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Review Article

REVIEW ARTICLE

Neglected filoviruses

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One sentence summary: While Ebola virus dominates the headlines, other filoviruses remain largely uncharacterized but may pose equal risks to humans.

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ABSTRACT

Eight viruses are currently assigned to the family *Filoviridae*. Marburg virus, Sudan virus and, in particular, Ebola virus have received the most attention both by researchers and the public from 1967 to 2013. During this period, natural human filovirus disease outbreaks occurred sporadically in Equatorial Africa and, despite high case-fatality rates, never included more than several dozen to a few hundred infections per outbreak. Research emphasis shifted almost exclusively to Ebola virus in 2014, when this virus was identified as the cause of an outbreak that has thus far involved more than 28 646 people and caused more than 11 323 deaths in Western Africa. Consequently, major efforts are currently underway to develop licensed medical countermeasures against Ebola virus infection. However, the ecology of and mechanisms behind Ebola virus emergence are as little understood as they are for all other filoviruses. Consequently, the possibility of the future occurrence of a large disease outbreak caused by other less characterized filoviruses (i.e. Bundibugyo virus, Lloviu virus, Ravn virus, Reston virus and Taï Forest virus) is impossible to rule out. Yet, for many of these viruses, not even rudimentary research tools are available, let alone medical countermeasures. This review summarizes the current knowledge on these less well-characterized filoviruses.

Keywords: cuevavirus; Ebola; ebolavirus; *Filoviridae*; filovirus; marburgvirus

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INTRODUCTION

Viruses with single-stranded negative-sense RNA genomes can be assigned to three unofficial supergroups based on whether these genomes are multisegmented, circular or unsegmented, and on evolutionary relationships of the genome-encoded RNA-dependent RNA polymerases (Li *et al.* 2015). All of the unsegmented viruses are currently assigned to the rapidly expanding order *Mononegavirales*. The family *Filoviridae* is one of the eight mononegaviral families (Afonso *et al.* 2016). *Filoviridae* members are differentiated from other mononegaviruses not only by genomic sequence, but also by the formation of characteristically shaped, filamentous virions. In addition, filoviruses are also differentiated from other mononegaviruses by comparatively long (≈19 kb) genomes containing gene overlaps, unique transcriptional initiation and termination signals, and an open reading frame (ORF) encoding a unique structural protein without obvious homologs in other mononegaviruses (i.e. VP24) (Kuhn *et al.* 2010, 2011). Filoviruses are further assigned to seven species included in the three genera *Cuevavirus*, *Ebolavirus* and *Marburgvirus* (Table 1) based on individual molecular properties (Kuhn *et al.* 2010, 2011; Bao, Chetvernin and Tatusova 2012; Lauber and Gorbalenya 2012). Six of the currently eight recognized filoviruses are known human pathogens causing two diseases officially recognized by the World Health Organization: Ebola virus disease (EVD) and Marburg virus disease (MVD). The remaining two filoviruses are known or suspected animal pathogens (Table 2). In general, filoviruses can be considered exotic pathogens. Human and/or animal filovirus disease outbreaks are rare. Only 37 outbreaks have been recorded in the almost 50 years since the discovery of filoviruses, and of those, only 11 included more than 100 cases each (Table 3) (Kuhn 2015).

Based on the publication records (Kuhn 2008), the filoviruses that are currently most characterized and understood are Ebola virus (EBOV), Sudan virus (SUDV) and Marburg virus (MARV). The filovirus best known and feared by the general public is EBOV. This notoriety is in part due to numerous popular science publications, fiction and movies (Semmler 1998; Kuhn 2008; Blakey *et al.* 2015) and in part because EBOV had caused the most recorded human filovirus infections of all filoviruses, i.e. 1101 until 2012 (Kuhn 2015). At the end of 2013, EBOV caused an unprecedented EVD outbreak in Western Africa, with thus far 28 646 human infections and 11 323 deaths (case-fatality rate [CFR] = 39.51%) (World Health Organization 2016). Unsurprisingly, this public-health emergency resulted in massively increased funding for basic and translational EBOV research, including the development and testing in clinical trials of potential medical countermeasures.

However, despite tremendous progress in understanding of filovirus infections *in vitro* and *in vivo*, where and how filoviruses are maintained in nature remain unclear. In addition, the circumstances or mechanisms that lead to the occasional filovirus emergence in human and other mammal populations are also unknown. To emphasize research more or less exclusively on EBOV, SUDV and MARV may quite literally be a fatal mistake (Anthony and Bradfute 2015; Kozak and Kobinger 2016). One cannot exclude the possibility of a future disease outbreak of the scope of the Western African outbreak caused by other filoviruses, including those viruses currently thought to be apathogenic for humans.

This review provides an overview of the current knowledge of these neglected filoviruses: Bundibugyo virus (BDBV), Lloviu virus (LLOV), Ravn virus (RAVV), Reston virus (RESTV) and Taï Forest virus (TAFV).

Bundibugyo virus

Bundibugyo virus (BDBV; pronounced ˌbʊndiːˈbʊdʒɔː vaɪrəs) was discovered in Eastern Africa during a human viral hemorrhagic fever (VHF) outbreak that probably began in or around August 2007 and lasted until January 2008. The outbreak affected the Bundibugyo and Kikyo townships in Bundibugyo District of Western Uganda Administrative Region, Uganda and ultimately amounted to 149 suspected cases and 37 deaths (CFR = 24.8%; alternative published statistics, based on different case definitions and index case identifications, are as follows: 116 cases and 39 deaths [33.6%]; and 131 cases and 42 deaths [32.1%]) (Towner *et al.* 2008; MacNeil *et al.* 2010; Wamala *et al.* 2010; Centers for Disease Control and Prevention 2015). A second EVD outbreak due to BDBV was recorded in August of 2012 around Isiro, Haut-Uele District, Province Orientale, in northeastern Democratic Republic of the Congo, roughly 400 km northwest from Bundibugyo (Fig. 1). When that outbreak was declared over in November 2012, 62 people had been infected and 34 had died (CFR = 54.8%; an alternative statistic, based on a different case definition, is 77 cases and 36 deaths [CFR = 46.8%]) (Albariño *et al.* 2013; Centers for Disease Control and Prevention 2015; Kratz *et al.* 2015). Therefore, the average CFR range of human BDBV infections (32.3%–41.1%; Table 3) is comparable to that of EBOV infections (≈41.4%). The often repeated notion that BDBV is 'less virulent' than EBOV in humans cannot be upheld based on available data.

A number of BDBV isolates have been obtained (Table 4), and Ugandan Bundibugyo virus/H.sapiens-tc/UGA/2007/Butalya-811250 (BDBV/But-811250) was designated the type BDBV isolate (Kuhn *et al.* 2014). To date, all published *in vitro* and *in vivo* experiments involving BDBV have been performed exclusively with this isolate (Albariño *et al.* 2013). BDBV isolates from Uganda and Democratic Republic of the Congo share ≈98.6% genome identity. Genomic analyses revealed the BDBV genome organization to be identical to that of other ebolaviruses (Fig. 2) (Towner *et al.* 2008; Albariño *et al.* 2013).

Genomic analyses also indicated that each of the two outbreaks was in all likelihood caused through singular introductions into the human populations with subsequent human-to-human transmission (Albariño *et al.* 2013). However, how these introductions occurred and why no further BDBV introductions have occurred since the end of the second outbreak in 2012 remain unclear. The Bundibugyo District borders the Democratic Republic of the Congo. A large portion of the district is part of the Rwenzori Mountains, the Semliki National Park and Game Reserve with its associated wildlife, including non-human primates, and domestic or economical activities, such as cocoa farming, hunting and fishing (Wamala *et al.* 2010). These potential contacts with wildlife suggest that the BDBV introduction into the human population was a zoonotic event. However, the precise beginning of the 2007 Ugandan EVD outbreak and the identity and history of the human index case is unclear (Towner *et al.* 2008; MacNeil *et al.* 2010; Wamala *et al.* 2010). Likewise, no data have been published on the behavior and recent history of the suspected human index case of the 2012 EVD outbreak.

