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Ebola virus - Epidemiology, Diagnosis, and Control: Threat to Humans, Lessons Learnt, and Preparedness Plans - an Update on Its 40 Year's Journey

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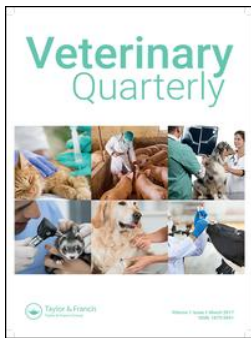
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Ebola virus – epidemiology, diagnosis, and control: threat to humans, lessons learnt, and preparedness plans – an update on its 40 year’s journey

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ABSTRACT

Ebola virus (EBOV) is an extremely contagious pathogen and causes lethal hemorrhagic fever disease in man and animals. The recently occurred Ebola virus disease (EVD) outbreaks in the West African countries have categorized it as an international health concern. For the virus maintenance and transmission, the non-human primates and reservoir hosts like fruit bats have played a vital role. For curbing the disease timely, we need effective therapeutics/prophylactics, however, in the absence of any approved vaccine, timely diagnosis and monitoring of EBOV remains of utmost importance. The technologically advanced vaccines like a viral-vectored vaccine, DNA vaccine and virus-like particles are underway for testing against EBOV. In the absence of any effective control measure, the adaptation of high standards of biosecurity measures, strict sanitary and hygienic practices, strengthening of surveillance and monitoring systems, imposing appropriate quarantine checks and vigilance on trade, transport, and movement of visitors from EVD endemic countries remains the answer for tackling the EBOV spread. Herein, we converse with the current scenario of EBOV giving due emphasis on animal and veterinary perspectives along with advances in diagnosis and control strategies to be adopted, lessons learned from the recent outbreaks and the global preparedness plans. To retrieve the evolutionary information, we have analyzed a total of 56 genome sequences of various EBOV species submitted between 1976 and 2016 in public databases.

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1. Introduction

The entry of Ebola virus disease (EVD) was first marked in the year 1976 (Vogel & Viale 2014) and since then it is a highly contagious virus infection leading to the death of large numbers of humans (Muyembe-Tamfum et al. 2012; Bausch & Schwarz 2014; Bellizzi 2014; Dhama, Malik, et al. 2015). The disease is zoonotic, and evidence suggests the involvement of fruit bats as the main reservoir (Towner et al. 2007; Towner et al. 2008; Spengler et al. 2016; Hassanin et al. 2016; Judson et al. 2016). Although it affects both human and non-human primates, the major outbreaks have been reported in humans, mostly in Western Africa (Weingart et al. 2013; Feldmann & Feldmann 2014; Shrivastava et al. 2015a, 2015b). The Ebola virus (EBOV) has been listed as Category A agent warranting biosafety containment level 4 (BSL4) for the handling of suspected samples. The disease symptoms start with fever, pain in stomach with vomiting and diarrhea,

bleeding, intravascular coagulation, multisystem organ failure, and hypovolemic shock and in terminal cases, the patient succumbs to death (Mendoza et al. 2015). The virus spreads from diseased to normal persons through direct contact and infected materials. This virus tends to persist in body leading to further complications such as loss of vision (Shantha et al. 2017), hearing (Hebert et al. 2017), etc. along with transmission through semen from male to female (Hartmann et al. 2017) and to fetus at the time of pregnancy (Friedrich 2016; Wiwanitkit 2016).

A major outbreak of EBOV, which occurred in 2014 in Western African countries, transpired to several other countries in a short span of time, culminating it as an alarming situation world over. The spread of EBOV was so rapid that family members and the community around infected people suffered sternly (Kuhn et al. 2010; Weingart et al. 2013; Gatherer 2014). Of note, affected young children showed comparatively

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shorter incubation period and rapid course of disease with high mortality (Team 2015). Owing to global trade and tourism, there is every possible opportunity that EBOV can spread to other continents where it may lead to massive outbreaks. The epidemics which occurred over the past few years should serve as an eye-opener to the world so that everyone is well prepared for the next pandemic if any occurs. In this direction, it is important that the nature of the agent, its evolution pattern, and pathway of spread must be understood at a subtle level. To deal with such a highly spreading disease we must be equipped with rapid state-of-art diagnostics which are useful at the bedside of the patient, ultimately benefiting in opting appropriate control and treatment measures. Likewise, there is an urgent need to have effective and adequate vaccines to provide protection to the population at high risk. Therapeutic strategies must be evaluated for their high potential.

Currently, there are five distinct EBOV species which have been described, and four of them (*Zaire ebolavirus*, *Sudanebolavirus*, *Tai Forest ebolavirus*, and *Bundibugyo ebolavirus*) cause disease in humans, whereas the *Reston ebolavirus* is known to cause disease in non-human primates solely. We here converse comprehensively over the developments ensued in understanding the etiopathology of EVD, ecology, prevention and control strategies along with various therapeutic alternatives.

2. The virus and its genome

EBOV belongs to the order *Mononegavirales* (single-stranded, non-segmented, negative-sense RNA virus) of the family *Filoviridae*, genus *Ebolavirus*. Other members of the family include *Marburgvirus* and *Cuevavirus*, of which *Marburgvirus* has also been implicated in causing hemorrhagic diseases similar to EBOV, and both of these are filamentous shape viruses. The genus *Marburgvirus* consists of a species *Marburg marburgvirus* with two viruses viz., Marburg virus (MARV) and Ravn virus (RAVV). The genus *Cuevavirus* consists of a single species – *Lloviu cuevavirus* with Lloviu virus (LLOV). The EBOV was first isolated in 1976 near Zaire valley in the Democratic Republic of Congo (Zaire) rooting its name from the place of isolation (Kuhn et al. 2010), initially named as an Ebola-like virus which was later changed into EBOV in the year 2002. The genus *Ebolavirus* consists of five species viz., (1) *Zaire ebolavirus* (*Zaire virus* – ZEBOV), (2) *Sudan ebolavirus* (*Sudan virus* – SUDV), (3) *Reston ebolavirus* (*Reston virus* – RESTV), (4) *Tai Forest ebolavirus* (*Tai Forest virus* – TAFV), and (5) *Bundibugyo ebolavirus* (*Bundibugyo virus* – BDBV) and Côte d'Ivoire ebolavirus (CIEBOV) (Kuhn et al. 2013; Bausch & Schwarz 2014; Bukreyev et al. 2014; Kuhn, Andersen, et al. 2014; Kuhn, Bào,

et al. 2014; <http://www.ictvonline.org/virusTaxonomy.asp>). The genetic diversity among species in the genus *Ebolavirus* ranges between 25% and 35% (Grard et al. 2011). Among the five species, ZEBOV and SUDV are extremely deadly, of which the former is most dangerous with more than 90% lethality (Bellizzi 2014; Toit 2014; Safari et al. 2015). ZEBOV was frequently documented in Central Africa and has caused the major outbreaks recently in Western African countries like Nigeria, Liberia, Guinea, Senegal, and Sierra Leone (Baize et al. 2014).

As shown in Figure 1, structurally the virions of filovirus show pleomorphism which includes long filaments (Latin-filum means thread), shaped like a '6', a 'U', or a circle. Viral filaments are 80 nm in diameter with up to 14,000 nm in length and are enveloped in a lipid membrane (<http://www.cdc.gov/vhf/virus-families/filoviridae.html>). The genome of EBOV is 19 kb in length, consists of seven genes which are arranged as – 3' (leader)- NP-VP35-VP40-GP-VP30-VP24-L-(trailer) 5' (Volchkov et al. 1999). These seven open reading frames encode structural nucleoprotein (NP), envelope glycoprotein (GP), and matrix proteins like viral proteins (VP24) (membrane-associated protein) and VP40. Except for GP, each of these genes encodes a single protein product. Apart from structural proteins, L (RNA polymerase), VP30 and VP35 (both polymerase matrix proteins) correspond to the major non-structural protein. These proteins exhibit different roles in virus pathogenesis like VP24 inhibits interferon signaling, VP35 is an antagonist of interferon, whereas VP40 has a role in budding and release of the virus (Feldmann & Geisbert 2011). VP40 has been recently found in the extracellular region like exosome that can affect the immune system of the host (Pleet et al. 2017). The GP gene codes for three different versions of GP, namely GP (contains GP1 attachment protein and the GP2 fusion/entry protein), soluble form (sGP – is produced from the unedited RNA transcript) and small soluble form (ssGP – is a truncated version of sGP). Most of these proteins have multiple functions. NP facilitates the genomic RNA encapsidation to form RNP complex (RNA + NP + + L), which plays an essential role in virus replication. The GP and VP40 are essential components of the viral envelope. GP has a role in virus attachment, entry, causing cell rounding, down-regulating host surface proteins, causes inflammation, cell damage, and cytotoxicity (Casillas et al. 2003; Geisbert et al. 2009; Olival et al. 2013). The nucleocapsid consists of the viral RNA complexed with five proteins, namely NP, VP24, VP30 and VP35, and L (Sanchez et al. 1993; Lee & Saphire 2009; Bharat et al. 2012; Basler 2014; Brauburger et al. 2014; Gallaher & Garry 2015; Dong et al. 2015; Jun et al. 2015). Unlike influenza A virus, the rate of genetic change is very slow in EBOV, but this virus diverged several thousand years ago (Suzuki & Gojobori 1997; Taylor et al. 2011).

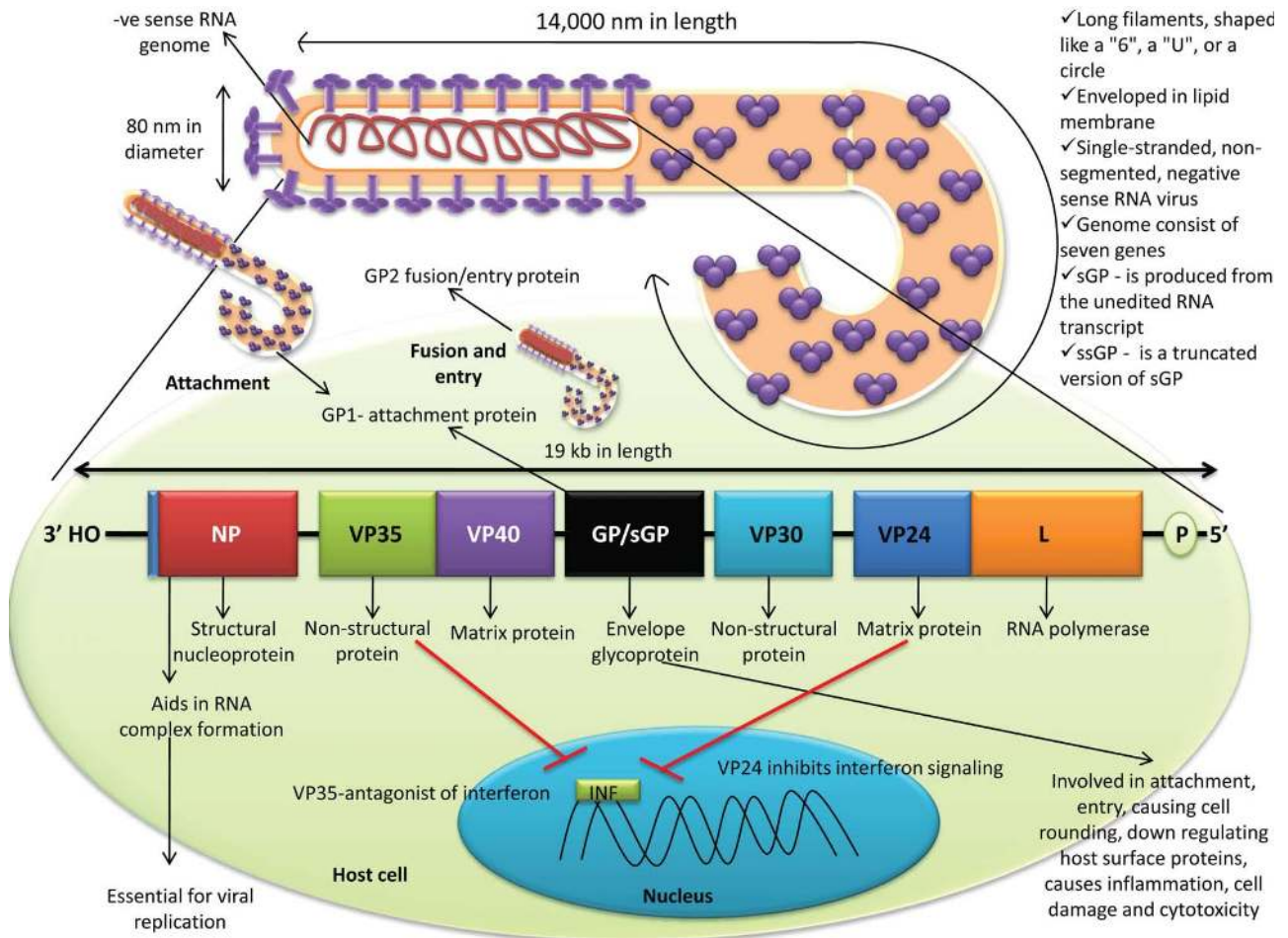


Figure 1. Structure of Ebola virus and its genome. Ebola virus possesses negative-sense RNA genome with exceptionally 14000 nm length with 3' nucleoprotein and 5' RNA polymerase end.

2.1. Evolutionary picture of Ebolaviruses

To retrieve the evolutionary information, we have analyzed a total of 56 genome sequences of various EBOV species submitted between 1976 and 2016 in public database, wherein the Zaire, Sudan, Reston, Taï, and Bundi EBOVs formed distinct clades (Figure 2). The ZEBOV strains organized into two distinct sub-clades, sub-clade 1 comprising of the isolates from Gabon and the Democratic Republic of Congo and sub-clade 2 clubbed Sierra Leone and Liberia isolates (Figure 2). The evolutionary divergence between the species was estimated using the Maximum Composite Likelihood model (Tamura et al. 2004). The number of transversal substitutions per site from the average of overall sequence pairs between groups was obtained. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). Codon positions included were 1st + 2nd + 3rd + Noncoding. There was a total of 17,999 positions in the final data-set. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013). Further, the analysis revealed that the Taï and Bundi EBOVs remain closely related to 17.8% mean group diversity; the ZEBOV had the mean group diversity of 26.1% and 26.8%, respectively with Taï and

Bundi EBOV. The SUDV outgroup from ZEBOV, Taï, and Bundi EBOVs revealed mean group diversity of 36.3%, 37.4%, and 37.6%, respectively. Similarly, RESTV outgroup from Zaire, Taï, and Bundi EBOVs came up with mean group diversity of 34.1%, 35.1%, and 34.8%, respectively. Likewise, the mean group diversity between Sudan and Reston EBOVs was found to be 36.6%.

