pourrut X, Kumulungui B, Wittmann T, Moussavou G, Delicat A, Yaba P, et al. The natural history of Ebola virus in Africa. Microbes Infect. 2005;7(7–8):1005–14.
10. Gatiso TT, Ordaz-Nemeth I, Grimes T, Lormie M, Tweh C, Kuhl HS, et al. The impact of the Ebola virus disease (EVD) epidemic on agricultural production and livelihoods in Liberia. PLoS Negl Trop Dis. 2018;12(8):e0006580.
11. World Bank Group. Update on the economic impact of the 2014 Ebola epidemic on Liberia, Sierra Leone, and Guinea. [cited Dec 31, 2018]. http://www.worldbank.org/content/dam/Worldbank/document/Economic%20Impact%20Ebola%20Update%202%20Dec%202014.pdf.
12. WHO. WHO statement on the meeting of the International Health Regulations Emergency Committee regarding the 2014 Ebola outbreak in West Africa. [cited Dec 31, 2018]. http://www.antarpath.com/wp-content/uploads/2018/12/whoStatement-Ebola.pdf.
13. You J, Mao Q. An improved word architecture for treatment of patients with Ebola virus disease in Liberia. Am J Trop Med Hyg. 2016;94(4):701–3.
14. Li C, Tong Y, Jiang T, Sun Y, Fang T. The role of international cooperation in the prevention and control of Ebola virus disease epidemic in Sierra Leone. Chin J Epidemiol. 2015;36(9):1026–8. (in Chinese)
15. Tong Y, Li C, Tan Y, He J, Gao B, Fang T. Ebola virus disease emergency response organization and command system in Sierra Leone. Chin J Epidemiol. 2015;36(9):1029–30. (in Chinese)
16. Baseler L, Chertow DS, Johnson KM, Feldmann H, Morens DM. The pathogenesis of Ebola virus disease. Annu Rev Pathol. 2017;12:387–418.
17. Merens A, Bigaillon C, Delaune D. Ebola virus disease: biological and diagnostic evolution from 2014 to 2017. Med Mal Infect. 2018;48(2):83–94.
18. Broadhurst MJ, Brooks TJ, Pollock NR. Diagnosis of Ebola virus disease: past, present, and future. Clin Microbiol Rev. 2016;29(4):773–93.
19. Zeitlin L, Whaley KJ, Olinger GG, Jacobs M, Gopal R, Qiu X, et al. Antibody therapeutics for Ebola virus disease. Curr Opin Virol. 2016;17:45–9.
20. Li J, Duan HJ, Chen HY, Ji YJ, Zhang X, Rong YH, et al. Age and Ebola viral load correlate with mortality and survival time in 288 Ebola virus disease patients. Int J Infect Dis. 2016;42:34–9.
21. Qin E, Bi J, Zhao M, Wang Y, Guo T, Yan T, et al. Clinical features of patients with Ebola virus disease in Sierra Leone. Clin Infect Dis. 2015;61(4):491–5.
22. Jiang T, Jiang JF, Deng YQ, Jiang BG, Fan H, Han JF, et al. Features of Ebola virus disease at the late outbreak stage in Sierra Leone: clinical, virological, immunological, and evolutionary analyses. J Infect Dis. 2017;215(7):1107–10.
23. Zhang X, Rong Y, Sun L, Liu L, Su H, Zhang J, et al. Prognostic analysis of patients with Ebola virus disease. PLoS Negl Trop Dis. 2015;9(9):e0004113.
24. Tong YG, Shi WF, Liu D, Qian J, Liang L, Bo XC, et al. Genetic diversity and evolutionary dynamics of Ebola virus in Sierra Leone. Nature. 2015;524(7563):93–6.
25. Ni M, Chen C, Qian J, Xiao HX, Shi WF, Luo Y, et al. Intra-host dynamics of Ebola virus during 2014. Nat Microbiol. 2016;1(11):16151.