MVD outbreaks due to MARV and RAVV could be directly linked to infected frugivorous bats from which replicating virus isolates could be obtained (Towner *et al.* 2009; Amman *et al.* 2012; Wahl-Jensen *et al.* 2013). EVD outbreaks due to EBOV have been loosely associated with bats via anti-EBOV antibody or EBOV genome fragment detection in bat sera in the absence of virus isolation (Leroy *et al.* 2005; Wahl-Jensen *et al.* 2013). Likewise, massive ape (central chimpanzee and western lowland gorilla) population declines have been temporary and spatially

Table 1. Official taxonomy of the family *Filoviridae* as of 2016 (Kuhn *et al.* 2010, 2011; Bukreyev *et al.* 2014).

Family	Genus	Species	Virus (abbreviation)[a]	Outdated designations and abbreviations
Filoviridae	*Cuevavirus*	*Lloviu cuevavirus*	Lloviu virus (LLOV)	None
	Marburgvirus	*Marburg marburgvirus*	Marburg virus (MARV)	Marburg virus (MBGV), Lake Victoria marburgvirus (LVMARV), Rhabdovirus simiae
			Ravn virus (RAVV)	Marburg virus (MBGV), Lake Victoria marburgvirus (LVMARV)
	Ebolavirus	*Bundibugyo ebolavirus*	Bundibugyo virus (BDBV)	Bundibugyo ebolavirus (BEBOV), Uganda ebolavirus (UEBOV)
		Reston ebolavirus	Reston virus (RESTV)	Reston ebolavirus (REBOV), Reston Ebola virus (REBOV), Ebola virus Reston (EBOV-R)
		Sudan ebolavirus	Sudan virus (SUDV)	Sudan ebolavirus (SEBOV), Sudan Ebola virus (SEBOV), Ebola virus Sudan (EBOV-S)
		Taï Forest ebolavirus	Taï Forest virus (TAFV)	Côte d'Ivoire ebolavirus (CIEBOV), Côte d'Ivoire Ebola virus (CIEBOV), Ivory Coast ebolavirus (ICEBOV)
		Zaire ebolavirus	Ebola virus (EBOV)	Zaire ebolavirus (ZEBOV), Zaire Ebola virus (ZEBOV), Ebola virus Zaire (EBOV-Z)

[a]Colors assigned to viruses in this table will be used in follow-up tables and figures as well: RAVV, purple; MARV, blue; LLOV, yellow; EBOV, red; BDBV, orange; TAFV, brown; SUDV, green; RESTV, gray.

Table 2. Official human filovirus disease classification and nomenclature as of 2016.

ICD-10 classification code: disease name (abbreviation) (World Health Organization 2015)	Outdated designations and abbreviations (Kuhn *et al.* 2011)	Etiological agents
A98.4: Ebola virus disease (EVD)	African hemorrhagic fever (AFHF), Ebola hemorrhagic fever (EHF)	BDBV, EBOV, SUDV, TAFV
A98.3: Marburg virus disease (MVD)	African green monkey disease, African hemorrhagic fever (AFHF), *Cercopithecus*-borne hemorrhagic fever (CBHF), Frankfurt-Marburg syndrome (FMS), green monkey disease, Marburg disease, Marburg fever, Marburg hemorrhagic fever (MHF), vervet monkey disease	MARV, RAVV
Not known to cause human disease		RESTV, LLOV

ICD-10, International Classification of Diseases, Tenth Revision (ICD-10, 1990–present).

associated with human EVD outbreaks, and while EBOV isolation was unsuccessful, EBOV genome fragments were detected in a low number of apes (Huijbregts *et al.* 2003; Walsh *et al.* 2003; Bermejo *et al.* 2006). However, neither bat nor ape associations could be established for BDBV thus far. Injection of BDBV into Egyptian rousettes (*Rousettus aegyptiacus*), the presumed natural bat reservoir of MARV and RAVV (Amman *et al.* 2012), did not result in replication (Jones *et al.* 2015). Therefore, the ecology of BDBV remains a mystery. Only one ecological survey for BDBV was reported. IgG antibodies against BDBV glycoprotein ($GP_{1,2}$) antigen were detected by ELISA and western blot in 9/353 (2.6%) of orangutans (*Pongo pygmaeus*) sampled on Kalimantan Island, Indonesia, in 2006. BDBV-specific IgM could not be detected (Nidom *et al.* 2012). These results may suggest that the BDBV distribution is not restricted to Eastern Africa. However, caution is advised as the inter-ebolavirus and non-filovirus $GP_{1,2}$ cross-reactivity of naturally occurring antibodies is undefined (antibodies to yet unknown filoviruses or unrelated antibodies may react with BDBV $GP_{1,2}$). For instance, IgG and IgM antibodies from survivors of the 2007 BDBV/EVD outbreak strongly and weakly cross-react with other, non-BDBV, ebolavirus antigen preparations, respectively (Macneil, Reed and Rollin 2011). In the absence of BDBV genome detection by deep sequencing or BDBV isolation in cell culture, serological results at best hint toward the whereabouts of BDBV in nature. Thus, at the moment, future EVD outbreaks due to BDBV can neither be geographically nor temporally anticipated, let alone be prevented.

The clinical presentation of EVD due to BDBV infection in Uganda in 2007 is summarized in Table 5 (MacNeil *et al.* 2010; Roddy *et al.* 2012). Based on these very limited data and additional statistics published for the 2012 outbreak (Kratz *et al.* 2015), the overall distribution of clinical signs and symptoms of BDBV infections appears highly similar to that of EBOV, SUDV and MARV infections (not reviewed here, see Siegert *et al.* 1967; Egbring, Slenczka and Baltzer 1971; Isaäcson *et al.* 1978; Piot *et al.* 1978; Smith, Francis and Simpson 1978; Bwaka *et al.* 1999; Bausch *et al.* 2006; Barry *et al.* 2014; Maganga *et al.* 2014; Schieffelin *et al.* 2014; Bah *et al.* 2015; Dallatomasina *et al.* 2015; Lado *et al.* 2015; Qin *et al.* 2015; Yan *et al.* 2015). Consequently, BDBV infection cannot be diagnosed based on clinical observation alone. During the 2007 EVD/BDBV outbreak, the mean incubation period was 6.3 days (the longest measured incubation period was

Table 3. Cumulative human filovirus disease cases (updated from Kuhn 2015, as of March 27, 2016).

Disease	Etiological agent	Location of index case	Numbers of deaths/numbers total of cases	CFR (%)	99%/95% confidence intervals
EVD	BDBV	Democratic Republic of the Congo, Uganda	71/211	33.7	±8.4/±6.4
	EBOV	Republic of the Congo, Gabon, Guinea, Zaire/Democratic Republic of the Congo	12 473/30 115	41.4	±0.7/±0.6
		[same dataset excluding the Western African/Guinea outbreak of 2013–2016]	1150/1469	78.3	±2.77/±2.11
	SUDV	Sudan/South Sudan, Uganda	412/779	52.9	±4.6/±3.5
	TAFV	Côte d'Ivoire	0/1	0	N/A
MVD	MARV	Angola, Democratic Republic of the Congo, Kenya, Uganda, Zimbabwe	383/474	80.8	±4.7/±3.6
	RAVV	Democratic Republic of the Congo, Kenya, Uganda	2/3	66.7	±70.1/±53.3

N/A, not applicable; CFR, case-fatality rate.