2.2. Epidemiology, transmission, and spread

It is evident that since the first report of EVD, the number of ratified cases has increased several folds. The causality cases of EVD almost doubled in Africa in comparison to the earlier reported cases of the last four decades (6458 deaths out of 12,299 cases; Muyembe-Tamfum et al. 2012; Tambo et al. 2014; Bellizzi 2014) and the cases reported between 2014 and 2016 (12,922 deaths out of 31,079 cases) (CDC 2015) (Table 1). The 2014–2015 outbreak was assumed to be the biggest epidemic in the history of this disease (Gatherer 2014; Zhang & Wang 2014; Elstona et al. 2017). Though several strains of EBOV have been identified in the past, the 2014–2015 outbreak affecting

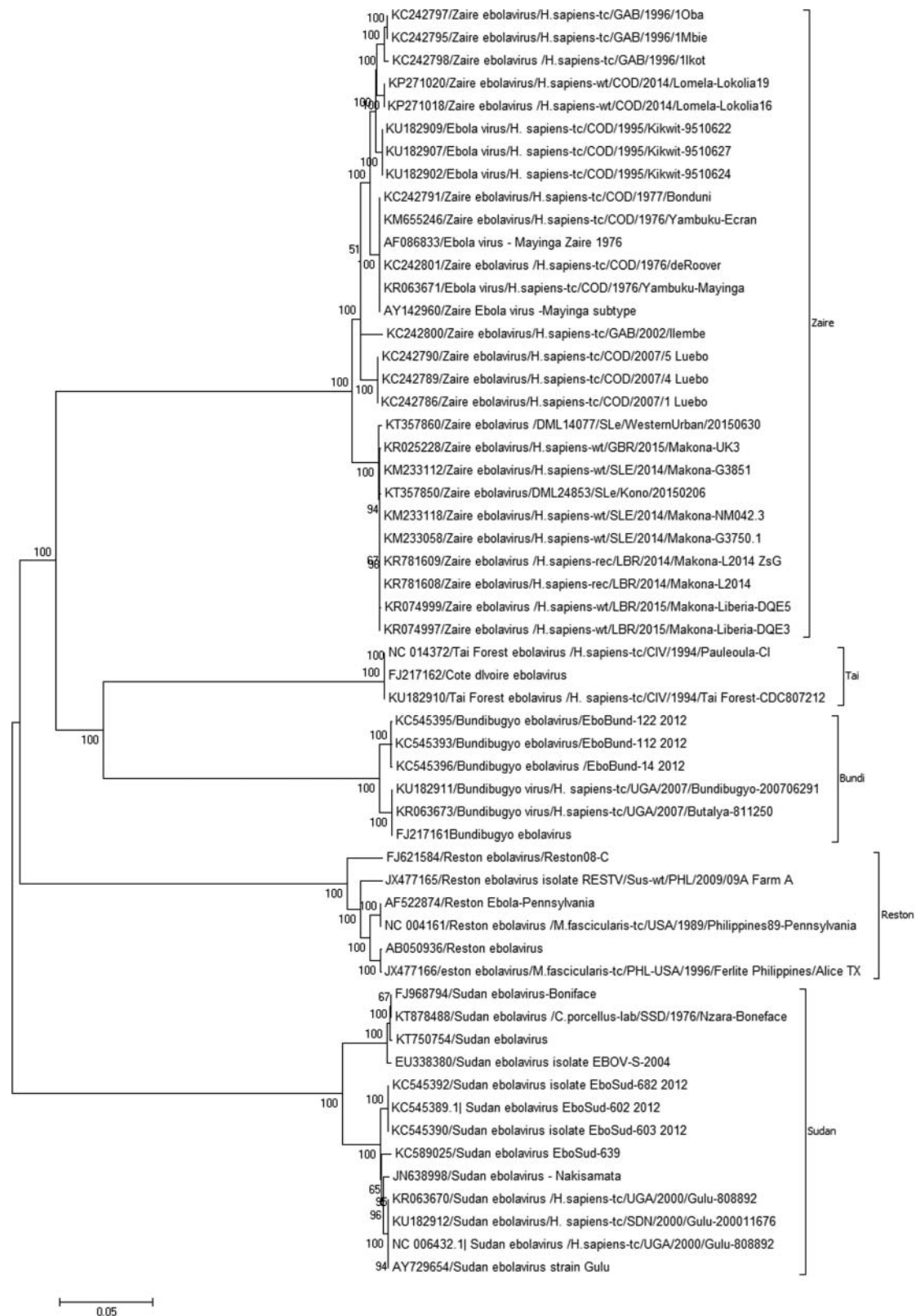


Figure 2. Evolutionary relationships of Ebola virus complete genomes. The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei 1987). The optimal tree with the sum of branch length = 1.06917633 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p -distance method (Nei & Kumar 2000) and are in the units of the number of base differences per site. The analysis involved 56 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + Noncoding. All positions containing gaps and missing data were eliminated. There was a total of 17,999 positions in the final data-set. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013).

Table 1. Outbreaks of *Ebolavirus* genus between 1976 and March 2016.

Ebola subtype	Number of transmission events	Country	Reported number of human cases	Reported number (%) of deaths among cases	Remarks
Recent outbreaks (since 2014)					
Ebolavirus	–	Multiple countries mainly three West African countries, namely Guinea, Liberia, Sierra Leone	28,652	11,325 (40)	Data between March 2014 and 27 March 2016. Seven countries (Italy, Mali, Nigeria, Senegal, Spain, the United Kingdom, and the United States of America) have previously reported a case or cases imported from a country with widespread and intense transmission.
Ebolavirus	1	DRC	66	49 (74)	August–November 2014; the outbreak was unrelated to the outbreak of Ebola in West Africa.
Outbreaks between 1976 and 2013					
Bundibugyo virus	2	DRC, Uganda	185	50 (27)	Four outbreaks in monkeys only; one outbreak in pigs with antibody detection in six workers; two outbreaks in monkeys with seven people developed antibodies.
Reston virus	7	Philippines, USA, Italy	13	0 (0)	
Sudan virus	8	South Sudan, Uganda, England	779	412 (53)	
Tai Forest virus	1	Côte d'Ivoire (Ivory Coast)	1	0 (0)	
Zaire virus	15	Republic of Congo, DRC, Russia, Gabon, South Africa	1383	1086 (79)	
Total			31079	12922	

Source: <http://www.cdc.gov/vhf/ebola/outbreaks/history/chronology.html>; <http://apps.who.int/ebola/current-situation/ebola-situation-report-30-march-2016>

mainly Western African countries (Guinea, Sierra Leone, Liberia, Senegal, and Nigeria) was confirmed due to ZEBOV (Bellizzi 2014). This strain was the first EBOV which caused the historic 1976 Ebola case affecting a middle age school teacher in the Democratic Republic of the Congo (Johnson 1978). Identifying the severity and out-of-control situation about EVD, the World Health Organization declared it as a 'Public Health Emergency of International Concern' (PHEIC). Owing to its high virulence and fast transmission capability, it is categorized under 'class A' bio-weapon organism (Balmith et al. 2016) and thus making it essential to have real-time monitoring of EVD cases and to look into any connectivity among the cases. For this, use of smart mobile phones reporting of EVD cases in West Africa was encouraged by epidemiologists as an effective technique. Use of social network analysis in this way will certainly help in understanding disease spread in a better way (Kangbai 2016). The case fatality rate observed in Ebola-affected cases in different countries varied between 25% and 100%.

The countries affected during 1976–2016 are depicted in Figure 3 and a historical timeline of EBOV is presented in Figure 4. The disease outbreaks caused by different EBOV species are given in Table 1 and chronological data of EBOV cases and fatality since its first incidence is summarized in Table 2.

2.2.1. Zaire ebolavirus (ZEBOV)

The first known Ebola outbreak occurred in 1976 in Sudan (later the causative agent was classified under Sudan virus – SUDV) and the Democratic Republic of

the Congo (DRC; previously known as Zaire) (Report of an International Commission 1978; Report of a WHO/International Study Team 1978) (Figures 3 and 4). In the DRC outbreak (between September and October 1976), 318 cases of acute viral hemorrhagic fever (VHF) with 280 deaths (case fatality rate – CFR 88%) occurred in and around the Yambuku region. The duration of the clinical disease was about one week with non-specific symptoms for the initial four days followed by a severe sore throat, maculopapular rash, abdominal pain, and bleeding from multiple sites – mainly from the gastrointestinal tract. The causative agent could be isolated from eight cases using Vero cell cultures and was named as Ebola virus (Report of an International Commission 1978). The index case (the first identified case) was a 44-year-old male instructor who has been treated for presumptive malaria at the Yambuku hospital, and from there the subsequent outbreaks emerged.

The first EBOV was isolated in 1976 (isolate E718) from the blood sample of a 42-year-old Belgian nursing sister (patient No. 718) who was working at Mission Hospital, Yambuku, DRC. The sister (Myriam Louise Ecran) was one of the first victims of Ebola hemorrhagic fever (EHF) and died on 30 September 1976 (CDC 2015; www.bbc.com/news/magazine-28262541). Researchers decided to name the virus to the nearby river – the Ebola, rather than the village name to avoid stigma to that village. In Belgian, the river named as l'Ebola (the indigenous Ngbandi name is Legbala) means 'white water' or 'pure water' (Report of an International Commission 1978; Report of a WHO/

Table 2. Chronological data of Ebola virus cases and fatality since its first incidence.

Years	Country	Cases reported	Deaths	Case fatality rate
1976	Zaire	318	280	88%
	Sudan	284	151	53.1%
	England	1	0	0%
1977	Zaire	1	1	100%
1979	Sudan	34	22	64.7%
1989–1990	Philippines	3	0	0%
1990	USA	4	0	0%
1994	Gabon	52	31	59.6%
	Côte d'Ivoire Ivory Coast	1	0	0%
1995	Democratic Republic of Congo	315	250	71.4%
1996	Gabon	37	21	56.7%
1996–1997	Gabon	60	45	75%
1996	South Africa	2	1	50%
2000–2001	Uganda	425	224	52.7%
2001–2002	Gabon	65	53	81.5%
	Republic of Congo	57	43	75.4%
	Republic of Congo	143	128	89.5%
2002–2003	Republic of Congo	35	29	82.8%
2003	South Sudan	17	7	41.1%
2004	Russia	1	1	100%
2007	Democratic Republic of Congo	264	187	70.8%
2007–2008	Uganda	149	37	24.8%
2008	Philippines	6	0	0%
2008–2009	Democratic Republic of Congo	32	15	46.8%
2011	Uganda	1	1	100%
2012	Uganda	11	4	36.3%
	Democratic Republic of Congo	36	13	36.1%
	Uganda	6	3	50%
2012–2013	United States of America	4	1	25%
	Spain	1	0	0%
	United Kingdom	1	0	0%
	Italy	1	0	0%
	Guinea	3814	2544	66.7%
	Sierra Leone	14124	3956	28%
	Senegal	1	0	0%
2014–2016	Mali	9	6	66.6%
	Liberia	10678	4810	45%
	Nigeria	20	8	40%
	Total	31013	12872	41.5%

Note: Data compiled from WHO situation reports apps.who.int/ebola/ebola-situation-reports?m=20141121 and CDC updates on Ebola virus <https://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/previous-updates.html>

The subsequent outbreak of EBOV occurred in June 1977 in Tanda (325 km from Yambuku), DRC and a nine-year-old girl died with typical clinical signs of EHF. No secondary cases were identified (Heymann et al. 1980). Later, a less extensive form of EBOV occurred, between July and October 1979, in Nzara and Yambio (25 km away from Nzara), wherein 22 deaths occurred out of 34 cases with CFR of 65% (Baron et al. 1983).

After 15 years of pause, three EBOV outbreaks occurred in northeastern Gabon between 1994 and 1997. The first epidemic (fall 1994) occurred in Mekouka along with co-infection of yellow fever virus. In this epidemic, 49 cases of EVD, with 29 deaths (CFR 59%) occurred. A second epidemic (spring 1996) in Mayibout caused 31 cases with 21 deaths (CFR 68%) and of note a chimpanzee was suspected as the index case. The third epidemic (fall 1996) occurred in Booué and caused 60 cases with 45 deaths (CFR 75%). Several chimpanzees died in this area, and EBOV-antigen could be detected from one of the skin samples by immunostaining (Georges et al. 1999). Partial GP gene sequence analysis revealed that the causative agent behind all these three outbreaks was EBOV (Georges-

Courbot et al. 1997). Additionally, this outbreak also led to the death of a nurse in South Africa which was transmitted from an EBOV-infected Gabonese physician working in Libreville and traveled to Johannesburg for treatment (WHO 1996; Pourrut et al. 2005).

Afterwards, one of the largest EBOV outbreaks occurred in the city of Kikwit, DRC (~500 km southeast of Kinshasa), between January and July 1995 with 255 deaths out of 315 cases (CFR 81%). Of note, a 42-year-old male charcoal worker was identified as index case who had acquired infection from some natural reservoir such as bats, as no great apes were found in that region (Guimard et al. 1999; Khan et al. 1999).

In Tai Forest, Côte d'Ivoire, between 1996 and 1997, autopsy samples from several species of vertebrates including bats, rodents, insectivores, monkeys, and birds were screened, and anti-EBOV IgG could be detected only from a Colobe bai monkey (red colobus, *Colobus badius*) (Pourrut et al. 2005). During ecological studies in the Central African Republic (CAR) in 1998, 242 vertebrates include bats, rodents and insectivores were captured and organs tested for the presence of EBOV nucleic acid by GP and L genes-based reverse

transcription-polymerase chain reaction (RT-PCR). Seven animals including six mice (*Mus setulosus* and *Praomys* spp.) and a shrew (*Sylvisorex ollula*) were found harboring EBOV (Morvan et al. 1999).

Between October 2001 and December 2003, at least five EBOV outbreaks occurred in the border areas of northeast Gabon and northwest Republic of Congo (RC) with 313 cases and 265 deaths (CFR 85%). The first outbreak (October 2001–May 2002) occurred in both Gabon and RC; the outbreak occurred as multiple independent epidemic chains with 92 cases and 70 deaths (CFR 77%). Epidemiologic investigations suggest the possible involvement of carcasses of wild animals (bushmeat) including duikers, chimpanzees, and gorillas in the index patients. In January 2002, in Franceville, a single case was reported in the south of Gabon (Nkoghe, Nnogue, et al. 2005) and the remaining four outbreaks occurred only in RC.

In a later outbreak (Entsiami – January 2002 to June 2002), a total of 30 cases with 25 deaths (CFR 83%) occurred; a gorilla and a duiker were suspected to be the source for the index cases. In the following outbreak (Oloba – May to June 2002), 13 cases with 12 deaths (CFR 92%) occurred, and a chimpanzee was identified as the source for index case. In another outbreak (Mbomo and Kéllé – December 2002 to April 2003) 143 cases with 129 deaths (CFR 90%) occurred; gorillas and duikers were suspected to be the source of infection for the index cases. The last outbreak (Mbanza and Mbomo – November 2003 and December 2003) caused 35 cases with 29 deaths (CFR 83%). However, the source of infection could not be traced (<http://www.who.int/wer/2003/en/wer7826.pdf>; Formenty et al. 2003; Leroy et al. 2004; Pourrut et al. 2005; Rouquet et al. 2005; Nkoghe, Formenty, et al. 2005). Sequencing analysis of GP gene of EBOV revealed that the above five human outbreaks originated from distinct animal sources and viral strains (Leroy et al. 2004). These Gabon-RC cross-border outbreaks were marked by large wildlife epizootics associated with over 80% mortality especially in great apes (Leroy et al. 2004; Rouquet et al. 2005; Bermejo et al. 2006; Lahm et al. 2007; Gard et al. 2011).

After the first outbreak in the forest zone of Gabon-RC, an Animal Mortality Monitoring Network was created. The analysis of the samples recovered between August 2001 and June 2003 revealed that 10 gorillas, 3 chimpanzees, and 1 duiker were positive for EBOV infection (Rouquet et al. 2005). Another group of researchers from Centre International de Recherches Médicales de Franceville, Gabon analyzed a total of 34 carcasses in the same period, and 14 (10 gorillas, 3 chimpanzees, and 1 duiker) were found positive for EBOV infection (Pourrut et al. 2005).

In the seroprevalence study of EBOV in 20 species of non-human primates between 1985 and 2000 in

Cameroon, Gabon, and the RC, the wild chimpanzees showed 12.9% positivity. Also, few other monkey species (five drills, one baboon, one mandrill, and one *Cercocebus* sp.) were also positive indicating the complexity of the EBOV circulation and the possibility of involvement of more reservoir species (Leroy et al. 2004; Pourrut et al. 2005).