26. Li T, Yao HW, Liu D, Ren HG, Hu Y, Kargbo D, et al. Mapping the clinical outcomes and genetic evolution of Ebola virus in Sierra Leone. JCI. Insight. 2017;2(15)
27. Deng L, Liu M, Hua S, Peng Y, Wu A, Qin FX, et al. Network of co-mutations in Ebola virus genome predicts the disease lethality. Cell Res. 2015;25(6):753–6.
28. Jiang S, Wang K, Li C, Hong G, Zhang X, Shan M, et al. Mathematical models for devising the optimal Ebola virus disease eradication. J Transl Med. 2017;15(1):124.
29. Xia ZQ, Wang SF, Li SL, Huang LY, Zhang WY, Sun GQ, et al. Modeling the transmission dynamics of Ebola virus disease in Liberia. Sci Rep. 2015;5:13857.
30. Fang LQ, Yang Y, Jiang JF, Yao HW, Kargbo D, Li XL, et al. Transmission dynamics of Ebola virus disease and intervention effectiveness in Sierra Leone. Proc Natl Acad Sci USA. 2016;113(16):4488–93.
31. Liu G, Wong G, Su S, Bi Y, Plummer F, Gao GF, et al. Clinical evaluation of Ebola virus disease therapeutics. Trends Mol Med. 2017;23(9):820–30.
32. Yang M, Ke Y, Wang X, Ren H, Liu W, Lu H, et al. Development and evaluation of a rapid and sensitive EBOV-RPA test for rapid diagnosis of Ebola virus disease. Sci Rep. 2016;6:26943.
33. Chen Z, Liang H, Chen X, Ke Y, Zhou Z, Yang M, et al. An Ebola virus-encoded microRNA-like fragment serves as a biomarker for early diagnosis of Ebola virus disease. Cell Res. 2016;26(3):380–3.
34. Li WG, Chen WW, Li L, Ji D, Ji YJ, Li C, et al. The etiology of Ebola virus disease-like illnesses in Ebola virus negative patients from Sierra Leone. Oncotarget. 2016;7(19):27910–5.
35. Bai CQ, Mu JS, Kargbo D, Song YB, Niu WK, Nie WM, et al. Clinical and Virological characteristics of Ebola virus disease patients treated with favipiravir (T-705)-Sierra Leone, 2014. Clin Infect Dis. 2016;63(10):1288–94.
36. Wang H, Wong G, Zhu W, He S, Zhao Y, Yan F, et al. Equine-origin immunoglobulin fragments protects nonhuman primates from Ebola virus disease. J Virol. 2018;
37. Shuai L, Wang X, Wen Z, Ge J, Wang J, Zhao D, et al. Genetically modified rabies virus-vectored Ebola virus disease vaccines are safe and induce effi-

cacious immune responses in mice and dogs. Antivir Res. 2017;146:36–44.
38. Zhu FC, Wurie AH, Hou LH, Liang Q, Li YH, Russell JB, et al. Safety and immunogenicity of a recombinant adenovirus type-5 vector-based Ebola vaccine in healthy adults in Sierra Leone: a single-centre, randomised, double-blind, placebo-controlled, phase 2 trial. Lancet. 2017;389(10069):621–8.
39. Gao R, Chen C, Wang F, Wang H, Jiang T, Sun Y, Fang T. Analysis of mechanism for international joint prevention and control of Ebola virus disease in Sierra Leone. Chin J Epidemiol. 2015;36(9):1031–3. (in Chinese)
40. Huang Q, Fu WL, You JP, Mao Q. Laboratory diagnosis of Ebola virus disease and corresponding biosafety considerations in the China Ebola treatment center. Crit Rev Clin Lab Sci. 2016;53(5):326–40.
41. Jiang H, Shi GQ, Tu WX, Zheng CJ, Lai XH, Li XX, et al. Rapid assessment of knowledge, attitudes, practices, and risk perception related to the prevention and control of Ebola virus disease in three communities of Sierra Leone. Infect Dis Poverty. 2016;5(1):53.