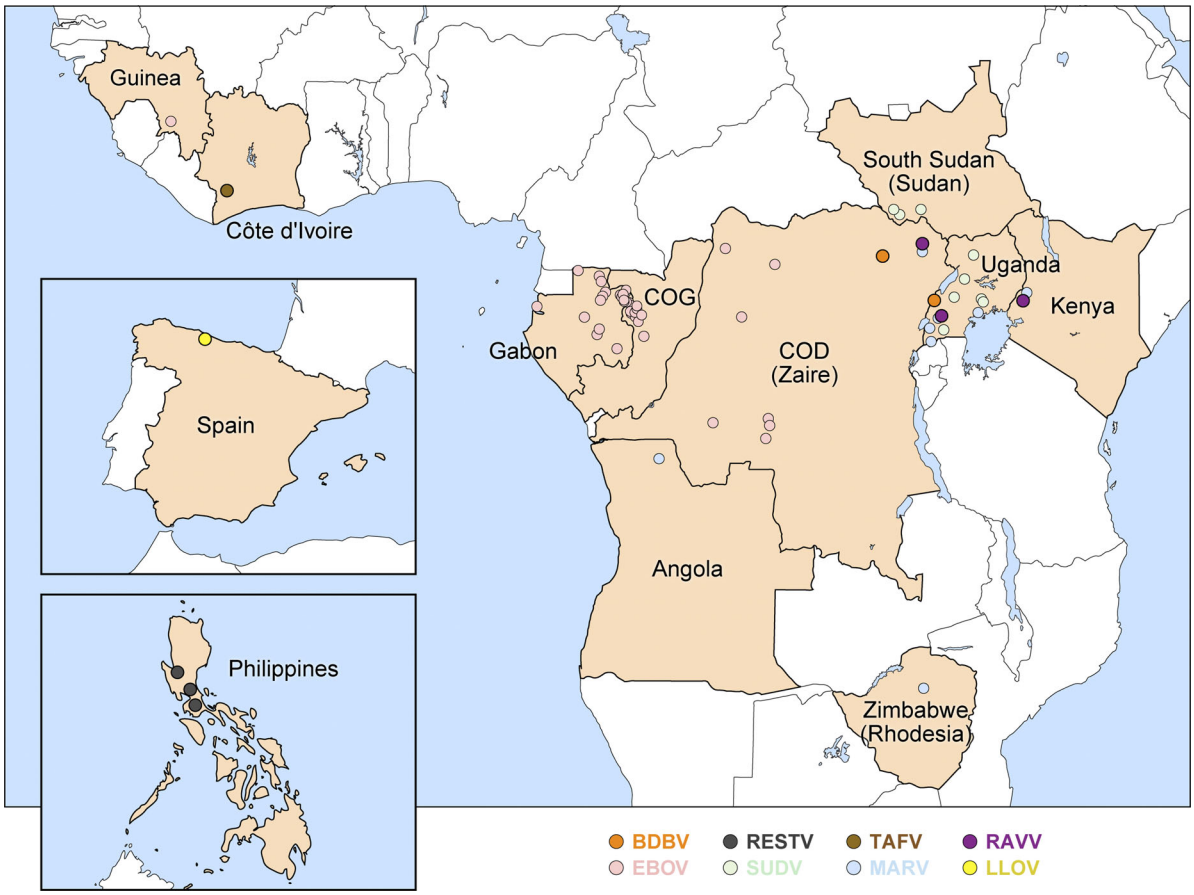

Figure 1. Geographical location of primary index cases causing disease outbreaks due to neglected filovirus infections. Countries with index cases are shown in light brown with outbreak locations marked as bold dots (disease outbreaks due to more prominent filoviruses, EBOV, SUDV or MARV, are indicated via faded dots for reference). Former country names are listed in parenthesis under present names. COD: Democratic Republic of the Congo; COG: Republic of the Congo. Adopted from (Kuhn 2015).

25 days) (MacNeil *et al.* 2010, 2011) and, therefore, maybe somewhat shorter than that of EBOV infection [12.7 ± 4.3 days in a 1995 EVD/EBOV outbreak (Eichner, Dowell and Firese 2011) and 11.4 days in the 2013–2016 EVD/EBOV outbreak in Western Africa (World Health Organization Ebola Response Team 2014)]. Asthenia, diarrhea and headache were the most frequent symptoms/signs. Hemorrhagic signs were observed in approximately half of the cohort (MacNeil *et al.* 2010; Roddy *et al.* 2012), which is typical for all ebolavirus infections. As has been reported for EBOV, SUDV and MARV infections (Baltzer *et al.* 1979; Bwaka *et al.*

Table 4. Neglected filovirus isolates and sequences.

Year of isolation (country)	Isolation host	Isolate designation (alternative designation)	Host (patient/animal) information	Complete or partial genomic sequence
		BDBV (Towner *et al.* 2008; Albariño *et al.* 2013)		
2007 (Uganda)	Human (clinical specimen #200706291)	811250 (BUN-038)[a]	52-year-old male; disease onset 3 November; hospitalized 10 November; clinical sample (blood) collected on 14 November; died 26 November	Complete sequence obtained from Vero E6 cell passage 2 material: • RefSeq #NC_014373.1 • GenBank #FJ217161.1 • GenBank #KR063673.1
	Human (clinical specimen #200706304)	Not available	Not available	Not available
	Human (clinical specimen #200706320)	Not available	Not available	Not available
	Human (clinical specimen #200706327)	Not available	Not available	Not available
2012 (COD)	Human	EboBund-14 2012 (Isiro-14)	Survivor. No other information available	Complete sequence: GenBank #KC545396.1
	Human	EboBund-112 2012 (Isiro-112)	Lethal case. No other information available	Complete sequence: GenBank #KC545393.1
	Human	EboBund-120 2012 (Isiro-120)	Lethal case. No other information available	Complete sequence: GenBank #KC545394.1
	Human	EboBund-122 2012 (Isiro-122)	Survivor. No other information available	Complete sequence: GenBank #KC545395.1
		LLOV (Negredo *et al.* 2011)		
2003 (Spain)	Schreibers's long-fingered bat	Bat86	Deceased bat found in cave	Coding-complete sequence: • RefSeq #NC_016144.1 • Genbank #JF828358.1
		RAVV (Johnson *et al.* 1996; Bausch *et al.* 2006; Lofts *et al.* 2007; Warfield *et al.* 2007; Towner *et al.* 2009; Adjemian *et al.* 2011; Amman *et al.* 2012, 2014)		
1987 (Kenya)	Human	810040 (01KEN87, M/Kenya/Kitum Cave/1987/Ravn, ravKEN87aug10)	15-year-old male; disease onset 10 August, hospitalized 13 August in Mombasa; transferred to Nairobi 18 August; died 20 August. Virus isolated from serum sample	Coding-complete sequence obtained from • SW-13 cell passage 2 + Vero E6 cell passage 5 material: RefSeq #NC_024781.1; GenBank #DQ447649.1. • Passage 1 in non-human primates: R1: GenBank #EU500827.1 • Strain adapted to guinea pigs: GenBank #EF446131.1 • Strain adapted to SCID mice: R2: GenBank #EU500828.1 • Strain adapted to BALB/c mice: R3: GenBank #EU500826.1
1999 (COD)	Human	09DRC99may26	Lethal case. Disease onset 26 May. No other information available	Coding-complete sequence: GenBank #DQ447652.1
2007–2008 (Uganda)	Human (clinical specimen #200703648)	811225 (02Uga2007)	Lethal case. 25-year-old male. Disease onset 14 September, sample collected 21 September	Coding-complete sequence: GenBank #FJ750953.1
	Egyptian rousette (sample specimen #200704525)	811274 (44Bat 2007)	Female adult	Coding-complete sequence: GenBank #FJ750954.1
	Egyptian rousette (sample specimen #200704669)	811275 (188Bat 2007)	Female adult	Coding-complete sequence: GenBank #FJ750955.1
	Egyptian rousette (sample specimen #200805444)	811391 (982Bat 2008)	Male adult	Coding-complete sequence: GenBank #FJ750956.1
	Egyptian rousette	276Bat 2007	Male adult	• Partial gene *NP*: GenBank #FJ74367.1 • Partial gene *VP35*: GenBank #FJ743678.1

Table 4. (*Continued*).