To identify the viral reservoir in the multiple outbreaks of Ebola in Gabon and RC between 2001 and 2005, the investigation was carried out from samples of over 1000 small vertebrates. Anti-EBOV IgG, as well as EBOV-specific RNA sequences, were detected from three different bat species (*Hypsignathus monstrosus*, *Epomops franqueti*, and *Myonycteris torquata*). The sequence confirmation of EBOV indicates that bats might serve as a natural reservoir of EBOV (Leroy et al. 2005; Pourrut et al. 2009). In a large-scale serological survey from these three bat species which were captured between 2003 and 2006 in Gabon and RC 5% EBOV infection positivity was shown, supporting their potential reservoir status (Pourrut et al. 2007; Pourrut et al. 2009). In a further extension of this study, samples were analyzed for EBOV from nine species of bats which were sampled between 2003 and 2008 and among these, 4% of the total population sampled belonged to six species (*Epomops franqueti*, *Hypsignathus monstrosus*, *Myonycteris torquata*, *Micropteropus pusillus*, *Mops condylurus*, and *Rousettus aegyptiacus*) which revealed anti-EBOV antibodies (Pourrut et al. 2009).

The EBOV reemerged in the Luebo region of DRC in 2007 and 2008, causing two successive outbreaks. The 2007 outbreak caused 264 cases with 187 deaths (CFR 71%) and re-occurred one year later with 32 cases with 15 deaths (CFR 47%). The suspected source of the Luebo 2007 outbreak was fruit bats (*Hypsignathus monstrosus* and *Epomops franqueti*). The index source of the 2008 outbreak could not be traced out. The analysis of whole-genome sequence results of viruses from these two outbreaks confirms them almost identical (Gard et al. 2011).

Following this, the EBOV outbreak occurred in multiple villages in the DRC between July and November 2014 in Équateur province with a total of 69 cases with 49 deaths (CFR 71%). A pregnant woman was the index case, and monkey meat was the suspected source of the infection. A complete genome sequence from this outbreak shared 99.2% and 96.8% identities with EBOVs of 1995 outbreak in the DRC and current outbreak in West Africa, respectively (Maganga et al. 2014).

The recent EBOV-epidemic burst in West Africa started in Guinea in late 2013 and spread to several countries within a few months period (Cenciarelli et al. 2015). As of 13 April 2016, a total of 31,013 cases with 12,872 deaths (CFR 41.5%) have been reported. The

widespread transmission occurred in three countries, namely Liberia, Sierra Leone and Guinea (28,616 cases with 11,310 deaths – CFR 40%). Very few travel-related cases (36 cases and 15 deaths) have been reported in seven countries, namely Italy, Mali, Nigeria, Senegal, Spain, the United Kingdom, and the United States of America) (<http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/case-counts.html>). The suspected index case for these outbreaks was a two-year-old child (<http://www.who.int/csr/disease/ebola/ebola-6-months/guinea/en/>). The whole-genome sequencing of 99 samples from 78 affected individuals in Sierra Leone suggested the involvement of the West African variant of EBOV in the current epidemic. The suspected source of the outbreak was from an unknown animal reservoir. The Sierra Leone outbreak originated from the introduction of two EBOV variants lineages (Gire et al. 2014).

Considering the highest risk to the national security and public health concern, the US-NIAID, classified the EBOV under Category A Priority Pathogens (<https://www.niaid.nih.gov/topics/biodefenselated/biodefense/pages/cata.aspx>). Laboratory-acquired EBOV infection also had been reported from Russia on two occasions. It had emerged that in 1996, a female lab technician carrying out the high-risk work, cut herself when she drew blood from a horse that had been infected with EVD and died quickly. In the second incident, a Russian lab worker died in 2004 while working with EBOV-infected guinea pigs (https://www.washingtonpost.com/national/health-science/2014/10/23/ce409716-5945-11e4-b812-38518ae74c67_story.html; <http://www.theaustralian.com.au/news/world/the-times/russians-died-in-ebola-weapons-lab/news-tory/e426b0bd0064da5fe8c78e4d485c162c>).

2.2.2. Sudan ebolavirus (*Sudan virus* – SUDV)

Sudan virus – SUDV (*Sudan ebolavirus*) and Ebola virus – EBOV (*Zaire ebolavirus*) are related regarding clinical disease but vary in serology, pathogenic potential, and virological properties (Richman et al. 1983). In Sudan, between June and November 1976, a large outbreak of EHF occurred in Nzara, Maridi, and the surrounding area and caused 284 cases with 151 deaths (CFR 53%). The clinical disease observed in this outbreak was associated with high mortality (CFR 53%) and a prolonged recovery period in survivors. A cotton factory worker in Nzara was identified as the index case (Report of a WHO/International Study Team 1978). The specimens collected during the Sudan and DRC outbreaks were sent to high-security laboratories in England (Microbiological Research Establishment, Porton Down), Belgium (Institute of Tropical Medicine, Antwerp), and the United States of America (CDC, Atlanta) for isolation and identification of the agent. All three laboratories isolated a virus that morphologically resembled Marburg virus but was serologically distinct (Bowen et al.

1977; Emond et al. 1977; Johnson et al. 1977). In the UK, one of the investigators accidentally pricked his thumb while handling the samples and developed an illness (Emond et al. 1977).

Between July and October 1979, SUDV outbreak occurred in Nzara and Yambio of southern Sudan and caused 34 cases with 22 deaths (CFR 65%). A 45-year-old man who had been employed in the Nzara textile factory was the index case (Baron et al. 1983), and the possible source of infection was bats (Pourrut et al. 2005).

An outbreak of VHF occurred between October 2000 and January 2012 from Gulu district (borders Sudan), Uganda, and 425 infected cases were reported with 224 deaths (CFR 53%). A concurrent outbreak of SUDV and measles occurred in Yambio County of southern Sudan between April and June 2004. A total of 17 VHF cases and 7 deaths (CFR 41%) were reported in Yambio payam. The CDC confirmed the presence of SUDV in this outbreak (WHO 2005). The index case was a radio technician, and the source of the outbreak was a baboon (*Papio anubis*) (WHO 2005). During 2008, SUDV outbreak recurred in Kaluamba with 37 cases and 16 deaths (CFR 44%). An 18-year-old girl appeared to be an index case, but the source was not known (Muyembe-Tamfum et al. 2012; Rewar & Mirdha 2014).

In May 2011, a 12-year-old girl from Luwero, Uganda, was admitted to the Hospital with symptoms of VHF and died within three hours of admission. Evidence of infection with SUDV was confirmed by RT-PCR, antigen-detection ELISA, and virus isolation. Analysis of complete genome sequencing of the above virus had 99.3% identity to the Gulu SUDV – 2000 virus. The circumstantial evidence suggests that a bat was the source of infection (Shoemaker et al. 2012).

In order to detect VHFs in Uganda, a surveillance program was initiated in 2010 by the CDC, Atlanta, USA, in collaboration with the Uganda Virus Research Institute and the Uganda Ministry of Health (MacNeil et al. 2011). Samples collected from the outbreak of Kibaale, Uganda between July and August 2012, yielded 11 laboratory confirmed cases (SUDV infection) with 4 deaths (CFR 36%). Complete genome analysis of the above four clinical samples showed ~99.9% sequence identity with each other and ~99.2% identity with the SUDV Nakisimata (Uganda – 2011), and SUDV Gulu (Uganda – 2000) isolates (Albarino et al. 2013). In the same surveillance, the samples collected from an outbreak in the relatively close districts of Luwero, Jinja, and Nakasongola, Uganda in November 2012 yielded six laboratory confirmed cases with three deaths (CFR 50%). Sequence analysis of NP gene from three serum samples revealed that they are nearly ~100% identical. Involvement of bats was suspected for its transmission (Albariño et al. 2013).

2.2.3. Reston ebolavirus (*Reston virus* – RESTV)

In October 1989, 100 cynomolgus monkeys (*Macaca fascicularis*) were transported from Manila, the Philippines to Reston, Virginia and placed in a quarantine facility. During the quarantine, numerous macaques died, due to concomitant infection of Ebola-related filovirus (as per the recent classification – Reston virus or RESTV) and by simian hemorrhagic fever virus (SHFV – an arterivirus) (CDC 1989). RESTV could be isolated at least from five monkeys (Jahrling et al. 1990). This was considered as the first known EBOV outbreak that occurred outside of Africa as well as in non-human primates (Miranda & Miranda 2011).

Between November 1989 and March 1990, active infection of RESTV occurred in seven shipments of cynomolgus monkeys and further transmission to monkeys in quarantine facilities and many of them died. Four animal handlers at a quarantine facility had anti-RESTV antibodies (CDC 1989; Jahrling et al. 1990; CDC 1990a). Among these four cases, viremia developed in one person on days 9, 10, and 11 post-inoculation (WHO 2009). CDC has screened ~2200 serum samples from different monkey species from a variety of settings, and ~10% had antibodies against at least one of four filovirus test antigens (EBOV, SUDV, RESTV, and Marburg virus) (CDC 1990b).

Studies were initiated to document transmission at export facilities located in the Philippines. At one export facility, within three months period (between March and May 1990), out of 403 monkeys, 161 (40%) died from RESTV as confirmed in these animals. The clinical manifestations observed in the outbreak included diarrhea (50%), respiratory illness (34%), and hemorrhage (1%) (Hayes et al. 1992). However, three workers in the animal facility developed antibodies against RESTV without any clinical signs (Miranda et al. 1991). The source of RESTV was not known (Rollin et al. 1999).

The next outbreak occurred in 1992. A batch of 55 cynomolgus monkeys was imported from a monkey-breeding company in the Philippines into Italy in March 1992. Four monkeys died within a month, and viruses isolated from three monkeys were confirmed as filoviruses by electron microscopy. They were shown to be antigenically related to EBOV by indirect immunofluorescence assay (WHO 1992). Although two people developed virus-specific IgG antibodies, clinical infections not observed (Miranda & Miranda 2011).

In March 1996, an imported cynomolgus monkey that was held in a quarantine facility in Texas developed non-specific signs such as anorexia and lethargy and died after three days. A second monkey also showed similar kind of symptoms, and it was euthanized. RESTV was isolated from both the animals. No viral antigen or RESTV-specific antibodies could be detected from animal handlers. Also, during the laboratory investigation, as in 1989 and 1990, SHFV could

have infected some of the animals (Rollin et al. 1999). GP gene of RESTV isolated from the first animal had 98.9% nucleotide identity with the 1989 RESTV (Sanchez et al. 1999). In a USA–Philippine Joint Investigation at the monkey export facility in the Philippines it revealed that 14 out of 21 animal houses had infected monkeys. Anti-RESTV antibodies and virus nucleic acid could be detected from 3 out of 1732, and 132 of 1011 monkeys, respectively. Seroprevalence on animal handlers from monkey facilities and people with occupational exposure to pigs in USA, Italy, and the Philippines, from 1989 to 2009 indicated that 9 (2%) out of 662 persons had detectable RSTV-specific IgG antibodies (Rollin et al. 1999; WHO 2009; Miranda & Miranda 2011).

In the Philippines, during 2008–2009, serum samples were collected from 141 wild-caught bats (17 species) and at different locations 7 out of 16 serum samples from *Rousettus amplexicaudatus* bats had anti-RESTV antibodies. These antibody-positive bats were captured near the RESTV positive cynomolgus monkeys and swine suggesting that bats are a possible natural reservoir of RESTV (Taniguchi et al. 2011). In the Philippines, between July 2007 and June 2008, there were multiple outbreaks of a respiratory and abortion disease syndrome in swine. The clinical signs resembled an infection caused by a highly pathogenic porcine reproductive and respiratory syndrome virus (PRRSV – an arterivirus), also referred to as ‘blue ear disease.’ The diagnostic investigation carried out at two USA laboratories (APHIS and FADDL), revealed concomitant infection of pigs with PRRSV and RESTV. Viral genomes from three samples revealed a very high inter-isolate divergence (3.93%) as compared to the original 1989 RESTV (2.5%). Anti-RESTV antibodies could be detected in 6 out of 141 people who worked on pig farms or with swine products (WHO 2009; Barrette et al. 2009).

In a multi-institutional study on bats in the Philippines, RESTV RNA was detected in oropharyngeal swabs taken from *Miniopterus schreibersii*, and sequencing of three samples showed high nucleotide identity with a pig isolate from Bulacan province. Also, four sera showed the presence of anti-EBOV antibodies, i.e. three from *Acerodon jubatus*, and one from *Pteropus vampyrus* (possibly infected with EBOV and RESTV), also suggesting that bats could be a natural reservoir for RESTV (Jayme et al. 2015).

In China, between February and September 2011, 137 PRRSV confirmed pig spleen samples were screened for the presence of RESTV, and four samples were found positive. The partial sequencing of L gene from four samples showed 95.1%–97.2% nucleotide identities with each other and 96.1%–98.9% identity with two RESTV variants of domestic pigs and cynomolgus macaques from the Philippines (Pan et al. 2014).

2.2.4. Tai Forest ebolavirus (*Tai Forest virus – TAFV*) and Bundibugyo ebolavirus (*Bundibugyo virus – BDBV*)

There was a single non-fatal human case of Tai Forest virus (TAFV – previously known as Côte d'Ivoire ebolavirus). In 1994, a Swiss ethnologist became infected by TAFV after conducting an autopsy examination on a wild chimpanzee in the Tai Forest, Cote-d'Ivoire (Le Guenno et al. 1995).

The first outbreak of VHF due to Bundibugyo virus – BDBV (*Bundibugyo ebolavirus*) occurred in Uganda between August and December 2007 and infested 131 cases with 42 deaths (CFR 32%) (MacNeil et al. 2011). The entire genome sequencing of BDBV and the TAFV were carried out by the Next Generation Sequencing, and the analysis revealed that the BDBV differs significantly from the other four Ebola virus species (Towner et al. 2008). Another outbreak of BDBV occurred in Isiro, DRC between June and November 2012 with 36 cases with 13 deaths (CFR 36%). The full genome sequence analysis revealed that the new BDBV was ~98.6% identical to those of the original BDBV-2007 isolate (Albariño et al. 2013).

Human outbreaks of EVD are hypothesized to be originated from direct contact with an infected animal or its body fluids. The human-to-human transmission occurred through the direct contact with blood from infected patients or other body fluids (Report of an International Commission 1978; Baron et al. 1983; Dowell et al. 1999; Muyembe-Tamfum et al. 1999; Roels et al. 1999; Francesconi et al. 2003; Muyembe-Tamfum et al. 2012; Lawrence et al. 2017). The major risk factors associated with virus transmission chains are gathering funerals of Ebola positive patients, close contact with family members of infected patients, and treating patients without adequate personal protective measures (Okware et al. 2002). People visiting or taking care of infected persons are at high risk of Ebola infections (Muyembe-Tamfum et al. 2012). Recently, a post-recovery sexual transmission of EBOV via semen has been documented in the West African outbreak (Mate et al. 2015). Food can contribute to EBOV transmission, particularly through harvesting of bush meat (meat of wildlife) (Mann et al. 2015). Transmission through fomites in a clinical setting is unlikely (Bausch et al. 2007). The experimental aerosol transmission has been demonstrated between monkeys (Jaxx et al. 1995; Johnson et al. 1995; Reed et al. 2011) and from pigs to monkeys (Weingartl et al. 2012). Similarly, airborne transmission among humans is hypothetical (Baron et al. 1983; Dowell et al. 1999).

3. Host range, carriers, and reservoirs

EBOV causes acute hemorrhagic fever in human and non-human primates like cynomolgus (*Macaca fascicularis*) and rhesus monkeys (*Macaca rhesus*), African

green monkey (*Cercopithecus aethiops*) and baboons (*Papio hamadryas*). Inoculation of infected material to laboratory animals had resulted in non-lethal febrile disease, and subsequent passages of splenic material in animals increase the virulence leading to the death of laboratory animals. Among mice, the newborn is more sensitive than adult ones. Stray dogs in Africa eating animals died due to EBOV remained asymptomatic. Furthermore, a survey in 2005 showed that around 30% of dogs had seropositivity for EBOV without any clinical signs (Allela et al. 2005; Osterholm et al. 2015). Pigs have been shown to acquire natural EBOV infection and can transmit EBOV to humans (Osterholm et al. 2015) and hence alarming the food safety and animal health officials (Feldmann & Feldmann 2014). However, limited information is available to predict or prove the role of other livestock species, like cattle, horse, sheep, etc. being a reservoir of EBOV (Mann et al. 2015). Apart from the transmission of the virus from reservoir animals, contaminated plant food products may also act as a source of infection to the susceptible population (Mann et al. 2015).