42. Chen T, Ka-Kit Leung R, Liu R, Chen F, Zhang X, et al. Risk of imported Ebola virus disease in China. Travel Med Infect Dis. 2014;12(6 Pt A):650–8.
43. Zawilińska B, Kosz-Vnenchak M. General introduction into the Ebola virus biology and disease. Folia Med Cracov. 2014;54(3):57–65.
44. Li Y, Ren X, Li D, Cheng Y, Gao F, Yu HJ. Ebola virus disease: epidemiology, ecology, diagnosis, treatment, and control. Sci Technol Rev. 2014;32(24):15–24. (in Chinese)
45. Goldstein T, Anthony SJ, Gbakima A, Bird BH, Bangura J, Tremeau-Bravard A, et al. The discovery of Bombali virus adds further support for bats as hosts of ebolaviruses. Nat Microbiol. 2018;3(10):1084–9.
46. Gire SK, Goba A, Andersen KG, Sealfon RS, Park DJ, Kanneh L, et al. Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak. Science. 2014;345(6202):1369–72.
47. Nakayama E, Saijo M. Animal models for Ebola and Marburg virus infections. Front Microbiol. 2013;4:267.
48. Weingartl HM, Embury-Hyatt C, Nfon C, Leung A, Smith G, Kobinger G. Transmission of Ebola virus from pigs to non-human primates. Sci Rep. 2012;2:811.
49. Yang XL, Ge XY, Hu B, Shi ZL, Zhang YZ, Fang LQ, et al. The epidemiology of Ebola virus disease. J ZheJiang U (Med Sci). 2014;43(06):621–45. (in Chinese)
50. Swanepoel R, Leman PA, Burt FJ, Zachariades NA, Braack LE, Ksiazek TG, et al. Experimental inoculation of plants and animals with Ebola virus. Emerg Infect Dis. 1996;2(4):321–5.
51. Leroy EM, Epelboin A, Mondonge V, Pourrut X, Gonzalez JP, Muyembe-Tamfum JJ, 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(6):723–8.
52. Pourrut X, Delicat A, Rollin PE, Ksiazek TG, Gonzalez JP, Leroy EM. Spatial and temporal patterns of Zaire ebolavirus antibody prevalence in the possible reservoir bat species. J Infect Dis. 2007;196(Suppl 2):S176–83.
53. Towner JS, Amman BR, Sealy TK, Carroll SA, Comer JA, Kemp A, et al. Isolation of genetically diverse Marburg viruses from Egyptian fruit bats. PLoS Pathog. 2009;5(7):e1000536.
54. Leroy EM, Kumulungui B, Pourrut X, Rouquet P, Hassanin A, Yaba P, et al. Fruit bats as reservoirs of Ebola virus. Nature. 2005;438(7068):575–6.
55. Ansumana R, Jacobsen KH, Sahr F, Idris M, Bangura H, Boie-Jalloh M, et al. Ebola in Freetown area, Sierra Leone—a case study of 581 patients. N Engl J Med. 2015;372(6):587–8.
56. Schieffelin JS, Shaffer JG, Goba A, Gbakie M, Gire SK, Colubri A, et al. Clinical illness and outcomes in patients with Ebola in Sierra Leone. N Engl J Med. 2014;371(22):2092–100.
57. Bwaka MA, Bonnet MJ, Calain P, Colebunders R, De Roo A, Guimard Y, et al. Ebola hemorrhagic fever in Kikwit, Democratic Republic of the Congo: clinical observations in 103 patients. J Infect Dis. 1999;179(Suppl 1):S1–7.
58. WHO Ebola Response Team. Ebola virus disease in West Africa—the first 9 months of the epidemic and forward projections. N Engl J Med. 2014;371(16):1481–95.