Year of isolation (country)	Isolation host	Isolate designation (alternative designation)	Host (patient/animal) information	Complete or partial genomic sequence
	Egyptian rousette	288Bat 2007	Female juvenile	• Partial gene *NP*: GenBank #FJ743672.1 • Partial gene *VP35*: GenBank #FJ743680.1
	Egyptian rousette	328Bat 2007	Male juvenile	• Partial gene *NP*: GenBank #FJ743674.1 • Partial gene *VP35*: GenBank #FJ743682.1
	Egyptian rousette	782Bat 2007	Male juvenile	Partial gene *NP*: GenBank #FJ743669.1
	Egyptian rousette	1013Bat 2008	Male adult	• Partial gene *NP*: GenBank #FJ743676.1 • Partial gene *VP35*: GenBank #FJ743684.1
2009 (Uganda)	Egyptian rousette	549 Qbat	No information available	• Partial gene *NP*: GenBank #JX462491.1 • Partial gene *VP35*: GenBank #JX462504.1
	Egyptian rousette	1304 Qbat	Female juvenile sampled on 9 November	Complete sequence: GenBank #JX458857.1
	Egyptian rousette	1431 Qbat	No information available	• Partial gene *NP*: GenBank #JX462499.1 • Partial gene *VP35*: GenBank #JX462508.1
2012 (Uganda)	Egyptian rousette	1407 Bat Uga 2012	No information available	Partial gene *VP35*: GenBank #KJ747241.1
		RESTV		
		(Sanchez *et al.* 1996; Ikegami *et al.* 2001; Groseth *et al.* 2002; Boehmann *et al.* 2005; Barrette *et al.* 2009; Carroll *et al.* 2013; Pan *et al.* 2014)		
1989–1990 (USA)	Crab-eating macaque	119810	No information available	Complete *GP* ORF: GenBank #U23152.1
	Crab-eating macaque	Pennsylvania	No information available	Complete sequence: • RefSeq #NC_004161.1 • GenBank #AF522874.1 • GenBank #AY769362.1
1992 (Italy, Philippines)	Crab-eating macaque	920084	No information available	Complete *GP* ORF: GenBank #U23416.1
	Crab-eating macaque	12552	No information available	Complete *GP* ORF: GenBank #U23417.1
1996 (USA, Philippines)	Crab-eating macaque	Calamba	No information available	Complete sequence: GenBank #AB050936.1
	Crab-eating macaque	Alice	No information available	Complete sequence: GenBank #JX477166.1
2008–2009 (Philippines)	Domestic pig	Reston08-E	Spleen sample. No other information available	Coding-complete sequence: GenBank #FJ621585.1
	Domestic pig	Reston08-C	Lymph node sample. No other information available	Coding-complete sequence: GenBank #FJ621584.1
	Domestic pig	Reston08-A	Lung sample. No other information available	Coding-complete sequence: GenBank #FJ621583.1
	Domestic pig	Reston09-A	No information available	Coding-complete sequence: GenBank #JX477165.1
2011 (China)	Domestic pig	43 L	Spleen sample from deceased animal. No other information available	Partial gene *L*: GenBank #JN872215.1
	Domestic pig	61 L	Spleen sample from deceased animal. No other information available	Partial gene *L*: GenBank #JN872216.1
	Domestic pig	79 L	Spleen sample from deceased animal. No other information available	Partial gene *L*: GenBank #JN872217.1

Table 4. (*Continued*).

Year of isolation (country)	Isolation host	Isolate designation (alternative designation)	Host (patient/animal) information	Complete or partial genomic sequence
	Domestic pig	104 L	Spleen sample from deceased animal. No other information available	Partial gene *L*: GenBank #JN872218.1
		TAFV (Towner *et al.* 2008)		
1994 (Côte d'Ivoire)	Human	807212	34-year-old female; disease onset 24 November; hospitalized 26 November; blood collected 27 November; evacuated to Switzerland 1 December; discharged 8 December	Complete sequence obtained from Vero E6 cell passage 7 material: • RefSeq #NC_014372.1 • GenBank #FJ217162.1 • GenBank #KU182910.1

[a]Note that clinical specimen # and isolate designations are often confused in the literature. COD, Democratic Republic of the Congo.

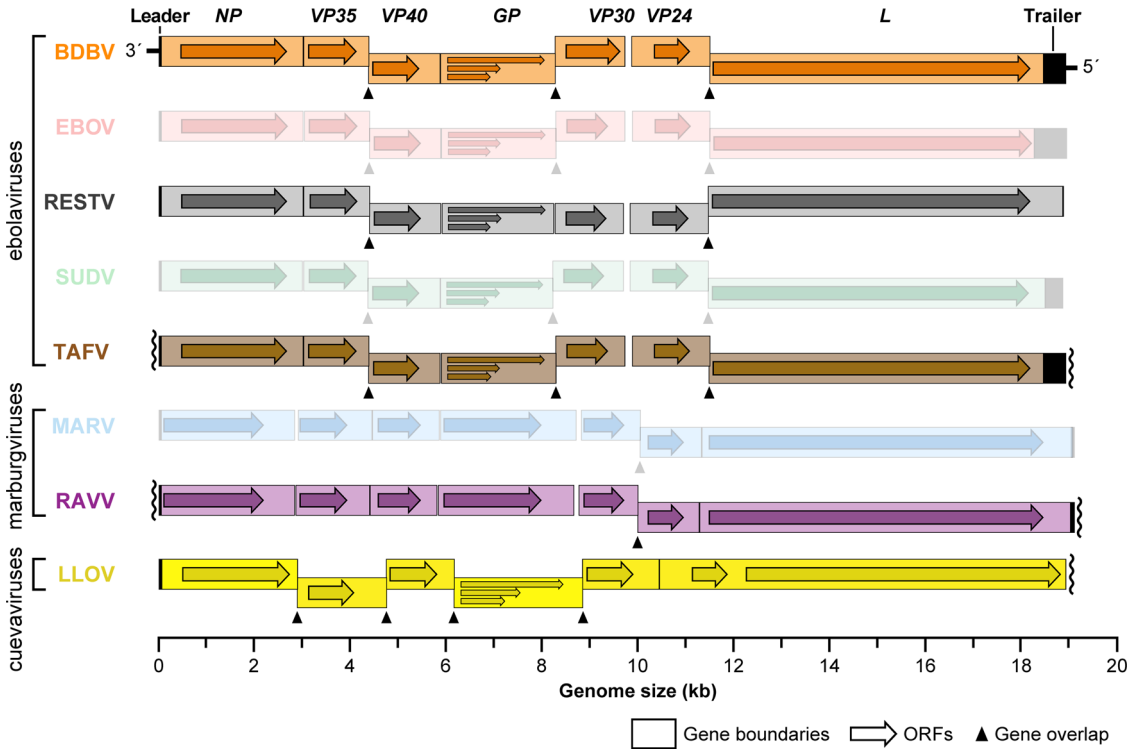

Figure 2. Organization of neglected filovirus genomes. Genes and open reading frames (ORFs) are shown as rectangles and horizontal arrows, respectively. Wavy lines indicate incomplete sequencing of 3′ leader or 5′ trailer sequences. Entries for prominent filoviruses, EBOV, SUDV or MARV, are muted.

1999; Kibadi *et al.* 1999; Rowe *et al.* 1999; Qureshi *et al.* 2015), survivors of disease caused by BDBV may suffer of long-term sequelae years after convalescence. These sequelae include arthralgia, difficulty swallowing, hearing loss, ocular deficits (blurred vision and retro-orbital pain) and sleeplessness (Clark *et al.* 2015). Whether BDBV is able to persist in convalescent patients, as has been reported for EBOV and MARV (Martini and Schmidt 1968; Nikiforov *et al.* 1994; Rodriguez *et al.* 1999; Mate *et al.* 2015; Varkey *et al.* 2015), is unclear.