Researchers predict that there may be a reservoir host for EVD, as it has a re-emerging pattern and hypothesize that three fruit bat species, namely *Epomops franqueti*, *Hypsignathus monstrosus*, and *Myonycteris torquata* are the important reservoir (Leroy et al. 2009). The African fruit bats have been suggested to be the natural reservoirs for EBOV (Zaire) and Marburg virus (Leroy et al. 2005; Barrette et al. 2009; Pourrut et al. 2009). In the Philippines, anti-RESTV antibody could be detected from bats which were captured near to the area where RESTV infections in cynomolgus monkeys and swine had been detected, suggesting that bats could be the possible natural reservoir (Taniguchi et al. 2011; Jayme et al. 2015). Finding of EBOV antibodies in bats from Bangladesh increased the speculation of the bat as a reservoir (Olival et al. 2013). In humans, there is no age and sex difference in susceptibility to EVD, and additionally, there is no report about humans to act as a reservoir. Recently, a study conducted to find any difference in the disease pathology between male and female man showed both had similar risk rate, yet females have higher survival rate after developing the disease (Team 2016). The virus can persist in the semen of men for a period of several months (~18 months) after recovery and can be transmitted to the female (Heeney 2015; Mackay & Arden 2015; Bausch and Crozier 2016; Fallah et al. 2016) which suggests that man may be a reservoir for the EBOV. To tackle this, WHO has recommended testing of semen by RT-PCR from EVD survivors for three months from onset of the disease along with counseling to encourage safe sexual practices until their semen is tested negative twice (Check 2016; Purpura et al. 2016). Death among chimpanzees and gorillas was always interposed with outbreaks in man, dead non-human

primate's contact, and also eating of fruit bats (Muyembe-Tamfum et al. 2012). Direct contact with secretions from infected persons and organ transplantation seems to be another important means of transmission. Body secretions from non-human primates also act as a potential source of infection to man (Feldmann & Feldmann 2014). Mucous membranes seem to be the major portal of entry of the organism. Aerosol infection in non-human primates raises concern about the chances of airborne infection in humans (Olival et al. 2013). Research findings show that the dried virus particle can travel longer distance compared to the virus in fluid form. EBOV needs a liquid medium for its survival. Hence, its transmission cannot be airborne. Studies reported that due to the increase in population and also increased movement of man and animals there is difference in the disease dynamics of recent outbreaks of EVD as compared to earlier outbreaks (Pigott et al. 2014).

3.1. Role of animals in transmission of EBOVs

The possible source and mode of transmission of EBOV for each outbreak are described earlier in the section on epidemiology. In this section, the role of animals in the transmission of EBOV is highlighted. Regarding the multiple outbreaks of EBOV between 2001 and 2003 in Gabon and RC, there is a link to handling/eating of bush meat. The majority of outbreaks started with the history of handling of infected wild animal carcasses, including gorillas, chimpanzees, duikers (small antelopes), and possibly monkeys by the index cases (WHO 2003; Leroy et al. 2004).

In 1994, in the Taï National Park, Côte d'Ivoire an Ebola outbreak occurred among chimpanzees (Formenty, Boesch, et al. 1999), and an ethologist was infected with TAFV while conducting a necropsy on a wild chimpanzee (Formenty, Hatz, et al. 1999). Investigation of a 2007 EBOV outbreak suggested that the possible source was fruit bats (Leroy et al. 2009). Since anti-EBOV antibodies and viral RNA have been detected in bats, there is a strong belief in the research community that fruit bats could be a primary natural reservoir for EBOV (Pourrut et al. 2009). In a large-scale survey conducted at Gabon and RC between 2003 and 2008, anti-EBOV antibodies could be detected from six bat species (Pourrut et al. 2009).

Multiple outbreaks of RESTV occurred between 1989 and 1996 in captive monkeys in the Philippines, the United States of America, and Italy. During these outbreaks, three animal handlers developed anti-RESTV antibodies without clinical symptoms. One became infected during necropsy on a monkey as evidenced by viremia and seroconversion. An additional, five monkey handlers in the Philippines developed anti-RESTV antibodies without significant illness (Miranda & Miranda 2011). Serological studies on animal handlers

from monkey facilities and people with occupational exposure to pigs in the USA, Italy, and the Philippines, from 1989 to 2009 indicated that 9 (2%) out of 662 persons had detectable RSTV-specific IgG antibodies (Rollin et al. 1999; WHO 2009; Miranda & Miranda 2011).

The RESTV infection was also identified in the Philippines (2007–2008) and China (2011) along with co-infection of PRRSV (Barrette et al. 2009; Pan et al. 2014). In the Philippines, six workers in contact with infected pigs had anti-RESTV IgG antibodies, suggesting that pigs can transmit RESTV to humans. Transmission from pigs may be due to direct contact with body fluids and respiratory routes (WHO 2009). Serum samples from wild-caught bats during 2008–2009 in the Philippines revealed anti-RESTV antibodies in *R. amplexicaudatus* bats. These antibody-positive bats were captured near to the area where REST infections were detected in cynomolgus monkeys and swine (Taniguchi et al. 2011). In another study, RESTV RNA was detected in oropharyngeal swabs taken from *Miniopterus schreibersii*, and the presence of the virus had also been identified in additional sympatric taxa (*Miniopterus australis*, *Cynopterus brachyotis*, and *Chaerephon plicata*) and additional locations (Puning Cave). Also, the presence of anti-RESTV antibodies was confirmed from *Acerodon jubatus* and *Pteropus vampyrus* suggesting that RESTV infection is widespread in bats (Jayme et al. 2015).

In a seroprevalence study conducted in the dog population during 2001–2002 outbreaks in Gabon, anti-EBOV virus antibodies could be detected from a significant number of samples which were collected from the human outbreak area. However, antibodies were detected in 2 out of 102 dog samples which were collected from the non-exposed country (Allela et al. 2005). Neither Ebola-like illness nor virus could be identified from dogs, and the validity of the immune assay for canine samples has been questioned (Osterholm et al. 2015). During ecological studies in the CAR in 1998, EBOV RNA could be detected from mice (*Mus setulosus* and *Praomys* spp.) and a shrew (*Sylvisorex ollula*) (Morvan et al. 1999).

3.2. Role of plants and food materials in transmission of EBOVs

Apart from the transmission of the virus from reservoir animals and contaminated meat, plant food products may also act as a source of infection to the susceptible population (Mann et al. 2015). A two-year-old child was identified as index case who was playing with mangoes partially eaten and dropped by bats (Coen & Henk, 2015; Mann et al. 2015). There are also reports of cases of Ebola infection and diseases acquired by eating fruits half-eaten by bats (Leroy et al. 2007). This may be due to the shedding of virus in the saliva of bats (Amman et al. 2015). It is evident from the study

conducted in which plants experimentally inoculated with the virus supported the replication of virus with or without the development of visible lesions (Swanepoel et al. 1996; Okware et al. 2002). The first victim of 2007 outbreak in the DRC was known to purchase fresh bat meat before developing the disease (Mann et al. 2015). But contradictory opinion occurs regarding the role of bushmeat as a source of infection (Mufunda et al. 2016). There is only indirect evidence available in this regard, and more needs to be investigated to confirm whether plants, plant products, and other food material can contribute towards spreading the disease. If this is so, then it is a serious concern as it would make the disease control more difficult. Furthermore, there is also fear that it might act as a weapon for bio-terrorism (Maras & Miranda 2016). Capacity to transmit the infection through food products depends on the ability of the virus to survive in the atmosphere for a certain period. It has been found that EBOV can survive up to a period of three weeks at a lower temperature in the atmosphere (Piercy et al. 2010).

4. Ebola virus disease (EVD)

EVD has an acute clinical outcome with signs like nausea, vomiting, diarrhea, pain in the abdomen, muscles,

and head; there is also the loss of appetite and enlargement of lymph nodes (Olival et al. 2013; Feldmann & Feldmann 2014). The incubation period ranges from 1 to 10 days, while exceptions may reach up to 21 days. Hemorrhages and sudden rise in temperature are the two common clinical signs noticed and other signs of chest pain, difficulty in breathing and swallowing. Blood may be noticed in feces and urine; there may also be coagulation problem during bruises or damage to blood vessels (Jahromi & Mood 2015). Bleeding from nostrils, gums, vaginal mucosa, and gastrointestinal (GI) tract have been a common sign in 40%–50% of the cases, and maculopapular rashes can be noticed in 50% of cases. Bleeding begins within seven days from the start of initial clinical symptoms, and it may be noticed both internally as well as subcutaneously. Hemorrhages may range from petechiae, purpura, and ecchymoses; hematomas can also occur due to bleeding in the subcutaneous tissue. Death can occur within 7–16 days from the start of clinical symptoms and occurs due to several organ dysfunctions and severe bleeding (Takada & Kawaoka 2001; Hoenen et al. 2006). Patients who survive from EVD will exhibit organ swelling, orchitis, and pain in the joints. An overview of EBOV transmission, pathogenesis, and clinical signs is depicted in Figure 5.

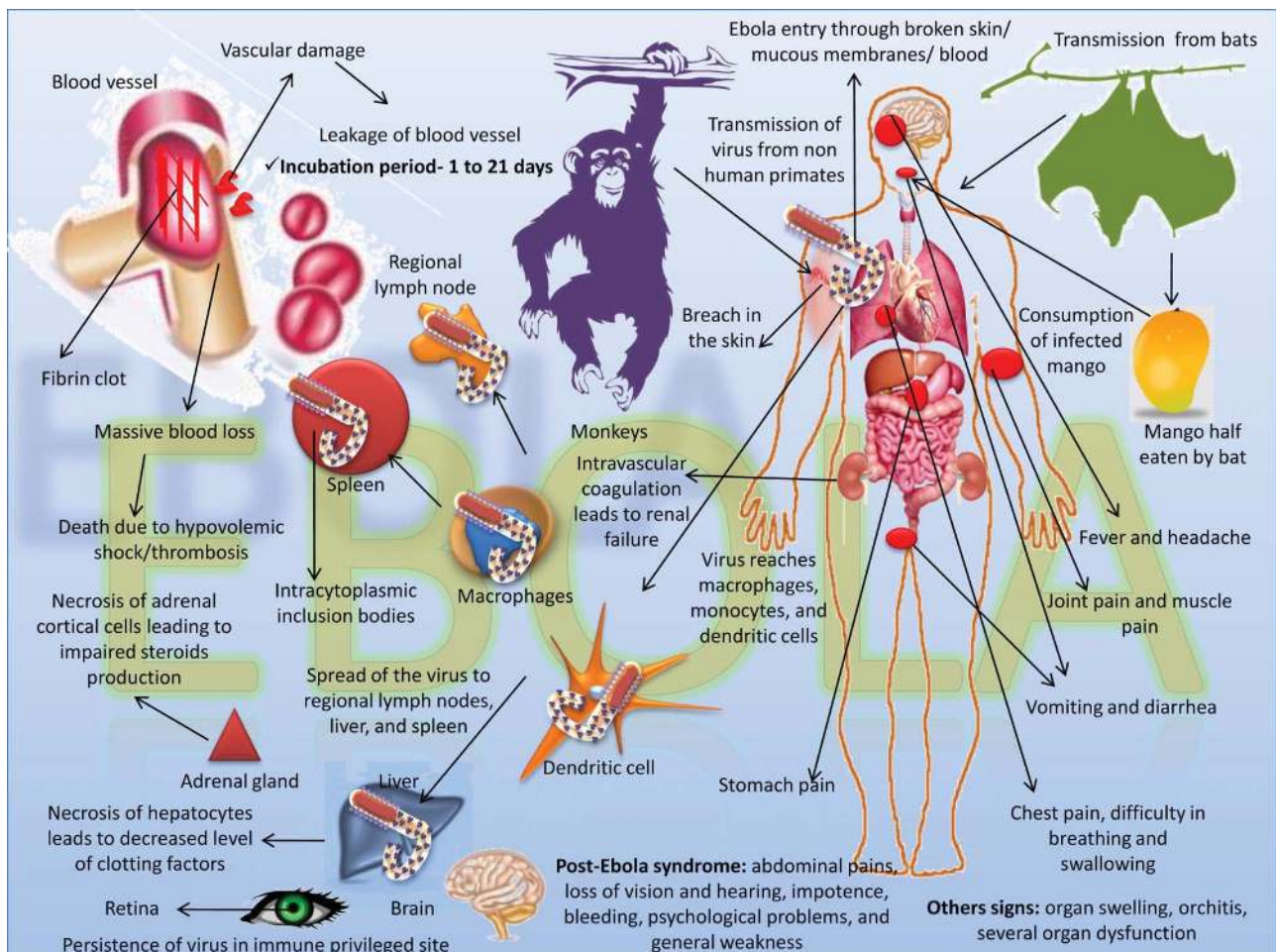


Figure 5. Transmission, pathogenesis, and clinical signs of Ebola virus.

The major route of infection is through the mucosa or skin from where it reaches the macrophages, monocytes, and dendritic cells, leading to spread of the virus to regional lymph nodes, liver, and spleen. Macrophages and monocytes stimulated by EBOV release cytokine storm thereby damaging tissues and blood vessels (Olival et al. 2013). Death occurs due to blood loss and/or coagulation (Casillas et al. 2003). Coagulopathy occurs due to thrombocytopenia, loss of anticoagulant protein C, destruction of clotting factors, and also due to the destruction of fibrin. Damage to blood vessels causes disseminated intravascular coagulation as well as renal failure (Geisbert et al. 2003a; Nyamathi et al. 2003). Antibodies developed against EBOV bind with the complement C1q and reach to the binding sites on dendritic cells and macrophages, leading to damage of these cells.

Lesions of EVD include extensive hemorrhages of the mucosa, necrosis of different organs like liver, kidney, testes, and ovaries. Necrotic foci with inflammatory cells can be noticed in hepatic lobules, and there may be multinucleated syncytia formation in the hepatic cells. Necrosis of red pulp and fibrin deposition are the characteristic lesions noticed in the spleen. Splenic macrophages reveal big, acidophilic particles in their cytoplasm which are similar to intracytoplasmic inclusion bodies. GI tract shows mononuclear infiltration into the submucosa and lamina propria. Mild emphysema, edema in the terminal alveoli, and stasis of blood can be noticed in the lung parenchyma (Wyers et al. 1999).

The clinical features observed in different EBOV and SUDV outbreaks were almost similar. The disease caused by EBOV and SUDV is known as 'Ebola virus disease' (EVD) – the term 'Ebola hemorrhagic fever' (EHF) was previously used. In EBOV outbreak in Zaire in 1976, the major clinical manifestations observed included a severe sore throat, maculopapular rash, intractable abdominal pain, and bleeding from multiple sites principally the gastrointestinal tract (malena, haematemesis, mouth/gingival, vaginal epistaxis, injection sites/scarification) (Report of an International Commission 1978). More detailed clinical manifestations were reported in SUDV outbreaks in Sudan in 1976. In this outbreak, EHF was a unique clinical disease with a CFR (53%) and a prolonged recovery period in those who survived. The clinical signs observed were fever, headache, and pains in joint and muscle, diarrhea, vomiting, chest pain, pain and dryness of the throat, rash, and hemorrhages (Report of a WHO/International Study Team 1978). In contrast to the above outbreaks, clinical manifestations observed in 2013–2016 West African outbreaks differed mainly due to less hemorrhage (less than 5% of patients before death). There were four clinical phases: (1) an early febrile phase (onset 0–3 days) – fever (up to 40 °C), malaise, fatigue, body aches etc., (2) gastrointestinal phase (onset 3–10 days) – epigastric

pain, nausea, vomiting, diarrhea, persistent fever, asthenia, headache, conjunctival injection, chest pain, abdominal pain, arthralgias, myalgias, hiccups, delirium etc., (3) shock or recovery phase (onset 7–12 days) diminished consciousness or coma, rapid thready pulse, oliguria, anuria and tachypnea, and (4) late complications phase (onset ≥ 10 days) associated with gastrointestinal hemorrhage, secondary infections, meningoencephalitis, and persistent neurocognitive abnormalities (Chertow et al. 2014; Chertow et al. 2016). The convalescent state is complicated by uveitis and viral shedding through ocular discharge (Varkey et al. 2015; Chancellor et al. 2016). In a study conducted by the WHO on EVD in West Africa, the most common symptoms observed included fever, fatigue, vomiting, diarrhea, loss of appetite, headache, abdominal pain, and hemorrhagic symptoms (WHO Ebola Response Team 2014, 2016).