59. Li LJ, Xu JG, Gao F. Ebola virus disease. Zhejiang: Zhejiang University Press; 2015. (in Chinese)
60. Heffernan RT, Pambo B, Hatchett RJ, Leman PA, Swanepoel R, Ryder RW. Low seroprevalence of IgG antibodies to Ebola virus in an epidemic zone: Ogooue-Ivindo region, northeastern Gabon, 1997. J Infect Dis. 2005;191(6):964–8.
61. Leroy EM, Baize S, Volchkov VE, Fisher-Hoch SP, Georges-Courbot MC, Lansoud-Soukate J, et al. Human asymptomatic Ebola infection and strong inflammatory response. Lancet. 2000;355(9222):2210–5.
62. Akerlund E, Prescott J, Tampellini L. Shedding of Ebola virus in an asymptomatic pregnant woman. N Engl J Med. 2015;372(25):2467–9.
63. Lotsch F, Schnyder J, Goorhuis A, Grobusch MP. Neuropsychological long-term sequelae of Ebola virus disease survivors - a systematic review. Travel Med Infect Dis. 2017;18:18–23.
64. Uyeki TM, Mehta AK, Davey RT Jr, Liddell AM, Wolf T, et al. Clinical management of Ebola virus disease in the United States and Europe. N Engl J Med. 2016;374(7):636–46.
65. Hunt L, Gupta-Wright A, Simms V, Tamba F, Knott V, Tamba K, et al. Clinical presentation, biochemical, and haematological parameters and their association with outcome in patients with Ebola virus

disease: an observational cohort study. Lancet Infect Dis. 2015;15(11):1292–9.
66. Smit MA, Michelow IC, Glavis-Bloom J, Wolfman V, Levine AC. Characteristics and outcomes of pediatric patients with Ebola virus disease admitted to treatment units in Liberia and Sierra Leone: a retrospective cohort study. Clin Infect Dis. 2017;64(3):243–9.
67. Cherif MS, Koonrungsesomboon N, Diallo MP, Le Gall E, Kasse D, Cherif F, et al. The predictor of mortality outcome in adult patients with Ebola virus disease during the 2014–2015 outbreak in Guinea. Eur J Clin Microbiol Infect Dis. 2017;36(4):689–95.
68. Bebell LM, Oduyebo T, Riley LE. Ebola virus disease and pregnancy: a review of the current knowledge of Ebola virus pathogenesis, maternal, and neonatal outcomes. Birth Defects Res. 2017;109(5):353–62.
69. Feldmann H, Jones S, Klenk HD, Schnittler HJ. Ebola virus: from discovery to vaccine. Nat Rev Immunol. 2003;3(8):677–85.
70. Osterholm MT, Moore KA, Kelley NS, Brosseau LM, Wong G, Murphy FA, et al. Transmission of Ebola viruses: what we know and what we do not know. MBio. 2015;6(2):e00137.
71. Leroy EM, Rouquet P, Formenty P, Souquiere S, Kilbourne A, Froment JM, et al. Multiple Ebola virus transmission events and rapid decline of central African wildlife. Science. 2004;303(5656):387–90.
72. Olival KJ, Hayman DT. Filoviruses in bats: current knowledge and future directions. Viruses. 2014;6(4):1759–88.
73. Dixon MG, Schafer IJ, CDC. Ebola viral disease outbreak—West Africa, 2014. MMWR Morb Mortal Wkly Rep. 2014;63(25):548–51.
74. Baize S, Pannetier D, Oestereich L, Rieger T, Koivogui L, Magassouba N, et al. Emergence of Zaire Ebola virus disease in Guinea. N Engl J Med. 2014;371(15):1418–25.
75. Larsen T, Stevens EL, Davis KJ, Geisbert JB, Daddario-DiCaprio KM, Jahrling PB, et al. Pathologic findings associated with delayed death in nonhuman primates experimentally infected with Zaire Ebola virus. J Infect Dis. 2007;196(Suppl 2):S323–8.