On the molecular level, EVD due to BDBV appears to differ from other ebolavirus infections. Whereas strong inflammatory responses ('cytokine storms') have been reported during EBOV infections and were associated with concomitant multiorgan dysfunction syndrome (Baize *et al.* 1999, 2002; Villinger *et al.* 1999), lethal human BDBV infections were associated with low concentrations of proinflammatory cytokines (interleukin [IL]-1α, IL-1β, IL-6, tumor necrosis factor-α) and high concentrations of antiinflammatory IL-10 (Gupta *et al.* 2012). These results indirectly confirm *in vitro* experiments with BDBV-infected peripheral blood mononuclear cells (PBMCs). BDBV-infected cells produced 10–100-fold less progeny virions and reacted with 2–10-fold lower expression of tumor necrosis factor-α, monocyte chemoattractant protein-1, IL-1β and macrophage inflammatory protein 1-α compared to EBOV-infected control cells. Interestingly, in contrast to the *in vivo* data, IL-10 expression was also low (Gupta *et al.* 2010).

Thus, pathogenetic mechanisms or properties from relatively well-characterized filoviruses (EBOV, SUDV and MARV) cannot be generalized to other, less characterized filoviruses. Consequently, promising medical countermeasures against one filovirus should not be assumed automatically to be promising avenues for related filoviruses. This lack of translatability is all

Table 5. Comparison of clinical signs or symptoms of human BDBV infection during the 2007 Uganda EVD outbreak.

Signs or symptoms of BDBV infection	Incidence of signs or symptoms (%) in survivors (MacNeil *et al.* 2010) versus (Roddy *et al.* 2012)	Incidence of signs or symptoms (%) in fatalities (MacNeil *et al.* 2010) versus (Roddy *et al.* 2012)
Abdominal pain	88 versus 42	93 versus 78
Anorexia/appetite loss	83 versus 58	80 versus 89
Anuria	NR versus 0	NR versus 22
Arthralgia or myalgia	83 versus 67	86 versus 89
Asthenia	NR versus 83	NR versus 89
Bleeding from any site	42 versus 8	53 versus 44
Chest pain	NR versus 17	NR versus 56
Conjunctival injection/conjunctivitis	NR versus 42	NR versus 44
Cough	NR versus 25	NR versus 11
Diarrhea	92 versus 58	87 versus 78
Difficulty breathing/distress	26 versus 8	57 versus 44
Fever	100 versus 0	100 versus 11
Headaches	84 versus 100	93 versus 89
Hiccups	17 versus 0	40 versus 11
Lumbar pain	NR versus 8	NR versus 33
Rash	35 versus 0 (non-bloody)	33 versus 0 (non-bloody)
Malaise or fatigue	96 versus NR	100 versus NR
Nausea and vomiting	92 versus 42	87 versus 89
Sore throat, odynophagia or dysphagia	43 versus 58	60 versus 89

NR, not reported.

the more concerning in regard to BDBV, as the virus itself is completely uncharacterized. With the exception of genomic sequence determination and functional deductions one can possibly make from alignment with other ebolavirus sequences, only three reports have been published addressing BDBV molecular biology directly. BDBV uses Niemann-Pick C1 (NPC1) as a cell-entry receptor (Ng *et al.* 2015); cell entry is dependent on cathepsin B *in vitro* (Misasi *et al.* 2012); and BDBV Δ-peptide has no effect on MARV replication (Radoshitzky *et al.* 2011).

The pathology of human BDBV infection remains unknown as neither autopsies nor biopsies were performed during the two disease outbreaks. Until future outbreaks might occur, the delineation of BDBV pathogenesis is solely dependent on animal models. Whereas numerous animal models are available for EBOV, SUDV and MARV infection (Table 6), the only established animal model for BDBV infection is based on intramuscular inoculation of crab-eating macaques (*Macaca fascicularis*). The conditions to achieve 100% fatality in this model have yet to be determined (typically, 25%–34% of infected animals survive) (Hensley *et al.* 2010; Falzarano *et al.* 2011; Mire *et al.* 2013). No detailed clinical or pathological descriptions of BDBV infection in crab-eating macaques are available. The establishment of a mouse model of BDBV infection was attempted using type 1 interferon α/β receptor knockout (IFN-$\alpha/\beta R^{-/-}$) mice (Table 6). When exposed to BDBV, these mice did not develop signs of clinical disease, weight loss or lethality (Brannan *et al.* 2015). However, in some of BDBV-infected mice, transient viremia and increased tissue titers (spleen and liver) were noted. Results from serial sampling experiments indicated hepatic disseminated intravascular coagulation (DIC). Splenic lymphoid hyperplasia occurred concurrently with mild lymphoid depletion, and immunohistochemical analysis detected viral antigen in red pulp macrophage-like cells. Platelets were transiently decreased, and white blood cells and absolute lymphocytes were transiently increased.

The absence of true rodent models for BDBV infection excludes the possibility of high-throughput *in vivo* drug screening and initial candidate countermeasure evaluation. The only partially lethal non-human primate model requires countermeasure evaluation to be performed with large animal numbers to achieve statistical significance of outcomes, which is both ethically problematic and in most cases prohibited by cost and logistics in limited biosafety level-4 space. Despite these obstacles, the BDBV crab-eating macaque model has been used for limited evaluation of candidate medical countermeasure platforms that are under advanced development for the prevention or treatment of EVD due to EBOV infection. At least two candidate vaccine platforms (i.e. DNA prime/recombinant adenovirus boost, recombinant vesicular stomatitis Indiana virus) appear promising against BDBV (Table 7) (Hensley *et al.* 2010; Falzarano *et al.* 2011; Mire *et al.* 2013). Theoretically, administration of a pan-filovirus vaccine comprised of mosaic ebolavirus proteins could elicit antibody responses to a number of ebolaviruses, including BDBV (Fenimore *et al.* 2012), but such a vaccine has not been tested in animals with BDBV infection.

Neither post-exposure prophylactics nor antivirals have yet been identified, let alone been tested, against BDBV infections *in vivo*. Consequently, treatment of human BDBV infections is limited to general supportive measures applied to other ebolavirus infections, such as nutritional supplementation, oral or intravenous fluid rehydration and medication against anxiety (e.g. diazepam), secondary infections (antibiotics, antimalarials, antimycotics), dyspepsia (e.g. cimetidine, ranitidine, omeprazole), nausea/vomiting (e.g., metaclopramide) and pain (e.g. acetominophen, morphine) (Roddy *et al.* 2012).

Diagnostic tests for BDBV infection are currently limited to various pan-filovirus reverse transcriptase-polymerase chain reaction (RT-PCR) or quantitative reverse transcriptase-PCR (qRT-PCR) protocols (Lu *et al.* 2015) and BDBV-specific sequence capture probes for next-generation sequencing (Koehler *et al.* 2014). A handful of BDBV-specific monoclonal or polyclonal murine, rabbit or crab-eating macaque antibodies (Ou *et al.* 2011; Holtsberg *et al.* 2015; Keck *et al.* 2015; Wang *et al.* 2015), BDBV

Table 6. Overview of animal models for filovirus infections.