5. Post-Ebola syndrome

Of note, thousands of patients have survived during the Ebola outbreaks and have shown symptoms that persisted or developed after hospital discharge, which could be due to the persistence of the virus in the immune privileged sites, such as brain and retina of the host (Fallah et al. 2016). Defective interfering particles of the virus might be responsible for virus persistence and chronic condition due to EBOV infection as observed under *in vitro* condition in cell culture condition (Calain et al. 2016). To note, this virus persists in the body longer than suspected by the scientists. However, there is a paucity of sequelae of data regarding post-Ebola syndrome (PES). In an SUDV outbreak in Uganda in 2000, 60 out of 257 people survived and the PES complications observed included abdominal pain, loss of vision and hearing, impotence, bleeding, psychological problems, and general weakness (Wendo 2001; Shantha et al. 2016). During 1995 EBOV outbreak in Kikwit DRC, the clinical signs observed among 19 survivors were arthralgia, ocular disease, parotitis, unilateral orchitis, hearing loss or tinnitus, and pericarditis (Kibadi et al. 1999). In the same outbreak, 29 Ebola convalescents and 152 household contacts (HHCs) were monitored for up to 21 months. Arthralgias and myalgia were the most commonly reported symptoms among convalescents. The other clinical signs observed during the first six months of follow-up were abdominal pain, extreme fatigue, and anorexia more frequently than did HHCs, whereas fever, headache, diarrhea, dyspnea, hiccups, and hemorrhage were the same in both groups (Figure 5) (Rowe et al. 1999).

In a recent outbreak occurred in Guinea in 2015, a survey was conducted on 105 EVD survivors using a standard data collection form revealing anorexia and arthralgia frequently (Qureshi et al. 2015). Other clinical signs reported in different outbreaks included pain,

weakness, hearing difficulty, and mental disturbances (Report of a WHO/International Study Team 1978; Okware et al. 2002). Recently, a very systematic cross-sectional survey of the symptoms of all survivors was conducted from the Ebola Treatment Unit at Freetown, Sierra Leone. The unit treated 88 EVD confirmed cases between 1 December 2014 and 31 March 2015, of whom 44 survived. The symptoms observed in the survivors included musculoskeletal pain (70%), headache (48%), and ocular problems (14%). A total of 117 separate complaints were reported, 31 patients (70%) had musculoskeletal pain, 21 (48%) had headaches, and 6 (14%) had ocular problems. Twenty-six (59%) of the 44 survivors reported other symptoms like cough, abdominal pain, chest pain, itching, insomnia, fever, loss of appetite, labored speech, epigastric pain, rash, weight loss, hiccups, increased appetite, chest pain, sneezing, diarrhea, vomiting, left sided weakness with facial nerve palsy, breathlessness, rash, dry flaky skin, earache, fever blister/cold sore, left scrotal swelling, nasal congestion, and tremors (Scott et al. 2016).

6. Pathogenesis

The EBOV viral proteins are involved in various stages of the pathogenesis (Figure 5), namely VP35 is essential for ribonucleoprotein complex (RNP) production, important for viral replication and transcription, and VP35 blocks the signaling of type I interferon (Muhlberger et al. 1999; Basler et al. 2000; Basler et al. 2003). By antagonizing the production of interferon, host cellular IFN- α/β response obstruction and inhibition of anti-viral protein production as a result of dsRNA of the virus are the mechanisms involved in pathogenesis caused by VP35 protein (Basler et al. 2003; Wong et al. 2014). VP40 matrix protein is involved in budding from the host cell and also involved in the formation of the virus-like particles (VLPs) (Harty et al. 2000; Jasenosky et al. 2001; Noda et al. 2002). Envelope GP and other viral proteins may have a role to inhibit immune mechanism thereby increasing the virulence of the virus (Takada & Kawaoka 2001). Lately, scientists have tried to elucidate the exact molecular pathogenesis of this virus (Shi & Shen 2013) where they found that caspase 8, FADD like apoptosis regulator (CFLAR), dystroglycan 1 (DAG1), and tissue factor pathway inhibitor were inhibited in human umbilical vein endothelial cells expressing EBOV GP.

The virus enters through mucosal surfaces, cut or lesions of the skin, or direct transfer. Cells like monocytes, macrophages, dendritic cells, hepatocytes, and endothelial cells are the targets of EBOV (Feldmann et al. 1996; El Sayed et al. 2016). Epithelial, endothelial fibroblasts, hepatocytes, and adrenal gland cells are also infected (Olejnik et al. 2011). Following infection, mononucleated cells are preferred by EBOV for replication in initial stages and a rapid viremia is generated

(Olejnik et al. 2011). EBOV destructs lymphocytes and monocytes and induces cytokine storm and coagulation anomalies. Also enhanced activation of B and T cell is evident with a higher magnitude of inflammatory cytokines (McElroy et al. 2015). Evidence suggests the pathological manifestations by EBOV via releasing immune mediators which are more critical. There is a rapid multiplication of virus inside the host, and it escapes the host defense mechanism. A major target of EBOV is antigen presenting cells mainly CD16+ monocytes which are not activated in an acute episode of EVD (Lüdtke et al. 2016). Both virus-encoded and host proteins are responsible for pathogenesis, releasing inflammatory cytokines like tumor necrosis factor alpha (TNF- α), IFN- α , IFN- γ , interleukin-2 (IL-2), and IL-10 that are responsible for massive hemorrhage and death (Villinger et al. 1999). This strongly activates the immune system, and mainly interferon-related genes are upregulated (such as ISG15, OAS1, etc.) (Caballero et al. 2016). Antibodies can be detected after six days' post infection and can be detected up to 90 days. Reports show that IgG response can be detected for around 400 days in animals while it can persist around 10 years in human sera (Ksiazek, Rollin, et al. 1999). Though antibodies can be detected in the serum against glycoproteins, they turned out to be not protective against infection and also failed to inhibit replication of the virus in cell culture. Passive administration of antibodies to animals prevented clinical manifestations but has not prevented death which hypothesizes that immunity is not mainly through antibodies but could also be cell-mediated. There is no report till to date regarding the re-infection of EBOV in a person who suffered earlier infection, showing that there is lifelong immunity against EBOV.

During the clinical course, the predominant clinicopathological findings included hypoalbuminemia, hyponatremia, hypokalemia, hypocalcemia, and hypomagnesemia. Aminotransferase activity peaked at a median of nine days after the onset of illness (Uyeki et al. 2016).

7. Experimental animal inoculation

Several experimental studies have been conducted on the animal-to-animal EBOV transmission using different animal species. One of the studies demonstrated transmission of EBOV from inoculated rhesus monkeys to control monkeys caged in the same room. They postulate the possible mode of transmission through aerosol, oral, or conjunctival exposure to virus-laden droplets (Jaax et al. 1995). However, there is a possibility for transmission through spitting and throwing feces by the monkeys (<http://www.cdc.gov/vhf/ebola/transmission/human-transmission.html>) or through routine animal husbandry practices (Osterholm et al. 2015). In another study, the transmission of EBOV from

infected pigs to uninoculated caged macaques was demonstrated when they were housed together (Weingartl et al. 2012). The route of inoculation also might play an important role in the transmission as in an experiment when monkeys were inoculated with EBOV via intramuscular route; no transmission occurred in uninoculated control monkeys (Alimonti et al. 2014).

Few studies have examined the role of route of exposures in transmission. One such study demonstrates that EBOV infection through oral, conjunctival, or intramuscular routes can cause the illness in rhesus monkeys (Jaax et al. 1996). In another study, aerogenic route of infection was demonstrated with different doses of antigen (Johnson et al. 1995). Another study investigated the replication, pathogenicity, shedding, and transmission efficacy of EBOV in pigs, wherein, following mucosal exposure, EBOV replicated in pigs, mainly in the respiratory tract, and induced severe lung lesions (Kobinger et al. 2011).

8. Clinical and laboratory diagnosis: a complex issue!

During several Ebola outbreaks since 1976, EVD has emerged as a direct challenge to manage (CDC 2015; Mattia et al. 2016; Racsa et al. 2016). If detected early, it gives good prognosis and lives can be saved (Okware et al. 2015). The diagnostic and therapeutic advances following the most recent outbreak have improved prognosis to some extent (Butler 2014; Gilbert 2015). In a September 2014 statement, the WHO said:

The Ebola epidemic ravaging parts of West Africa is the most severe acute public health emergency seen in modern times. Never before in recorded history has a biosafety level four pathogen infected so many people so quickly, over such a broad geographical area, for so long. (WHO 2014)

This grim situation triggered a search for reliable and sensitive laboratory tests to identify Ebola and diagnose EVD as early as possible after exposure (Ayukekbong 2016; Uyeki et al. 2016). Historically, Dr Ngoy Mushola recorded the first clinical description of the EVD in his clinical log during the Zaire outbreak: 'The illness is characterized by a high temperature of about 39 °C, hematemesis, diarrhea with blood, retrosternal abdominal pain, prostration with "heavy" articulations, and rapid evolution of death after a mean of three days' (Olupot-Olupot 2015; Shah et al. 2015). At present WHO/CDC defines that:

any illness with onset of fever and no response to treatment for the usual causes of fever in the area, along with at least one of the following signs: bloody diarrhea, bleeding from gums, bleeding into skin (purpura), and bleeding into the eyes and urine

for a suspected Ebola case.

To proceed for diagnosis, first of all, patients' history such as travel, occupation, and exposure to wild animals must be recorded. The most difficult task in EVD exposure is to specifically and differentially diagnose the EVD at the onset of disease symptoms. The EVD-infected individuals are presented clinically as very sick with muscular and abdominal pain, cramps and hiccups with vomition and diarrhea, severe headache, weakness followed by bruising and petechial bleeding. With the severity of disease, mucosal and frank bleeding occurs in the form of epistaxis and bloody stool. The EVD symptoms are quite similar with several other infectious diseases such as Typhoid fever, Lassa fever, Meningococemia, Malaria, Influenza, Measles, Marburg virus disease, Shigellosis, Leptospirosis, Yellow fever, fulminant viral Hepatitis, and Travelers' diarrhea (Hartman et al. 2010; Uyeki et al. 2016) and thus need to be differentially diagnosed. The CDC/WHO recommend for immediate clinical risk analysis for EBOV infection in any person with the symptoms described above. Currently, there is no specific treatment existing except that an early diagnosis of EBOV will ensure fast supportive care before the development of irreversible shock and necessary preventive measures and patient isolation can be instituted. Since major Ebola outbreak in 2014, researchers are tirelessly working to develop a specific test to confirm Ebola exposure before and after the onset of clinical symptoms. This initiative is also critical as most of the body fluids are highly infectious due to the persistent presence of Ebola for a long time. The abrasions on the skin and exposed mucosa are the most likely route of infection after direct contact of EBOV-containing body fluids from an infected person. The WHO/CDC characterized blood, feces, and vomit as the most infectious body fluids. Furthermore, EBOV also been detected in saliva (Formenty et al. 2006; Spengler et al. 2015), breast milk (Nordenstedt et al. 2016), aqueous humor (Varkey et al. 2015), semen, vaginal fluid, and urine (Deen et al. 2015; Chughtai et al. 2016; Fischer et al. 2016; Pettitt et al. 2016; Thorson et al. 2016).

After the EVD-outbreak in 2014, there is a critical urgency for the availability of robust diagnostic and prognostic tools for infectious diseases, particularly, in the resource-poor areas (Ghani et al. 2015). Diagnostic measures must be employed as soon as clinical symptoms become evident. In the early stage of infection, the presence of EBOV-antigen must be confirmed, followed by antibody detection at the late stage of infection (Olival et al. 2013; Okeke et al. 2014). In the year 1979, the first report on the quantitative measurement of antibodies against EBOV in human sera from Northwest Zaire was published (Van der Groen & Pattyn 1979). Subsequently, an immunofluorescence focus assay (Truant et al. 1983) and formalin-fixed tissue specimens by enzyme treatment (Kurata et al. 1983) were developed to detect EBOV. Since EBOV equally

infect primates, an enzyme immunosorbent assay was employed (Ksiazek et al. 1992) for the detection of EBOV in the infected primate tissues. For rapid diagnosis (within 30 minutes), EBOV-antigen detection based on the immune-filtration technique was also developed with limited detection of the Zaire and Sudan species only (Lucht et al. 2007). Ultimately, the confirmatory diagnosis relies on the isolation of virus on Vero or Vero E6 cell lines (Steele et al. 2001; Sanchez 2007; Makino & Kawaoka 2009). However, handling of infected samples and isolation of virus essentially requires BSL 4 infrastructure due to very high infectivity and mortality of lab personnel. Various other diagnostic platforms include electron microscopy, immunohistochemistry, and IgG and IgM antigen capture enzyme-linked immunosorbent assay (ELISA) has also been evaluated (Mishra 2014). Serum, plasma, and whole blood have been used with good results for detection of EBOV using antigen capture ELISA by using a semi-synthetic repertoire of llama single-domain antibodies (Sherwood et al. 2007).

RT-PCR seems to be a good diagnostic tool for detection of the virus (Drosten et al. 2002; Towner et al. 2004). Given the complex nature of EVD and rapid transmission, it is essential to deploy highly sensitive and specific EBOV detection assays. A reverse transcription loop-mediated isothermal amplification (RT-LAMP) has been developed to detect EBOV within 26 minutes (Kurosaki et al. 2007) without RT-PCR instruments. Recently, RT-LAMP for detecting EBOV directly from blood has been described for the GP gene of EBOV. It detects EBOV within 40 minutes with high sensitivity of detection limit to identify 2.8×10^2 pfu/test and 1×10^3 pfu/test of EBOV-Kikwit and EBOV-Makona, respectively (Benzine et al. 2016). Further, using DNA-intercalating dye SYBR green based or TaqMan probe-based real-time RT-PCR, highly sensitive detection of EBOV-antigen is possible (Huang et al. 2012; Liu et al. 2012). A multiplex real-time PCR has also been developed to detect Ebola and Marburg viruses in a single step (Yang et al. 2012). Similarly, several companies around the world are marketing lateral flow-like kits that can detect EBOV-antigens. Corgenix (Broomfield, CO, USA) and Veda Lab (Alençon, France) have developed kits which use a small amount of sample (blood, plasma, or urine) from infected persons (Baker 2014).