76. WHO. What we know about transmission of the Ebola virus among humans. [cited Dec 31, 2018]. https://www.who.int/mediacentre/news/ebola/06-october-2014/en/.
77. Bausch DG, Towner JS, Dowell SF, Kaducu F, Lukwiya M, Sanchez A, et al. Assessment of the risk of Ebola virus transmission from bodily fluids and fomites. J Infect Dis. 2007;196(Suppl 2):S142–7.
78. Brainard J, Hooper L, Pond K, Edmunds K, Hunter PR. Risk factors for transmission of Ebola or Marburg virus disease: a systematic review and meta-analysis. Int J Epidemiol. 2016;45(1):102–16.
79. Francesconi P, Yoti Z, Declich S, Onek PA, Fabiani M, Olango J, et al. Ebola hemorrhagic fever transmission and risk factors of contacts. Uganda Emerg Infect Dis. 2003;9(11):1430–7.
80. Ajelli M, Parlamento S, Bome D, Kebbi A, Atzori A, Frasson C, et al. The 2014 Ebola virus disease outbreak in Pujehun, Sierra Leone: epidemiology and impact of interventions. BMC Med. 2015;13:281.
81. Busico KM, Marshall KL, Ksiazek TG, Roels TH, Fleerackers Y, Feldmann H, et al. Prevalence of IgG antibodies to Ebola virus in individuals during an Ebola outbreak, Democratic Republic of the Congo, 1995. J Infect Dis. 1999;179(Suppl 1):S102–7.
82. Becquart P, Wauquier N, Mahlakoiv T, Nkoghe D, Padilla C, Souris M, et al. High prevalence of both humoral and cellular immunity to Zaire ebolavirus among rural populations in Gabon. PLoS One. 2010;5(2):e9126.
83. Gonzalez JP, Nakoune E, Slenczka W, Vidal P, Morvan JM. Ebola and Marburg virus antibody prevalence in selected populations of the Central African Republic. Microbes Infect. 2000;2(1):39–44.
84. Hogardt M, Wolf T, Kann G, Brodt HR, Brandt C, Keppler OT, et al. Management of microbiological samples in a confirmed case of Ebola virus disease: constraints and limitations. J Clin Microbiol. 2015;53(11):3396–400.
85. Lancet T. Ebola: protection of health-care workers. Lancet. 2014;384(9961):2174.
86. Mate SE, Kugelman JR, Nyenswah TG, Ladner JT, Wiley MR, Cordier-Lassalle T, et al. Molecular evidence of sexual transmission of Ebola virus. N Engl J Med. 2015;373(25):2448–54.
87. Colebunders R, Borchert M. Ebola haemorrhagic fever—a review. J Infect. 2000;40(1):16–20.
88. Rowe AK, Bertolli J, Khan AS, Mukunu R, Muyembe-Tamfum JJ, Bressler D, et al. Clinical, virologic, and immunologic follow-up of convalescent Ebola hemorrhagic fever patients and their household contacts, Kikwit, Democratic Republic of the Congo. Commission de Lutte contre les Epidemies a Kikwit. J Infect Dis. 1999;179(Suppl 1):S28–35.
89. Deen GF, Broutet N, Xu W, Knust B, Sesay FR, McDonald SLR, et al. Ebola RNA persistence in semen of Ebola virus disease survivors—final report. N Engl J Med. 2017;377(15):1428–37.
90. WHO. Sexual and reproductive health interim advice on the sexual transmission of the Ebola virus disease. [cited Dec 31, 2018]. https://www.who.int/reproductivehealth/topics/rtis/ebola-virus-semen/en/.
91. Salvaggio MR, Baddley JW. Other viral bioweapons: Ebola and Marburg hemorrhagic fever. Dermatol Clin. 2004;22(3):291–302.