Experimental animal (species)	Virus/variant isolate (route of administration)	Outcome	Selected references
		Bats	
Egyptian rousette (*Rousettus aegyptiacus*)	MARV/371bat-811277 (SC)	Replication in absence of disease	Amman *et al.* (2015); Jones *et al.* (2015)
		Carnivores	
Domestic ferret (*Mustela putorius furo*)	EBOV, RESTV, SUDV, MARV (isolate and route unknown)	Lethal disease	Yet to be published
		Leporids	
Chinchilla rabbits	Guinea pig-adapted EBOV/Yam-Ecr (IM)	Partially lethal disease	Ryabchikova *et al.* (1996a)
		Non-human primates	
Common marmoset (*Callithrix jacchus*)	EBOV/Kik-13625 (aerosol), EBOV/Kik-9510621 (IM)	Lethal disease	Carrion *et al.* (2011); Smither *et al.* (2015)
	MARV/Hes-Pop (aerosol), MARV/MtE-Mus (IM)	Lethal disease	Carrion *et al.* (2011); Smither *et al.* (2013)
Common squirrel monkey (*Saimiri sciureus*)	MARV (isolate unspecified; IP)	Lethal disease	Simpson (1969a,b)
Crab-eating macaque (*Macaca fascicularis*)	BDBV/But-811250 (IM)	Partially lethal disease	Hensley *et al.* (2010); Falzarano *et al.* (2011); Mire *et al.* (2013)
	EBOV/Kik-9510621 (aerosol, IM)	Lethal disease	Jahrling *et al.* (1996a); Geisbert *et al.* (2003a,b); Reed *et al.* (2011)
	MARV/Ang (isolate unspecified; IM or aerosol), MARV/MtE-Mus (IM)	Lethal disease	Daddario-DiCaprio *et al.* (2006a); Fritz *et al.* (2008); Alves *et al.* (2010); Hensley *et al.* (2011)
	RAVV/KiC-810040 (IM)	Lethal disease	Daddario-DiCaprio *et al.* (2006a)
	SUDV/Nza-Bon (aerosol)	Lethal disease	Fisher-Hoch *et al.* (1992); Zumbrun *et al.* (2012b)
	SUDV/Nza-Bon (IP)	Partially lethal disease	
	RESTV/Phi-H28 (SC)	Partially lethal disease	Jahrling *et al.* (1996b)
	TAFV/Pau-CI (IM)	Partially lethal disease	Geisbert *et al.* (2009)
Grivet/African green monkey (*Chlorocebus aethiops*)	EBOV/Kik-9510621 (aerosol), EBOV/Yam (isolate unspecified; IP), EBOV/Yam-Ecr (SC)	Lethal disease	Baskerville *et al.* (1978); Ryabchikova, Kolesnikova and Luchko (1999); Reed *et al.* (2011)
	MARV/Hes (isolate unspecified; IC, IN, IP, or SC), MARV/Hes-Pop (IM)	Lethal disease	Simpson (1969a); Bazhutin *et al.* (1992)
	SUDV/Nza-Bon (aerosol)	Lethal disease	Fisher-Hoch *et al.* (1992); Zumbrun *et al.* (2012b)
	SUDV/Nza-Bon (IP)	Replication in absence of disease	
Hamadryas baboon (*Papio hamadryas*)	EBOV/Yam-Ecr (SC)	Lethal disease	Mikhailov *et al.* (1994); Ryabchikova, Kolesnikova and Luchko (1999); Ryabchikova and Price (2004)
	MARV/Hes-Pop (SC)	Lethal disease	Ryabchikova and Price (2004)
Rhesus monkey (*Macaca mulatta*)	EBOV/Kik-9510621 (aerosol, IM)	Lethal disease	Baskerville *et al.* (1978); Ellis *et al.* (1978); Fisher-Hoch *et al.* (1985); Jaax *et al.* (1995); Johnson *et al.* (1995); Reed *et al.* (2011)
	EBOV/Yam-Ecr (IP)		
	EBOV/Yam-May (aerosol, IM) MARV/Ang (isolate unspecified; IM), MARV/Hes (isolate unspecified; IC, IM, IN, IP, or SC), MARV/MtE-Mus (IM)	Lethal disease	Simpson (1969a); Daddario-DiCaprio *et al.* (2006b); Geisbert *et al.* (2007)

Table 6. (*Continued*).

Experimental animal (species)	Virus/variant isolate (route of administration)	Outcome	Selected references
	RAVV/KiC-810040 (IM)	Lethal disease	Johnson *et al.* (1996)
	SUDV/Nza-Bon (aerosol)	Lethal disease	Ellis *et al.* (1978); Zumbrun *et al.* (2012b)
	SUDV/Nza-Bon (IP)	Significant disease with partial lethality	
		Rodents	
Golden hamster (*Mesocricetus auratus*)	Mouse-adapted EBOV/Yam-May (IP)	Lethal disease	Ebihara *et al.* (2013)
	Guinea pig- and hamster-adapted MARV/Hes (isolate unspecified; IC or IP)	Lethal disease	Simpson (1969c); Zlotnik and Simpson (1969)
	RESTV/Phi89-Pen (IP) or RESTV/Phi08-08-A (IP)	Replication in absence of disease	de Wit *et al.* (2011)
Guinea pig (*Cavia porcellus*)	Guinea pig-adapted EBOV/Yam-Ecr (IP), guinea pig-adapted EBOV/Yam-May (aerosol, SC)	Lethal disease	Ryabchikova *et al.* (1996a); Connolly *et al.* (1999); Geisbert *et al.* (2006); Subbotina *et al.* (2010); Twenhafel *et al.* (2015)
	Guinea pig-adapted MARV/Ang (isolate unspecified; IP), MARV/Hes (isolate unspecified; IC, IP, IV), MARV/Hes-Ci67 (IP), MARV/Hes-Pop (aerosol), MARV/MtE-Mus (SC)	Lethal disease	Bechtelsheimer, Korb and Gedigk (1970); Korb *et al.* (1971); Ryabchikova *et al.* (1996b); Hevey *et al.* (1997); Lofts *et al.* (2007); Cross *et al.* (2015)
	Guinea pig-adapted RAVV/KiC-810040 (SC, IP)	Lethal disease	Hevey *et al.* (1997); Wang *et al.* (2006); Lofts *et al.* (2007); Swenson *et al.* (2008b); Ursic-Bedoya *et al.* (2014); Cross *et al.* (2015)
	RESTV/Phi08-08-A (IP), RESTV/Phi89-Pen (IP)	Replication in absence of disease	de Wit *et al.* (2011)
Laboratory mouse (immunocompetent)	Mouse-adapted EBOV/Yam-May (IP)	Lethal disease in BALB/c, C57BL/6 mice and collaborative-cross mice	Bray *et al.* (1998, 2001); Gupta *et al.* (2001); Zumbrun *et al.* (2012a); Rasmussen *et al.* (2014)
	Mouse-adapted MARV/Ang (isolate unspecified; IP)	Lethal disease in BALB/c and C57BL/6 mice	Qiu *et al.* (2014)
	Mouse-adapted RAVV/KiC-810040 (IP)	Lethal disease in BALB/c mice	Warfield *et al.* (2009)
	RESTV/Phi08-08-A (IP), RESTV/Phi89-Pen (IP)	Replication in absence of disease	de Wit *et al.* (2011)
Laboratory mouse (immunodeficient)	BDBV/But-811250 (IP) in IFN-$\alpha/\beta R^{-/-}$ mice	Non-lethal disease with evidence of DIC, hepatocellular necrosis, splenic lymphoid hyperplasia	Brannan *et al.* (2015)
	BDBV/But-811250 (IP) re-exposure (≥29 days after initial exposure) in IFN-$\alpha/\beta R^{-/-}$ mice	No clinical signs	Brannan *et al.* (2015)
	EBOV/Yam-May or EBOV/Kik-9510621 (SC or IP) in IFN $\alpha/\beta R^{-/-}$ or STAT1$^{-/-}$ mice, EBOV/Yam-May (aerosol) in perforin KO, IFN$\gamma^{-/-}$, or SCID mice	Lethal disease	Bray (2001); Raymond, Bradfute and Bray (2011); Lever *et al.* (2012); Zumbrun *et al.* (2012a); Brannan *et al.* (2015)

Table 6. (*Continued*).