ELISA tests can be used for detection of antibodies of EBOV though this is not promising as antibodies develop only in the late phase of the infection and a chance for a person to escape the initial phase without death is less (Ksiazek, West, et al. 1999; Mishra 2014). Similarly, there is cross-reaction of antibodies with other pathogens. Therefore, antibody detection tests are less effective for EBOV infection. Specific detection of EBOV in clinical laboratories is currently being carried out largely through the analysis of the virus's nucleic acid (genetic material), using commercial or in-

house tests (Dhama, Malik, et al. 2015). Although these nucleic acid tests (NATs) are highly specific they require well-established laboratories and fully trained personnel due to their complex nature. Also, the turn-around time of the final results may take more than 12–24 hours. During Guinea Ebola outbreak, patient's swab samples were used to detect EBOV by a rapid reverse transcription recombinase polymerase amplification (RPA) assay (EBOV-RT-RPA). This highly efficient assay produced results in 30–60 minutes (Faye et al. 2015; Dedkov et al. 2016). A new test comparable to ELISA is developed by using Fe₃O₄ magnetic nanoparticle as a nanozyme probe (nanozyme-strip) to specifically detect the EBOV-glycoprotein as low as 1 ng/mL and is 100-fold more sensitive than the standard strip method (Duan et al. 2015). The WHO/CDC has officially approved (19 February 2015) the **ReEBOV*TM Antigen Rapid Test Kit (Corgenix, Broomfield, CO, USA) as eligible for procurement and to be used in Ebola-affected countries (Dhillon et al. 2015; Flint et al. 2015). The *ReEBOV*TM Antigen Rapid Test is an immune-chromatographic dipstick immunoassay point-of-care test based on detection of the Ebola protein (VP40 antigen) rather than nucleic acid and can provide results within 15 minutes. The *ReEBOV*TM Antigen Rapid Test kit can correctly identify about 92% of Ebola-infected patients and 85% of those not infected with the virus compared to other available NAT and currently being used in the field (RealStar® Filovirus Screen RT-PCR Kit 1.0, Altona Diagnostics GmbH, Hamburg, Germany). Despite the fact that *ReEBOV*TM Antigen Rapid Test kit is less accurate, it is easy to perform and does not require electricity. It can, therefore, be used at lower health care facilities or in mobile units for patients in remote areas (Broadhurst et al. 2015). It is always better to confirm the data from *ReEBOV*TM Antigen Rapid Test Kit by testing a new blood sample using an approved Ebola NAT. To test the sensitivity and versatility, comparative studies on 11 different procedures have been performed for currently available RT-PCR assays (Nouvellet et al. 2015; Cherpillod et al. 2016). However, the detection limit of *ReEBOV*TM test is not sufficiently sensitive to identify all the EBOV-suspected cases. Another significant obstacle to the use of these RT-PCR tests is to detect novel filovirus species and lineages which have emerged due to rapid mutation rates and genetic diversity of RNA viruses. More recently, a real-time RT-PCR assay was developed as a point-of-care test that does not require RNA extraction and thus the entire process completes within 1.5 hours (Zhang et al. 2017).

The chronology of diagnostic tests developed to detect EBOV in various situations has been summarized in Table 3 and the diagnostic kits developed for EBOV detection are presented in Table 4.

In recent times, new nucleic acid sequencing technologies (referred to as 'next-generation' sequencing (NGS) have evolved which provide more efficient and

Table 3. Chronology of Ebola diagnosis efforts.

S. No.	Technique(s)	Input molecule (starting material)	Target to be used for detection	Advantage(s)	Reference/year
(1)	Formalin-fixed tissue-specimens examination after enzyme treatment.	Formalin-fixed tissue slides	–	Advantages are minimized due to arrival of sophisticated and efficient diagnostic techniques.	Kurata et al. (1983)
(2)	Immunofluorescence focus assay.	Serum	Ebola antigen		Truant et al. (1983)
(3)	Enzyme immunosorbent assay for primates.				Ksiazek et al. (1992)
(4)	Immuno-enzyme test system.				Borisevich et al. (1996)
(5)	Transmission electron microscopic study of the hemorrhagic spots.	Squamous epithelial cells and the capillary endothelial cells on mucous membrane of mucous membrane of soft palate	Direct virus visualization	Visualization of virus in the enlarged Golgi apparatus.	Wang et al. (1997)
(6)	Immuno-histochemical and electron microscopic examination techniques.	Tissue	Viral antigens and particles directly visualized	For safe, sensitive, and specific detection of Ebola virus in formalin-fixed skin.	Zaki et al. (1999)
(7)	Diagnosis by RT-PCR in an epidemic setting.	Peripheral blood mononuclear cells (PBMC)	Nucleic acid	PCR-based diagnostic assay.	Leroy et al. (2000)
(8)	Development and evaluation of a fluorogenic 5' nuclease assay.	Cultured virus in Vero cells	Glycoprotein (GP) antigens	Detect and differentiate between Zaire and Sudan subtypes.	Gibb et al. (2001)
(9)	5' nuclease probe detection by real-time reverse transcription-PCR.	Serum	Nucleic acid	Detection limit: 9–16 RNA copies per reaction.	Drosten et al. (2002)
(10)	Antigen capture ELISA directed against the envelope glycoprotein of Ebola virus.	Serum	Glycoprotein (GP) antigens	Sensitivity up to $\sim 10^3$ pfu/ml. Able to detect even SDS-inactivated virus.	Lucht et al. (2004)
(11)	Rapid diagnosis of Ebola hemorrhagic fever by reverse transcription-PCR in an outbreak setting and assessment of patient viral load as a predictor of outcome.	Serum, blood, or plasma	Nucleoprotein (NP)	One-step fluorogenic Q-RT-PCR assay	Towner et al. (2004)
(12)	Immune-filtration based antigen-detection assay for rapid detection (only Zaire and Sudan strain-specific).	Serum	Matrix protein (VP40)	Sensitivity similar to ELISA and rapid assay without need of the higher technical skill.	Lucht et al. (2007)
(13)	Reverse transcription-loop-mediated isothermal amplification assay (Zaire strain-specific).	Vero cells cultured virus	NP	Portable amplification-based platform. Negative for other febrile disease viruses such as Lassa virus, Rift Valley fever virus, Yellow fever virus.	Kurosaki et al. (2007)
(14)	Quantitative 5' nuclease assay for NP/-	Vero cells cultured virus	NP	Evaluation of commercial mastermixes for sensitivity and reaction time.	Stephens et al. (2010)
(15)	SYBR Green I real-time RT-PCR.	Cell cultured virus	NP	No cross reactivity with Marburg, Dengue, Xinjiang hemorrhagic fever virus, Japanese encephalitis virus, Influenza virus (H1N1 and H3N2) and Porcine reproductive and respiratory syndrome viruses.	Liu et al. (2012, 2014)
(16)	Multiplex RT-PCR for simultaneous detection of Marburg virus and Ebola virus.	Cell cultured virus	Nucleic acid	Sensitive with ability to test two hemorrhagic viruses in a single assay.	Yang et al. (2012)
(17)	Indirect ELISA for detecting Abs specific to Ebola and Marburg viruses.	Cell culture supernatant	NP	Very sensitive assay.	Huang et al. (2014)
(18)	Novel biomarker soluble CD40 ligand (sCD40L) correlates of EVD clinical outcome.	Serum	Different 26 cytokines	Simultaneous detection of 26 proinflammatory cytokines. Increased soluble CD40 ligand (sCD40L) level is indicator of diseases severity.	McElroy et al. (2014)
(19)	Comprehensive multiplex one-step TaqMan qRT-PCR assay.	Cell cultured virus	Nucleic acid	In a single qRT-PCR assay, 28 hemorrhagic viruses may be detected.	Pang et al. (2014)
(20)	Aptameric sensing technology for rapid pandemic monitoring.	Serum	Protein	Aptamers are bio-affinity probes with improved pathogen detection ability.	Acquah et al. (2015)

(continued)

Table 3. (Continued)

S. No.	Technique(s)	Input molecule (starting material)	Target to be used for detection	Advantage(s)	Reference/year
(21)	GeoSentinel surveillance network. Differential diagnosis of illness in travelers arriving from Sierra Leone, Liberia, or Guinea.	General health surveillance of physical illness	Patients	Based on clinical data collected on GeoSentinel global surveillance network platform (57 specialized travel and tropical medicine clinics).	Boggild et al. (2015)
(22)	Antigen capture ELISA and lateral flow immunodiagnostic.	Serum	Matrix VP40 and glycoprotein (GP) antigens	Sensitivity sufficient for clinical diagnosis.	Boisen et al. (2015)
(23)	Optofluidic analysis system for amplification-free, direct detection of Ebola.	Serum	Nucleic acid	Chip-scale approach Single nucleic acid detection in 10 minutes.	Cai et al. (2015)
(24)	Fe3O4 magnetic nanoparticle-based immuno-chromatographic strip (Nanozyme-strip)	Serum	Glycoprotein (GP) antigens	Comparable to ELISA, but faster and simpler.	Duan et al. (2015)
(25)	Rapid recombinase polymerase amplification Ebola virus detection assay.	Serum and swab	Nucleic acid	Assay can combine extraction and detection in a single step.	Faye et al. (2015)
(26)	Real-time EBOV-L-RT-qPCR for detection of Zaire Ebola virus.	Serum or blood	L gene	No false positives with serum or EDTA bloods samples.	Jääskeläinen et al. (2015)
(27)	The Ebola Spatial Care Path™ (SCP): accelerating point-of-care testing (POCT).	Whole blood	Platelet count, white blood cell count, granulocyte count and percentage, and lymphocyte/monocyte count and percentage, prothrombin time, virus-specific IgM and IgG, hemoglobin and hematocrit	Ebola molecular diagnostics kits for rapid POCT. The reader/processor/cartridges in sealed, disposable pouches containing the biological and chemical reagents.	Kost et al. (2015)
(28)	Ultrafast, sensitive and large-volume on-chip real-time PCR.	(General pathogen detection)		Ultrafast RT-PCR system using microfluidic thermalization can detect Ebola in 7.5 minutes.	Houssin et al. (2016)
(29)	Prototype Cepheid GeneXpert Ebola Assay.	Stored serum and plasma samples	GP and NP gene	No cross-reactivity. Less input sample volume.	Pinsky et al. (2015); Jensen et al. (2016)
(30)	RT-loop-mediated isothermal amplification (RT-LAMP) assay	Cultured virus	Untranscribed trailer region or nucleoprotein coding region of the viral RNA	Higher sensitivity to nanoscale. No cross-reactivity to other hemorrhagic fever viruses and arboviruses.	Kurosaki et al. 2016
(31)	Upconversion nanoprobe/nanoporous membrane system.	Inactivated Ebola virus samples	Ultrasensitive detection of Ebola virus oligonucleotide	Detection up to pico-mole level. Low cost, rapid, and ultrasensitive detection.	Tsang et al. (2016)
(32)	Biofire Film-Array BioThreat-E Test (v2.5).	EDTA-blood, serum or plasma	–	Whole blood may be used.	Leski et al. (2015); Weller et al. (2016)
(33)	Zaire Ebola virus (ZEBOV)-specific enzyme-linked immunosorbent assay (ELISA) using inactivated ZEBOV isolate Makona	Serum	Glycoprotein (GP) antigens	Simple and cost effective.	Krähling et al. (2016)
(34)	RT-LAMP	Blood	Untranscribed trailer region or nucleoprotein coding region of the viral RNA.	Whole blood used to detect GP gene of EBOV high sensitivity Result within 40 minutes	Benzine et al. (2016); Kurosaki et al. (2016)
(35)	ReEBOV Antigen Rapid Test (ReEBOV RDT)	Whole blood or serum	VP antigen (anti-EBOV VP40)	Highly specific Point of care test for EVD	Cross et al. (2016)

specific viral diagnostics. The technology of NGS provides rapid high-throughput DNA sequence data in large volumes. The NGS has been successfully tested for its efficiency during the 2014 Ebola outbreak in Guinea and Sierra Leone (Gire et al. 2014). This study was able to identify the virus sequence differences and similarities to previous outbreak variants and to elaborate its emergence from the natural reservoir. Further, data helped the researchers to understand how EBOV has moved across African nations during the months of the outbreak (Gire et al. 2014). A new approach for direct detection of EBOV infection has been tested. This approach is amplification-free and based on the

optofluidic analysis (Cai et al. 2015). Recently, a research team from Marburg, Germany (Krahling et al. 2016), has developed a Zaire Ebola virus (ZEBOV)-specific ELISA using inactivated ZEBOV-Makona virus isolate. The ZEBOV-ELISA is highly specific and sensitive with superior reproducibility. This newly developed test is suitable both for the detection of the ZEBOV surface glycoprotein in vaccinated individuals and for detection of specific antibody responses directed against different ZEBOV proteins in EVD patients (Krahling et al. 2016). The possibility of EBOV-encoded miRNA-like fragment as a specific biomarker for early EVD confirmation has also been explored (Chen et al.

Table 4. Diagnostic kits developed for Ebola virus (EBOV) detection.

S. No.	Kit name	Type of the test	Input material(s)	Company name, country
(1)	–	Antigen detection: LAMP	Serum, plasma, amniotic, or synovial fluid	Lucigen, USA
(2)	ABICAP-rapid-Ebola test	Antigen detection – antibody immuno-column for analytical purpose	Whole blood, serum, urine, stool, tears, and saliva as well as in environmental samples such as water, air, and soil	Senova GmbH, Germany
(3)	AccuPower EBOV Quantitative RT-PCR Kit (EBO-1111A)	Nucleic acid detection: RT- PCR	Serum or plasma	Bioneer, Korea
(4)	eZYSCREEN kit	Antigen detection	Blood or serum	French Atomic Energy Commission (CEA), France
(5)	Liferiver™ Ebola Virus (EBOV) Real Time RT-PCR Kit	Real-time reverse transcription PCR	Blood, serum, plasma (non-heparin anticoagulant)	Shanghai ZJ BioTech Co., Ltd., China
(6)	LightMix Modular Ebola virus Zaire	Real-time reverse transcription PCR	Whole Blood	Tib-molbiol/Roche, Germany
(7)	Lipsgene SEBOV/ZEBOV Kit	Real-time reverse transcription PCR	Blood, serum, or plasma	Lipsdiag, Germany
(8)	OraQuick® Ebola Rapid Antigen Test Kit	Antigen detection	Cadaveric oral fluid/whole blood	OraSure Technologies, Inc., USA
(9)	Rapid Response Ebola Virus Test Cassette	Antigen-detection chromatographic immunoassay	Whole blood/serum/plasma	BTNX Inc., Canada
(10)	RealStar Filovirus Screen RT-PCR Kit 1.0	Real-time reverse transcription PCR	EDTA plasma	Altona Diagnostics, Germany
(11)	ReEBOV Antigen Rapid Test Kit	Antigen detection: immuno-chromatographic dipstick immunoassay	Blood/serum/plasma	Corgenix, USA
(12)	SD Q Line Ebola Zaire Ag	Antigen detection – lateral flow chromatographic immunoassay	Blood or serum	SD Biosensor Inc., GyeongGi-do, Republic of Korea
(13)	Xpert® Ebola Test	Real-time reverse transcription PCR in self-contained cartridge system	Whole blood EDTA or buccal swab specimens	Cepheid AB, Sweden

2016). The high potential of the recent advances in the field of diagnosis including molecular tools, LAMP, lateral flow assay, biosensors, biochips, microarrays, recombinant protein, and nanotechnology-based detection methodologies must be exploited to their full perspectives for rapid detection and monitoring of EBOV infections (Kurosaki et al. 2007; Belak et al. 2009; Dhama et al. 2014; Benzine et al. 2016).

9. Global EBOV monitoring and disease surveillance: did we learn new lessons?

Due to social conflicts, globalization and climate change, intercontinental migration of humans has been rampant. The uncontrolled movements and demographic transitions demand a serious preparedness for unseen lethal epidemics of uncommon viral diseases. It is no more an exotic infection for any country but has taken the nature of a global problem (Arwady et al. 2015). Thus, surveillance of emerging infectious diseases is extremely vital for the early identification and preparedness of public health threats. Fortunately, particularly after the 2014 outbreak, several new rapid molecular diagnostic assays have become available for precise surveillance of highly infectious diseases in real-time manner (Benowitz et al. 2014; Curran et al. 2016).

The last EBOV outbreak in West Africa is a serious warning to the public health system and awakening to the communities for better preparedness to combat such large-scale epidemic. More than 12,000 deaths

were reported during 2014 EBOV-epidemics. The EBOV and related viruses are extremely lethal and managing such diseases is beyond our capacity if not prepared (Walker & Whitty 2015; Woolhouse et al. 2015; Jacobsen et al. 2016). New technologies have the capacity to simulate and predict the future epidemics and can direct the public health authorities to respond in a swifter way to infectious outbreaks of all kinds. A multi-disciplinary approach has the potential to manage the spread of pathogens and reduce the morbidity and mortality. These novel tools can be harnessed for mathematical modeling, risk mapping, phylogenetic analysis, pathogen genome sequencing, and rapid on-site diagnosis (Woolhouse et al. 2015). During 2001–2003, a study in Gabon and RC recommended a method for predicting and possibly prevent human Ebola outbreaks by determining a correlation of wild animal mortality with human Ebola outbreaks (Rouquet et al. 2005). To monitor the disease outbreak effectively, it should be considered to gather data from contaminated raw food products derived from bushmeat and plants also (Mann et al. 2015).