92. Leffel E, Reed D. Marburg and Ebola viruses as aerosol threats. 2004.
93. Wang ZY, Nie X, Guo ZD, Li JJ, Cheng HL, et al. Advances in research on the aerosol infection and transmission of Eboa virus. J Pathog Biol. 2018;13(08):914–6. (in Chinese)

94. Medina-Rivera M, Centeno-Tablante E, Finkelstein JL, Rayco-Solon P, Peña-Rosas JP, Garcia-Casal MN, et al. Presence of Ebola virus in breast milk and risk of mother-to-child transmission: synthesis of evidence. Ann N Y Acad Sci. 2021;1488(1):33–43.
95. Wikipedia. List of Ebola outbreaks. [cited Dec 31, 2018]. https://en.wikipedia.org/wiki/List_of_Ebola_outbreaks#cite_note-77.
96. Ebola Outbreak Epidemiology Team. Outbreak of Ebola virus disease in the Democratic Republic of the Congo, April–May, 2018: an epidemiological study. Lancet. 2018;392(10143):213–21.
97. Murray MJ. Ebola virus disease: a review of its past and present. Anesth Analg. 2015;121(3):798–809.
98. Pan Y, Zhang W, Cui L, Hua X, Wang M, Zeng Q. Reston virus in domestic pigs in China. Arch Virol. 2014;159(5):1129–32.
99. Jahrling PB, Geisbert TW, Dalgard DW, Johnson ED, Ksiazek TG, Hall WC, et al. Preliminary report: isolation of Ebola virus from monkeys imported to USA. Lancet. 1990;335(8688):502–5.
100. Philippine monkeys infected with Ebola not lethal to humans. [cited Dec 31, 2018]. https://www.philstar.com/headlines/2015/09/05/1496305/philippine-monkeys-infected-ebola-not-lethal-humans.
101. Hayes CG, Burans JP, Ksiazek TG, Del Rosario RA, Miranda ME, Manaloto CR, et al. Outbreak of fatal illness among captive macaques in the Philippines caused by an Ebola-related filovirus. Am J Trop Med Hyg. 1992;46(6):664–71.
102. Barrette RW, Metwally SA, Rowland JM, Xu L, Zaki SR, Nichol ST, et al. Discovery of swine as a host for the Reston ebolavirus. Science. 2009;325(5937):204–6.
103. Nkoghe D, Padilla C, Becquart P, Wauquier N, Moussavou G, Akue JP, et al. Risk factors for Zaire ebolavirus—specific IgG in rural Gabonese populations. J Infect Dis. 2011;204(Suppl 3):S768–75.
104. Muoghalu IS, Moses F, Conteh I, Swaray P, Ajudua A, Nordstrom A. The transmission chain analysis of 2014-2015 Ebola virus disease outbreak in Koinadugu District, Sierra Leone: an observational study. Front Public Health. 2017;5:160.
105. Wendelboe AM, McCumber M, Erb-Alvarez J, Mould N, Childs RW, Regens JL. Managing emerging transnational public health security threats: lessons learned from the 2014 west African Ebola outbreak. Glob Health. 2018;14(1):75.
106. Bausch DG, Schwarz L. Outbreak of ebola virus disease in Guinea: where ecology meets economy. PLoS Negl Trop Dis. 2014;8(7):e3056.
107. Pigott DM, Golding N, Mylne A, Huang Z, Henry AJ, Weiss DJ, et al. Mapping the zoonotic niche of Ebola virus disease in Africa. elife. 2014;3:e04395.
108. Walsh MG, Haseeb M. The landscape configuration of zoonotic transmission of Ebola virus disease in West and Central Africa: interaction between population density and vegetation cover. PeerJ. 2015;3:e735.
109. Ng S, Cowling BJ. Association between temperature, humidity and ebolavirus disease outbreaks in Africa, 1976 to 2014. Euro Surveill. 2014;19(35)
110. Groseth A, Feldmann H, Strong JE. The ecology of Ebola virus. Trends Microbiol. 2007;15(9):408–16.
111. Tambo E, Ugwu EC, Ngogang JY. Need of surveillance response systems to combat Ebola outbreaks and other emerging infectious diseases in African countries. Infect Dis Poverty. 2014;3:29.