Experimental animal (species)	Virus/variant isolate (route of administration)	Outcome	Selected references
	EBOV/Yam-May (IP) re-exposure 49 days after initial exposure with SUDV/Nza-Bon in IFN-$\alpha/\beta R^{-/-}$ mice	No clinical signs	Brannan *et al.* (2015)
	EBOV/Yam-May (IP) re-exposure 49 days after initial exposure with BDBV/But-811250 in IFN-$\alpha/\beta R^{-/-}$ mice	Complete protection	Brannan *et al.* (2015)
	EBOV/Yam-May (IP) re-exposure 49 days after initial exposure with TAFV/Pau-CI in IFN-$\alpha/\beta R^{-/-}$ mice	Partially lethal disease	Brannan *et al.* (2015)
	EBOV/Yam-May (IP) re-exposure 49 days after initial exposure with RESTV/Phi89 (isolate unspecified) in IFN-$\alpha/\beta R^{-/-}$ mice	Partially lethal disease	Brannan *et al.* (2015)
	EBOV/Yam-May (aerosol)	Partially lethal disease in DBA/2 mice	Zumbrun *et al.* (2012a)
	EBOV/Yam-May (aerosol or IP) in IFN-$\alpha/\beta R^{-/-}$ mice; SC or IP in STAT1$^{-/-}$ mice	Lethal/partially lethal disease in IFN-$\alpha/\beta R^{-/-}$ and STAT1$^{-/-}$ mice	Bray (2001); Lever *et al.* (2012); Brannan *et al.* (2015)
	EBOV/Yam-May (IP) re-exposure (≥29 days after initial exposure in IFN-$\alpha/\beta R^{-/-}$ mice	No clinical signs	Brannan *et al.* (2015)
	EBOV/Yam-May (aerosol) in SCID and STAT1$^{-/-}$ mice	Partial lethality	Zumbrun *et al.* (2012a)
	EBOV/Yam-May (aerosol)	Varying lethality using different BXD mouse strains	Zumbrun *et al.* (2012a)
	MARV/Hes-Pop (aerosol) or MARV/MtE-Mus (IP) in IFN-$\alpha/\beta R^{-/-}$ mice; MARV/Hes-Ci67 or MARV/MtE-Mus (IP) in SCID mice	Lethal disease	Bray (2001); Warfield *et al.* (2007); Lever *et al.* (2012)
	RAVV/KiC-810040 (IP or SC) in IFN-$\alpha/\beta R^{-/-}$ mice; IP in SCID mice	Lethal disease	Bray (2001); Warfield *et al.* (2007); Raymond, Bradfute and Bray (2011)
	Guinea pig-adapted RAVV/KiC-810040 (IP) in STAT-1 mice	Hepatocellular degeneration and necrosis, weight loss	Raymond, Bradfute and Bray (2011)
	RESTV/Phi08-08-A (IP) or RESTV/Phi89-Pen (IP)	Lethal/non-lethal disease, or no significant disease (contradictory reports) in IFN-$\alpha/\beta R^{-/-}$ and STAT1$^{-/-}$ mice	Bray (2001); de Wit *et al.* (2011); Raymond, Bradfute and Bray (2011); Lever *et al.* (2012); Brannan *et al.* (2015)
	RESTV/Phi89 (isolate unspecified; IP) re-exposure (≥29 days from initial exposure)	No clinical signs in IFN-$\alpha/\beta R^{-/-}$ mice	Brannan *et al.* (2015)

Table 6. (*Continued*).

Experimental animal (species)	Virus/variant isolate (route of administration)	Outcome	Selected references
	SUDV/Nza-Bon (IP)	Lethal/non-lethal disease (contradictory reports) in IFN-$\alpha/\beta R^{-/-}$, SCID and STAT1$^{-/-}$ mice	Bray (2001); Raymond, Bradfute and Bray (2011); Lever *et al.* (2012); Brannan *et al.* (2015)
	SUDV/Nza-Bon (IP) re-exposure (≥29 days from initial exposure in IFN-$\alpha/\beta R^{-/-}$ mice	No clinical signs	Brannan *et al.* (2015)
	TAFV/Pau-CI (IP)	No clinical signs or significant disease in IFN-$\alpha/\beta R^{-/-}$ mice	Raymond, Bradfute and Bray (2011); Brannan *et al.* (2015)
	TAFV/Pau-CI (IP) re-exposure (≥29 days from initial exposure)	No clinical signs	Brannan *et al.* (2015)
		Suids	
Domestic pig (*Sus scrofa*)	EBOV/Kik-9510621 (oro-nasal or IN, IO, and PO combination)	Non-lethal disease	Kobinger *et al.* (2011); Weingartl *et al.* (2012)
	RESTV/Phi08 (isolate unspecified; oro-nasal combination)	Replication in absence of disease	Marsh *et al.* (2011)

IC, intracranial(ly); IM, intramuscular(ly); IN; intranasal(ly); IO, intraocular(ly); IP, intraperitoneal(ly); IV, intravenous(ly); PO, oral(ly); SC, subcutaneous(ly).

cross-reactive murine antibodies (Fusco *et al.* 2015; Wang *et al.* 2015; Furuyama *et al.* 2016), BDBV cross-reactive murine single-chain variable domain fragments (scFv) and thermostable single-domain nurse shark antibodies (IgNAR V) raised against inactivated EBOV particles (Goodchild *et al.* 2011) are available, but have not yet been tested in diagnostic serological assays.

Lloviu virus

Lloviu virus (LLOV; pronounced j'ɔːvju vaɪrəs) was discovered in 2002 in Cueva del Lloviu in Spain. Next-generation sequencing of tissues collected from several of hundreds of deceased (insectivorous) Schreibers's long-fingered bats (*Miniopterus schreibersii*) within the cave revealed infection with this novel filovirus (Fig. 1) (Negredo *et al.* 2011). Therefore, LLOV is only the third (LLOV, MARV and RAVV) of the eight known filoviruses to unambiguously infect bats. However, analysis of available data does not permit drawing conclusions on whether LLOV caused the bats' deaths. Similar to MARV- and RAVV-infected bats, LLOV-infected bats could have been subclinically and persistently infected with LLOV and died of other causes. As LLOV has not yet been rediscovered, Schreiber's long-fingered bats may not be the natural host reservoir of LLOV.

A single coding-complete genomic sequence of LLOV, Lloviu virus/M.schreibersii-wt/ESP/2003/Asturias-Bat86 (LLOV/Ast-Bat86; Table 4) was assembled from one of the Spanish samples (Negredo *et al.* 2011) and was designated the type LLOV 'isolate' (Kuhn *et al.* 2014). The LLOV genome is highly reminiscent in organization of other filovirus genomes containing the same overall linear ORF arrangement (Fig. 2). However, LLOV appears to express seven structural proteins (nucleoprotein [NP], VP35, VP40, GP, VP30, VP24, L) from six genes rather than the filovirus-typical seven genes (Negredo *et al.* 2011). All attempts failed to isolate this virus in culture (Negredo *et al.* 2011), and all sample material was depleted. Because the 5′ genomic terminus of the virus could not be sequenced, it is unclear whether a functional LLOV genome could be synthesized for *in vitro* rescue. Consequently, only four studies are published targeting LLOV specifically. These studies relied on recombinantly expressed LLOV proteins (Maruyama *et al.* 2014; Feagins and Basler 2015) or on virus surrogate systems such as vesiculoviral pseudotypes (Maruyama *et al.* 2014), retroviral pseudotypes (Ng *et al.* 2014) or recombinant vesiculoviruses (Ng *et al.* 2014, 2015) to study parts of the presumed LLOV replication cycle. Results indicate that the LLOV surface $GP_{1,2}$ mediates LLOV cell entry by binding to the universal endosomal filovirus receptor NPC1 (Ng *et al.* 2014, 2015) in a pH-, cathepsin L-dependent (not cathepsin B-dependent) manner reminiscent of EBOV (Maruyama *et al.* 2014; Ng *et al.* 2014). As shown for other filoviruses, co-expression of LLOV $GP_{1,2}$ and matrix protein VP40 results in the formation of filamentous filovirion-like particles (Maruyama *et al.* 2014). In terms of interferon response inhibitory functions, LLOV proteins VP35, VP40 and VP24 are functional analogs of EBOV rather than MARV (Feagins and Basler 2015). Finally, LLOV Δ-peptide inhibits cell transduction of retroviral particles pseudotyped with MARV $GP_{1,2}$ just like EBOV and MARV Δ-peptides (Ng *et al.* 2014).