In recent times, Internet-based surveillance tools and epidemic simulation-based prediction can efficiently facilitate the potential risk assessment and outbreak detection in infectious disease emergencies which can prevent future infectious disease threats (Christaki 2015). The widespread transmission that occurred in the three most affected countries (Guinea, Liberia, and Sierra Leone) in West Africa and the epidemic's designation as a 'Public Health Emergency of

International Concern (PHEIC) insisted the formulation of specific guidelines to efficiently manage the EBOV epidemic. In 2014 CDC, issued interim USA guidance for 'Monitoring and Movement of Persons with Potential Ebola Virus Exposure' to guide CDC staff and public health partners engaged in the response. The guidance provided by public health authorities and collaborative agencies with recommendations for monitoring travelers who may be potentially exposed to Ebola and therefore authorities can enforce the restriction in movement. In humans, EBOV spreads through direct contact with infected body fluids (blood, vomitus, feces, semen, vaginal fluid, and urine) with broken skin or exposed mucous membranes, and possibly through sexual contact. Because of the risk for importing disease from infected travelers, in October 2014 the CDC recommended that 'all travelers to the United States of America from Ebola-affected countries receive enhanced entry screening and post-arrival active monitoring for Ebola signs or symptoms until 21 days after their departure from an Ebola-affected country (CDC 2015). On 19 February 2016, the CDC-guidance expired as Guinea was declared free of EBOV transmission (after more than 45 days had passed since the last EBOV case reported and the human-to-human transmission ended).

New technology platforms are now being employed vigorously for monitoring and surveillance of EBOV (Cross et al. 2016). During 2014 EBOV outbreak, genomic surveillance was performed using 'next-generation' sequencing (NGS) which helped in identifying the EBOV origin and transmission (Gire et al. 2014). Further, several start-ups are seriously designing the efficient and portable devices for EBOV genomic surveillance. In this line, a novel portable Nanopore DNA sequencing instrument (Pennisi 2016; Quick et al. 2016) was developed and used in EBOV genome sequencing. The Nanopore real-time genomic surveillance system was transported in standard airline luggage to Guinea and used for real-time genomic surveillance of the ongoing epidemic during April 2015. Once received an Ebola-positive sample, this portable system produced results in less than 24 hours and genomic sequencing within 15–60 minutes. Such innovations warrant the use of genomic surveillance in resource-limited settings in a real-time manner. Rapid monitoring of potential outbreaks, using such portable sequencer, is now a reality in remote areas (Quick et al. 2016). Further, a handy, portable, and compact (13.5 cm × 8.5 cm × 2.5 cm) infection monitoring system was recently introduced to monitor mass gathering places such as airports (Sun et al. 2015). This highly mobile and hand-held system can rapidly screen the non-contact vital signs using two integrated sensors. One sensor which is a 24-GHz microwave radar can measure the heart and respiration rates and another sensor is a thermopile array for capturing facial temperature. After collecting vital signs

data, the system detects infected individuals using a linear discriminant function. Such devices can be very useful in providing real-time updates and essential resources for managing current Ebola outbreak with the help of Global Ebola Response Monitoring and Mapping System (WHO) and United Nations Mission for Ebola Emergency Response (UNMEER) (Sun et al. 2015).

The global revolution of the Internet and social media is a very powerful tool in disease surveillance. Short message service (SMS)-based system has been established to facilitate the active surveillance of individuals who are either returning from EVD-affected countries or in contacts with EBOV-exposed local confirmed cases (Tracey et al. 2015). This SMS-system collects the information on symptoms and temperature twice daily and communicates to the public health authorities (Tracey et al. 2015). During the August 2015 epidemic of EBOV in Sierra Leone, toll-free, nationwide Ebola call centers were established by Sierra Leone's Emergency Operations Center. This 'alert' system proved to be very effective in providing health education about EBOV to the callers and boosting public reporting to public health officials about possible Ebola cases and deaths (Miller et al. 2015). Based on a study in southern Sierra Leone, the effectiveness of the cell phone messaging technology has been proved to be highly effective as a rapid communication tool for community epidemic surveillance from peripheral health care facilities to higher levels (Jia & Mohamed 2015). One recent report suggested that the utility of hybrid information network in facilitating the quicker public health measures may be possible, by integrating both hierarchical and formalized command control-driven and community-based, or ad hoc emerging networks, which can efficiently employ social media to facilitate the robust preparedness for the potential outbreak and improving the capability of EBOV-identification and diagnosis (Hossain et al. 2016).

A community-based effort in Liberia has been very successful to defeat the EVD in recent times (Shrivastava et al. 2016). These coordinated efforts involved strong leadership and clear vision by the policy-maker by declaring the disease as a public health priority to all the concerned departments; actively involving the community leaders by developing a sense of need among them; increasing the level of faith and neutralizing any rumors about the disease among the local people – by building see-through walls in treatment centers so that people can watch the proceeding or developing mechanism to respond promptly to transport calls or by sensitizing the health professionals to build confidence among local residents; strengthening of the treatment facilities (by creating medical units, treatment centers, logistics support) and laboratory network by establishing five dedicated Ebola laboratories; courageous attitude of the local volunteers;

enormous support from the international community; and well-coordinated national and international response (Shrivastava et al. 2016). In response to the 2014 EVD-outbreak, a common international code, ICD-10-CM code 'A98.4' (International Classification of Diseases -10th Version-Clinical Modification) has been implemented in all – in all health care institutions for Ebola bio-surveillance specifically. This health care coding system will facilitate real-time tracking of EBD-outbreaks (Chabra 2015). Since EBOV is a mutating RNA virus, a rapid monitoring system using aptameric sensing technology has been proposed for pandemic surveillance (Acquah et al. 2015). Computer simulation and mathematic modeling have been proposed to quantify the West Africa Ebola epidemic (Chretien et al. 2015). International Society of Travel Medicine and CDC collectively operate a global GeoSentinel surveillance network of travel and tropical medicine clinicians that collects de-identified demographic, diagnostic, and travel information on ill travelers who cross international borders. GeoSentinel is composed of 63 clinical sites in 29 countries on 6 continents. Through the efforts of GeoSentinel surveillance network, differential diagnosis of EVD in travelers arriving from Liberia, Sierra Leone, or Guinea was conducted to track the outbreak (Boggild et al. 2015). A similar approach 'Surveillance and Outbreak Response Management System (SORMAS)' has also been suggested to support the control of the EVD-outbreak (Fähnrich et al. 2015). In USA, to understand and address the doubts and concerns of the citizens' life twitter chat was conducted thus such platforms can be very effective in future to collect and disseminate the information in future at the time of such outbreaks (Lazard et al. 2015).

Finally, the lessons learned from such epidemic with high mortality emphasize that the efficient surveillance system and coordinated global efforts involving every social and political leader can be a best possible scenario to prevent such deadly viral disease (Carney & Weber 2015). Though CDC/WHO has officially declared the end of the Ebola outbreak, the residual risks of Ebola reintroduction or re-emergence are still haunting the human population worldwide. According to the Ebola Situation Report – 16 March 2016, the WHO has strongly recommended the implementation of superior EBOV-monitoring systems in Guinea, Liberia, and Sierra Leone to alert authorities for the EVD-clinical cases or EVD-related death. Developed rich nations must effectively implement a high-quality, robust emergency response system, and provide the unconditional support to the establishment of sustainable health care services in West Africa (Quaglio et al. 2016). Only in this way we together can avert the next humanitarian crisis due to EBOV or related viral diseases.

10. Prevention and control

Currently, there is no robust and potent vaccine to control this lethal infection leaving prevention to be the best way to escape from infection. Monitoring, surveillance, isolating infected persons and treating them separately, travel to the endemic area during outbreaks should be discouraged, are the limited and effective ways to prevent further spread of infection (Tambo et al. 2014). For EVD-outbreak prevention, a new organization named as 'United Nations Mission for Ebola Emergency Response (UNMEER)' has been created during the 2014 outbreak of EBOV in Africa.

Various strategies like reporting of EVD-outbreak, proper treatment to infected persons, proper burial of dead persons, disinfection of the areas, and proper planning to prevent further spread are the measures taken to control EVD at national and international level. Hospitals, where infected persons are treated, should also be disinfected along with sterilization of all instruments that come in contact with the infected persons. Both incineration and autoclave can easily destroy the virus. Fecal wastes should also be deactivated to destroy the virus. Places, where there are no proper facilities for incineration or autoclave should pack the materials in a leak proof triple-layered container and these should be transported to other places for sterilization and disposal.

There is an urgent need for creating awareness among the public about this deadly disease so that spread of Ebola can be prevented (Salman et al. 2017). Proper monitoring at airports and public places where there are all possible chances for spread of infection is of utmost importance (Brown et al. 2014; Read et al. 2015). People who fall ill should be kept under the scanner for at least 21 days to rule out the infection. Health care workers and doctors are also at higher risks as they come in direct contact with the infected persons. Hence, they should wear all protective wears like gowns, gloves, face mask, goggles, and respirators and also should follow barrier nursing. Collected samples for diagnosis should be transported under high precaution and handled only in the BSL4 lab. Climate change has a huge role in the dissemination of various diseases, and EBOV can also be transmitted to non-human primates due to movement of bats as a result of climate change (Dhama, Tiwari, et al. 2013). Since bushmeat has been found to be the potential and important source of many epidemics in the past, it is important to monitor their illegal export to the naïve countries (Mann et al. 2015).

11. Vaccines

Although EBOV was discovered in 1976 until now, there is no effective vaccine or post-exposure

treatment available. After the recent outbreaks, the EVD management plan relies majorly on supportive therapies. Since 2014 EBOV-outbreaks, the scientific community is working tirelessly to develop immunization regimen to control virus spread (Geisbert & Jahrling 2003). The prime-boost approach of DNA vaccine followed by adenovirus vectored ZEBOV GP has resulted in good cellular and humoral response in cynomolgus macaques during experimental studies. DNA vaccines are also in phase I of clinical trials. The glycoprotein of EBOV has been a good target for eliciting humoral immunity and surface protein targeted human monoclonal antibody has provided a ray of hope for a better vaccine (Nyamathi et al. 2003). These DNA vaccines contain GP and NP of any of the five strains of EBOV. It is administered intramuscularly and followed by booster it generated an immune response in 70% of volunteers with poor T cell response. DNA vaccination may be further improved by vaccinating through electroporation, injecting higher quantities, or prime boosting with other antigen delivery platforms (Sarwar et al. 2015). The problem in developing a peptide-based vaccine to protect from this infection is due to the vast similarity that exists between it and human peptides. So, it is necessary to identify certain peptides that are unique and conserved in EBOV to develop peptide-based subunit vaccines (Kanduc, in press). Experiments with lab animals like mice and guinea pigs revealed that DNA vaccine protected them against challenge with wild-type Mayinga 1976 strain of ZEBOV (Gulland 2014).

Recombinant virus vectors are a good choice of antigen delivery as they induce the cell-mediated immune response, due to intracellular expression and processing of antigen. Of note, Ad5 serotype is a popular adenovirus-based vector used for such purposes due to ease in genetic manipulations and to grow to high titers. In this approach, an E1 region of adenovirus is replaced with GP of Ebola, and in non-human primates, it offered 100% protection in a single dose. However, preexisting neutralizing antibodies limit the utility of Ad vectors (Ledgerwood et al. 2010). Immunization in individuals having anti-Ad5 antibodies before vaccination showed reduced cellular and humoral response. Adenovirus-based vaccine (cAdVaxE) is a bivalent vaccine against GP of ZEBOV and SEBOV which has protected 100% of mice against challenge (Wang et al. 2016). Another virus used include vesicular stomatitis virus (VSV) which is an enveloped single-stranded RNA virus. The recombinant VSV is produced by replacing GP of VSV with GP of EBOV. When recombinant VSV-based filovirus vaccine is used in prime-boost approach, it can give good protection. The VSV-vectored vaccine expressing GP of filovirus has protected non-human primates against three species of filovirus and Marburg virus (Mire et al. 2013). Furthermore, it has entered in phase III efficacy trial in Guinea.

It conferred protection in non-human primates using different routes of immunization like mucosal, oral, or intramuscular (Geisbert et al. 2009) with advantages like transient viremia and self-limited febrile illness (Sridhar 2015). Likewise, the recombinant human parainfluenza virus 3 (HPIV-3), a single-stranded, negative-sense RNA virus, has been used as a vaccine candidate owing to its ability to accommodate several GPs in the viral backbone (Skiadopoulos et al. 2002). Intranasal immunization with EBOV GP expressed with replication-competent HPIV3 showed better protection against challenge with a higher parental dose of EBOV (Yang et al. 2008). Other vectors like cytomegalovirus (CMV)-based vaccine protected mice against EBOV, and this has the potential to be used against EVD in humans (Tsuda et al. 2015).

VLPs produced by expressing GP and VP40 in mammalian cells can also stimulate protective immune response making better vaccine options (Cazares et al. 2016). GP-based fusion vaccines developed by EBOV GP fused with Fc fragment of human IgG1 adjuvanted with poly-ICLC have shown to induce a strong humoral immune response and protect guinea pigs against lethal EBOV challenge (Konduru et al. 2016). Subunit vaccine contained GP protein expressed in *Autographa californica* multicapsid nucleopolyhedrovirus, a baculovirus expression system in SF9 insect cells. Infected cells are lysed and adjuvanted with Matrix-M, a phospholipid constituent of synthetic cholesterol and saponin. This vaccine generated higher cellular and humoral activity (Lovgren et al. 2011; Martins et al. 2016). With the use of reverse genetics, defective EBOV without VP30 was found 100% effective in protecting guinea pigs and mice (Hoenen et al. 2012). Replication-defective vaccine by deleting VP30 has also been considered as an effective vaccination strategy to prevent the infection (Saphire 2015).

Although WHO reported two vaccine candidates, namely chimpanzee adenovirus-based, and VSV-based EBOV vaccines, as are under phase III clinical trials, there is a need to search for a safe and effective vaccine (Cohen 2014; Gulland 2014). Heterologous VSV- and Ad5-vectored Ebola vaccine, GamEvac-Combi was used in phases I and II trial which induced both humoral and cellular immunity in all the 84 volunteers (Dolzhikova et al. 2017). Presently, seven vaccines have entered clinical trials (Chappell & Watterson 2017). Scientists from the University of Texas, USA developed a new EBOV inhalant vaccine that provides 67%–100% protection in non-human primates. Most recently, a recombinant VSV-Zaire ebolavirus vaccine (rVSV-ZEBOV) is being tested under 'Sierra Leone Trial to Introduce a Vaccine against Ebola (STRIVE)' program. This promising candidate vaccine, which is in phase 2/3 human trial, is the result of collaborative efforts between the College of Medicine and Allied Health Sciences, University of Sierra Leone, the Sierra Leone

Ministry of Health and Sanitation, and the CDC. This new vaccine is based on Zaire ebolavirus immunogen and can only protect against Zaire ebolavirus, the major agent behind EVD in West Africa (Folayan et al. 2016). Progress and advances in the area vaccines and vaccination comprising of edible vaccine, peptide vaccine, DNA vaccine, immunomics-based vaccine, and others need to be optimally utilized for designing and developing effective vaccines along with exploiting immunomodulatory approaches employing novel adjuvants and vaccine delivery systems for improving the efficacy of the EBOV vaccines (Dhama, Wani, et al. 2013; Delany et al. 2014; Sarwar et al. 2015; Singh et al. 2015).