112. Varma JK, Prezant DJ, Wilson R, Quinn C, Asaeda G, Cagliuso NV, et al. Preparing the health system to respond to Ebola virus disease in New York City, 2014. Disaster Med Public Health Prep. 2017;11(3):370–4.
113. CDC. Experimental Ebola drug may have helped 2 US patients. [cited Dec 31, 2018]. http://www.cidrap.umn.edu/news-perspective/2014/08/experimental-ebola-drug-may-have-helped-2-us-patients.
114. Dornemann J, Burzio C, Ronsse A, Sprecher A, De Clerck H, Van Herp M, et al. First newborn baby to receive experimental therapies survives Ebola virus disease. J Infect Dis. 2017;215(2):171–4.
115. Henao-Restrepo AM, Longini IM, Egger M, Dean NE, Edmunds WJ, Camacho A, et al. Efficacy and effectiveness of an rVSV-vectored vaccine expressing Ebola surface glycoprotein: interim results from the Guinea ring vaccination cluster-randomised trial. Lancet. 2015;386(9996):857–66.
116. Gsell PS, Camacho A, Kucharski AJ, Watson CH, Bagayoko A, Nadlaou SD, et al. Ring vaccination with rVSV-ZEBOV under expanded access in response to an outbreak of Ebola virus disease in Guinea, 2016: an operational and vaccine safety report. Lancet Infect Dis. 2017;17(12):1276–84.
117. Nkengasong JN, Onyebujoh P. Response to the Ebola virus disease outbreak in the Democratic Republic of the Congo. Lancet. 2018;391(10138):2395–8.
118. Zhang Z, Hou LH. Advances in clinical trials of Ebola vaccine. Infect Dis Inform 2018; 31(05):430–565. (in Chinese).
119. Fischer WA 2nd, Vetter P, Bausch DG, Burgess T, Davey RT Jr, Fowler R, et al. Ebola virus disease: an update on post-exposure prophylaxis. Lancet Infect Dis. 2018;18(6):e183–e92.
120. Shultz JM, Espinel Z, Espinola M, Rechkemmer A. Distinguishing epidemiological features of the 2013–2016 West Africa Ebola virus disease outbreak. Disaster Health. 2016;3(3):78–88.
121. Martinez MJ, Salim AM, Hurtado JC, Kilgore PE. Ebola virus infection: overview and update on prevention and treatment. Infect Dis Ther. 2015;4(4):365–90.
122. Shears P, O'Dempsey TJ. Ebola virus disease in Africa: epidemiology and nosocomial transmission. J Hosp Infect. 2015;90(1):1–9.

123. Bogoch II, Creatore MI, Cetron MS, Brownstein JS, Pesik N, Miniota J, et al. Assessment of the potential for international dissemination of Ebola virus via commercial air travel during the 2014 west African outbreak. Lancet. 2015;385(9962):29–35.
124. WHO, CDC. Infection control for viral haemorrhagic fevers. [cited Dec 31, 2018]. https://www.who.int/csr/resources/publications/ebola/whoemcesr982sec1-4.pdf.
125. Brolin Ribacke KJ, Saulnier DD, Eriksson A, von Schreeb J. Effects of the West Africa Ebola virus disease on health-care utilization - a systematic review. Front Public Health. 2016;4:222.
126. Liu W, Fang TY, Cao WC. The major progress on the Ebola virus disease study and international Ebola virus testing in Sierra Leone. Chin J Epidemiol. 2015;36(9):1023–5. (in Chinese)
127. Jiang T, Zhao G, Han J, Xin W, Zhuang D, Tan Y, He J, Gao R, Wang H, Chen C, et al. Comparison of international Ebola virus testing laboratories in Ebola virus disease outbreak in Sierra Leone. Chin J Epidemiol. 2015;36(9):1034–7. (in Chinese)