12. Treatment

The recommended symptomatic treatments include anti-inflammatory drugs for fever and pain, and intravenous fluids to maintain body osmotic balance. As the kinome analysis revealed the involvement of mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinases (PI3Ks), and calcium/calmodulin kinases in EBOV infectivity, the PI3Ks inhibitor LY294002 and CAMK2 inhibitor KN-93 were tested and found effective in reducing EBOV infection in Vero E6 cells. The p38 MAP kinase has a role in activating the signaling cascade leading to produce interleukins and TNF α . The p38 MAPK inhibitor like SB202190 helps in ameliorating EBOV-mediated cytokine storm in human monocyte-derived dendritic cells (Johnson et al. 2014). Artemisinin, an antimalarial drug is also beneficial regarding reducing the acute inflammatory response (Ho et al. 2014). FTY720 is a drug used to treat patients with autoimmune disorders and prevents transplant rejection. Its sphingosine analog AAL-R helped in limiting the inflammation in mice challenged with H1N1 influenza virus (Walsh et al. 2011).

As of now, to kill EBOV, we do not have a specific anti-viral drug. Many anti-viral drugs are being tested. Therefore, the current treatment relies only on supportive therapy to revive. Recently, it has been discovered that antibodies can effectively block the entry of this virus so it can be employed as therapy for control of Ebola infection in humans (Corti et al. 2016). There is a search for a new drug to control lethality caused by this virus (Choi & Croyle 2013; Kilgore et al. 2015). Clomiphene and Toremifene are estrogen receptor drugs which were found to inhibit EVD infection in mice. These drugs inhibit virus entry and also inhibit EBOV fusion to host cells (Johansen et al. 2013). Ion channel blocking agents like amiodarone were reported to inhibit entry of EBOV into the cells (Gehring et al. 2014). These drugs combined with anti-viral drugs can be candidates for the treatment of EVD in places where medical facilities are less. Recently, a group of researchers has tested the anti-flu drug Favipiravir in EVD

treatment (Nagata et al. 2015; Sissoko et al. 2016) which indicated its use in patients with medium to high viremia. However, it turned out to be not effective in the case of very high viremia (Sissoko et al. 2016). Convalescent whole blood (CWB) and convalescent plasma (CP) therapy intends to transfuse the blood/plasma from disease survivors and helps in neutralizing and reduce virus load, till the time patients own immune system can destroy the invader (Van Griensven et al. 2015). Up to 10 ml/kg BW of CP can be obtained on 15 days interval from a plasma donor. It has longer shelf-life. However, CP usage is superior to CWB as there might be a problem of the immune response against leukocyte antigens and febrile transfusion reactions with the risk of bloodborne pathogen transmission (Erhabor & Adias 2011). WHO also recommended the use of whole blood products and convalescent serum (Gulland 2014) during critical conditions. Before transfusion, exhaustive testing of plasma is required for residual EBOV RNA, and other potential viruses like HIV, HBV, HCV, and syphilis. Purified IgG from survivors given to challenged non-human primates two days post infection, prevented death, showing the importance of antibody preparations (Dye et al. 2012). RNAi has been a promising technology that can be used for the treatment of EBOV (Warren et al. 2010). A pyrazine carboxamide derivative T-705 (Favipiravir) is in the last phase of the clinical trial and has yielded satisfactory results in a mouse model against EBOV. Favipiravir neither inhibits the DNA or RNA synthesis (inhibit RNA-dependent RNA polymerase) in cell lines of mammalian origin, nor it is toxic to cells (Oestereich et al. 2014). Brincidofovir is a cidofovir analogue conjugated with lipid and also possesses anti-viral activity. It has activity against dsDNA viruses but how it prevents Ebola replication is still a question (Florescu & Keck 2014; Dunning et al. 2016). The broad spectrum anti-viral agent alisporivir which inhibits the host protein cyclophilin A (CypA) seems to have not much effect on Ebola strains (Makona and Mayinga) as this virus does not require CypA for replication (Chiramel et al. 2016).

BCX4430, viral RNA polymerase inhibitor was protective against EBOV in mice (Fauci 2014; Warren et al. 2014). A protein named double-stranded RNA binding protein 76 (DRBP76) interacted with viral proteins, with RNAs and with PKR, an interferon-induced anti-viral kinase and also inhibited polymerase function of EBOV (Shabman et al. 2011). The siRNA technology targeting L-protein of RNA polymerase was used to inhibit EBOV replication hence can be used as a post-exposure treatment to EBOV infection (Geisbert et al. 2010). The siRNAs and phosphorodiamidate morpholino oligomers, PMO aimed at ZEBOV RNA polymerase L-protein prevented EVD in non-human primates (Warren et al. 2010; Geisbert et al. 2010). PMOs tend to target VP35 and VP24 that protects from the disease (Warren et al. 2015). Two new drugs based on antisense PMOs (AVI-

Table 5. Different available therapies for Ebola virus treatment.

Type of therapy	Name of molecule/modality	Modus operandis	Model organism (onto which the test has been conducted)	Company	References
siRNA therapy	TKM-Ebola	Cocktail of three siRNAs targeting L, VP24, and VP35 genes	Monkeys	Tekmira, Canada	Geisbert et al. (2010)
Antiviral drug	AVI-7537	Through gene silencing	Mice and monkeys	Sarepta Therapeutics	Warren et al. (2010); Iversen et al. (2012); Heald et al. (2014)
	Favipiravir (T-705), a pyrazinecarboxamide derivative	Inhibit viral RNA-dependent RNA polymerase activity and induce lethal RNA mutations	Mice	Toyama Chemical, Japan	Smither et al. (2014); Oestereich et al. (2014)
	Brincidofovir (CMX001)	Improved bioavailability and activity against dsDNA viruses	Human (but not for ebola-specific)	Chimerix of Durham, North Carolina	Florescu et al. (2015)
ZMapp	Combination of humanized monoclonal antibodies (c13C6 + c2G4 and C4G7)	Conformational epitopes of GP2 or GP trimer stem region are targeted	Macaques	Mapp Biopharmaceutical, San Diego, California	Haque et al. (2015); Kilgore et al. (2015)
Nucleotide analogue	BCX4430	Nucleoside RNA polymerase inhibitor, incorporate into growing RNA and lead to premature termination	Mice	BioCryst Pharmaceuticals, Inc., Durham, North Carolina	Warren et al. (2014)
Induction of Niemann-Pick C1 mutant phenotype	U18666A/clomiphene/toremiphene induce cholesterol accumulation in lysosomes and inhibit Ebola virus infection	Membrane fusion and virus escape is prevented	<i>In vitro</i>	A chemical supplied by several vendors across the world	Carette et al. (2011)
Repurposed drug	FTY720 (FDA-approved drug for multiple sclerosis)	Prevent cytokine storm and sepsis, reduce inflammation	Human	Sigma/Novartis	Japtok and Kleuser (2009)
	FX06 (anticoagulant therapy)	Fibrin-derived peptide help in vascular leakage	Human	MChE-F4Pharma, Vienna, Austria	Wolf et al. (2015)
	Rosuvastatin, atorvastatin, and pravastatin (lipid lowering drugs)	Reduction of cholesterol content affect the biosynthesis machinery in Ebola virus	Human	By several vendors across the world	Calza et al. (2012); Haque et al. (2015)
	Chloroquine, hydroxychloroquine (antimalarial/lupus erythematosus /rheumatoid arthritis treatment with its anti-inflammatory properties)	Prevent endosome acidification and Ebola virus is trapped inside endosome	Human	By several vendors across the world	Madrid et al. (2013); Olsen et al. (2013); Kuehne et al. (2016)
	rNAPc2 (recombinant nematode anticoagulant protein c2)	Modulation of coagulation factors	Primates	ARCA biopharma, Colorado	Geisbert et al. (2003)
	Suramin (anti-trypanosomal agent)	Anti-heparin activity, interfere with viral replication and viral entry into the cell	<i>In vitro</i>	Germanin, Bayer	Henß et al. (2016)
Convalescent plasma (CP) therapy	Plasma for Ebola survivors	Providing highly specific antibodies and replenish lost serum components	Rhesus macaques	NA	Jahriling et al. (2007); Dye et al. (2012); Mendoza et al. (2015)
Viral-vectored vaccines	Recombinant chimpanzee adenovirus serotype 3 vector	GP gene	Human	NA	Choi et al. (2015)
	Recombinant Vesicular stomatitis virus		Cynomolgus macaques	NA	Jones et al. (2005); Qiu et al. (2009); Falzarano et al. (2011)
DNA vaccine	Venezuelan equine encephalitis virus (VEEV) replicons	GP/NP	Mice and guinea pigs	NA	Pushko et al. (2003)
	Inserted NP/GP	Antigen presentation by both MHC-I and II	Guinea pigs or chimpanzees	NA	Sullivan et al. (2009); Hoenen et al. (2012)
Virus-like particles (VLPs)	DNA vectors expressing GP and VP40 proteins in human 293T cells	Morphologically similar to virus and provoke protective immune response	Mice	NA	Ye and Yang (2015); Carra et al. (2015)
Trojan-horse antibodies	Bispecific antibody	Specific for NCP1 or GP receptor binding site coupled to mAb against conserved surface exposed GP epitope	Mice	NA	Wec et al. (2016)
Human transbodies	Cell penetrable human ScFv against VP40	Inhibit egress of EBOV VLPs from hepatocytes	<i>In vitro</i>	NA	Teimoori et al. (2016)
TmPyP4	Cationic porphyrin	Inhibition of L gene of EBOV	<i>In vitro</i>	NA	Wang et al. (2016)

6002, AVI-6003) and lipid nanoparticles (LNP)/siRNA (TKM-Ebola) are in phase I clinical trials. Encapsulation with LNP gives an effective drug delivery, but they need several dose regimens to achieve efficacy (Choi & Croyle 2013; Thi et al. 2015). The miRNA technology has also shown good results during *in vitro* trials (Sheng et al. 2014). Understanding the mechanism of virus entry through two-pore channels paved the way in testing certain molecules such as Tetrandrin that inhibits or halts virus entry into the cells and prevents the disease (Sakurai et al. 2015).

Meanwhile searching for new drugs, some of the drugs have been repurposed for therapy (Table 5). Chloroquine and its structural analogs including hydroxychloroquine, pamaquine, primaquine, and plasmaquine, etc. are antimalarial drugs. These are also considered as a lysosomotropic agent that prevents endosomal/lysosomal acidification. Based on the literature data, such endosomal/lysosomal acidification inhibition by potential therapeutic agents such as chloroquine are highly effective against viral infections and associated pathologies such as of EBOV. Intraperitoneal administration of chloroquine protected 100% of monkeys challenged with EBOV with a dose of 90 mg/kg BW, 4 hours before infection (Olsen et al. 2013). Of note, in Liberia when the mass administration of the antimalarial drug was adopted, reduction in fever status was observed during Ebola outbreak (Kuehne et al. 2016). Rosuvastatin, atorvastatin, and pravastatin are the statins which reduce inflammatory action and are known to reduce C-reactive protein (CRP) and TNF α . It also hampers cholesterol supported EBOV membrane biosynthesis (Haque et al. 2015). EBOV infection leads to overexpression of the procoagulant tissue factor and endothelial cells also participate in coagulation disharmony characteristic of Ebola infection. In macaque model recombinant nematode anticoagulant protein c2 (rNAPc2) improved survival and provided an additional therapeutic regimen which can be adapted along with several other therapies (Geisbert et al. 2003b). Suramin which is used as an anti-trypanosomal agent can also be used as an anti-viral agent to treat EVD and Chikungunya due to its competitive anti-heparin activity. It also can interfere with viral replication and viral entry into the cell (Henß et al. 2016). In the face of unavailability of any suitable treatment procedures for curing the affected persons of this dreaded disease, use of passive immunization (neutralizing antibodies) protocol was opted by transferring the sera from healthy persons which recovered from EVD (Saphire 2013). But it is not sufficient alone to give 100% protection after the onset of viremia (Mire et al. 2016). Additionally, ZMapp which possess humanized mouse antibodies showed promising results in non-human primates (Wong, Qiu, et al. 2014; Zhang et al. 2014) and consequently, on 12 August 2014, the WHO approved the use of ZMapp for the treatment of EVD.

These mAbs are expressed in the glycomodified *Nicotiana benthamiana* and found to be carrying better anti-EBOV efficiency in animal models (Zhang et al. 2014). Bispecific Trojan-horse antibodies that can broadly neutralize all known filoviruses were found to protect mice from multiple EBOV infection (Wec et al. 2016). Recently, attempts have been made to evaluate the anti-Ebola potential of human scFvs against VP40 envelope protein of EBOV that plays an important role in its life cycle. These antibodies were found to block the Ebola VLPs in hepatic cells which are transduced with pseudo-Lentivirus particles (Teimoori et al. 2016). This suggests that these transbodies might be effective against EBOV as well interfering with their replication in the cell. Also, it has been found that there exists a conserved guanine-rich sequence within the L gene of EBOV that can form quadruplex RNA and it can be targeted by cationic porphyrin TmPyP4 that leads to inhibition of L gene expression (Wang et al. 2016).

Progress is on the way towards developing effective therapeutics as some lead has been obtained by identifying the weak spots in EBOV structure. Looking into the demand of ZMapp and existing limited manufacturing facilities, an alternative approach such as the use of the plant as bioreactors for the bulk production of ZMapps, have been proposed. Certain host factors are also responsible for virus replication and survival. To identify them insertional mutagenesis can be employed which is a high-throughput method where host genes are disrupted. This helps in identifying those genes required for virus replication and can be used as a drug target in future (Cheng et al. 2016). This gives direction to further identification of viral targets or host targets which could be used to control or inhibit this virus disease in the patient. Molecular docking studies of EBOV-glycoproteins have been carried out recently which could provide insights into drug designing (Ahmad et al. 2016; M Alam El-Din et al. 2016). Advances in designing effective therapeutic agents and drugs by utilizing emerging and upcoming options, namely cytokines, recombinant proteins, RNAi technology, Toll like Receptors, avian egg antibodies, nano-medicine, immunomodulators, probiotics herbs, need to be explored to the desired potential to counter EBOV (Geisbert et al. 2010; Dhama, Chakraborty, et al. 2013; Dhama, Saminathan, 2015; Malik et al. 2013; Gao & Yin 2014; Arntzen, 2015; Streatfield et al. 2015; Yao et al. 2015; Cross et al. 2016; Iqbal et al. 2016).

13. Conclusions and future perspectives

EVD is one of the most common examples to refer emerging or re-emerging zoonoses globally. Nonetheless, the disease is known since 1976, its severity, exceptional infectious or contagious nature, acquiring pandemic status during 2014–2015, has raised numerous questions before the health professionals and

policy-makers. The emergence of Ebola as a threat has cautioned all of us to get ready with arsenals for contending Ebola-like diseases through multidisciplinary and collaborative approaches overcoming the national boundaries. This is the right time to tackle the EVD emergency through the development and use of state-of-art tools and techniques befitting for confirmatory diagnosis, developing internationally suited surveillance, monitoring, and networking systems as well as identifying the animal reservoirs. The urgency for developing and use of both, the safe and efficacious vaccine and affordable anti-viral drugs are desperately needed. Kinome, transcriptome, and exome analyses may be helpful in identifying the genomic targets up or down-regulated and also insight into siRNA and miRNA generated during infection helping in developing efficacious therapeutic regimens. This is an apt time to think of for 'One Health' concept and come out with a strategy to safeguard human, animal, and environment. Realizing the eventual impact of the threat imposed by EVD, all pinpointed efforts must be focused on achieving the goal of preventing and controlling the existence of EVD timely. The Ebola epidemic has been a humanitarian crisis in the three worst affected countries in West Africa. Although WHO has declared the end of Ebola, there is still a potential risk of sporadic transmission of EBOV which could be largely due to residual virus persistence in some survivors in West Africa (Brainard et al. 2016). The WHO is urging public health authorities to remain on high alert and ready to respond in any emergency (Stamm 2015). Further, strong surveillance and emergency response capacity need to be upheld, while care, screening, and counseling also need to be provided for survivors.

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