The potential of LLOV to infect humans is unknown. Because of the absence of a replicating LLOV isolate, no animal model of LLOV infection is available. Consequently, possible persistence of LLOV in animals, pathogenicity/virulence, pathogenesis or potential countermeasures against infection are not known. Specific diagnostic tests for LLOV infection have not yet been reported. However, a recently established, novel system using recombinant EBOV expressing LLOV $GP_{1,2}$ instead of EBOV $GP_{1,2}$ aided in the identification of LLOV $GP_{1,2}$-specific antibodies that could possibly be used in diagnostic assays such as immunofluorescent assay (IFA), enzyme-linked immunosorbent

Table 7. Overview of candidate vaccine development against neglected filoviruses.

Candidate vaccine (Reference(s))	Antigen: vaccination regimen	Efficacy in experimental animals	Efficacy in control animals
	DNA vaccines		
Naked DNA encoding codon-optimized gene (Grant-Klein *et al.* 2012)	MARV (unspecified isolate) $GP_{1,2}$: IM electroporation twice at 3-week intervals (5 μg) in BALB/c mice	10/10 (100%) survival of mice exposed IP to 1000 PFU of mouse-adapted RAVV/KiC-810040 4 weeks after the 2nd immunization	5/10 (50%) survived infection
Naked DNA encoding codon-optimized gene (Grant-Klein *et al.* 2012)	RAVV/KiC-810040 $GP_{1,2}$: IM electroporation twice at 3-week intervals (5 μg) in BALB/c mice	10/10 (100%) survival of mice exposed IP to 1000 PFU of mouse-adapted RAVV/KiC-810040 4 weeks after final immunization	5/10 (50%) survived infection
Naked DNA encoding codon-optimized genes (Grant-Klein *et al.* 2012)	MARV (unspecified isolate) $GP_{1,2}$ + RAVV/KiC-810040 $GP_{1,2}$ + empty vector: IM electroporation twice at 3-week intervals (20 μg)	10/10 (100%) survival of BALB/c laboratory mice exposed IP to 1000 PFU mouse-adapted RAVV/KiC-810040 4 weeks after final immunization	5/10 (50%) survived infection
Naked DNA (Riemenschneider *et al.* 2003)	RAVV/KiC-810040 $GP_{1,2}$: ID gene-gunning 4 times at 4-week intervals (10 μg)	6/6 (100%) survival of Hartley guinea pigs exposed SC to 1000 PFU of guinea pig-adapted RAVV/KiC-810040 at week 16	0/6 (0%) survived infection
	Replication-incompetent recombinant adenovirus vector vaccines		
Recombinant human adenovirus 5 vectors (CAdVax panfilo vaccine) (Swenson *et al.* 2008a)	Individual vectors each containing 1), EBOV/Kik-9510621 NP; 2), EBOV/Kik-9510621 $GP_{1,2}$ + SUDV/Nza-Bon $GP_{1,2}$; 3), MARV/Hes-Ci67 $GP_{1,2}$ + RAVV/KiC-810040 $GP_{1,2}$; or 4), MARV/MtE-Mus $GP_{1,2}$ + NP: IM twice at 63-day intervals (4×10^{10} PFU total)	• 5/5 (100%) survival of crab-eating macaques exposed to 1000 PFU IM EBOV/Kik-9510621 at day 106 • Possible background immunity to vector; high dose necessary	0/1 (0%) survived infection
Recombinant human adenovirus 5 vectors (CAdVax) (Swenson *et al.* 2008a)	Four vectors each containing (1), EBOV/Kik-9510621 NP; (2), EBOV/Kik-9510621 $GP_{1,2}$ + SUDV/Nza-Bon $GP_{1,2}$; (3), MARV/Hes-Ci67 $GP_{1,2}$ + RAVV/KiC-810040 $GP_{1,2}$; or (4), MARV/MtE-Mus $GP_{1,2}$ + NP: IM twice at 63-day intervals (4×10^{10} PFU total)	• 5/5 (100%) survival of crab-eating macaques exposed SC to 1000 PFU of MARV/MtE-Mus at day 105 • 1/5 (20%) of vaccinated animals had increased liver enzyme concentrations • Possible background immunity to vector; high dose necessary	0/1 (0%) survived infection
Recombinant human single adenovirus 5 vector (CAdVaxM(fus)) (Wang *et al.* 2006)	MARV/Hes-Ci67-MARV/MtE-Mus $GP_{1,2}$ fusion protein: SC twice at 4-week intervals (5×10^6 PFU)	5/6 (83%), 4/6 (67%), and 6/6 (100%) survival of guinea pigs exposed SC to 2000 LD_{50} of guinea pig-adapted RAVV/KiC-810040, MARV/MtE-Mus, or MARV/Hes-Ci67 at week 8, respectively	0/6 (0%) in each group survived
Recombinant human adenovirus 5 vector (CAdVaxM(fus)) (Wang *et al.* 2006)	MARV/Hes-Ci67-MARV/MtE-Mus $GP_{1,2}$ fusion protein: SC twice at 4-week intervals (5×10^7 or 5×10^8 PFU)	6/6 (100%) of each group of guinea pigs survived after exposure SC to 2000 LD_{50} of guinea pig-adapted RAVV/KiC-810040, MARV/MtE-Mus, or MARV/Hes-Ci67 at week 8	0/6 (0%) in each group survived
	Prime/boost vaccines		
Naked DNA/recombinant adenovirus 5 (rAD5) vector (Hensley *et al.* 2010)	Prime: EBOV/Yam-May $GP_{1,2}$ + SUDV/Nza-Bon $GP_{1,2}$ (in individual vectors): IM Mixture of 2 mg of each plasmid at 0, 4, 8, and 14 weeks Boost: IM 10^{11} particle units of EBOV/Yam-May $GP_{1,2}$ at 12 months	4/4 (100%) survival of crab-eating macaques exposed to 1000 $TCID_{50}$ of BDBV/But-811250 given IM at 7 weeks after boost	1/4 (25%) survived infection, but developed fulminant disease

Table 7. (*Continued*).

Candidate vaccine (Reference(s))	Antigen: vaccination regimen	Efficacy in experimental animals	Efficacy in control animals
Naked DNA/recombinant adenovirus 5 (rAD5) vector (Sullivan *et al.* 2000)	Prime: EBOV/Yam-May NP + EBOV/Yam-May $GP_{1,2}$ + TAFV/Pau-CI $GP_{1,2}$ + SUDV/Nza-Bon $GP_{1,2}$ (in individual vectors): IM or ID mixture of 1 mg of each plasmid at 4-week intervals Boost: IM 10^{10} PFU of EBOV/Yam-May $GP_{1,2}$ at 20 weeks	• 4/4 (100%) survival of crab-eating macaques exposed to 6 PFU of EBOV/Yam-Ma given IP at 32 weeks • Small, transient rise in viremia in 1 animal without symptoms	0/4 (0%) survived
	Replication-competent vesicular stomatitis Indiana virus vector vaccines		
Vesicular stomatitis Indiana virus (VSIVΔG) vector (Mire *et al.* 2013)	Prime: IM 1×10^7 PFU of SUDV/Nza-Bon $GP_{1,2}$ Boost: IM 1×10^7 PFU of EBOV/Yam-May $GP_{1,2}$ 14 days later	3/3 (100%) survival of crab-eating macaques exposed IM to 1000 PFU of BDBV/But-811250 22 days after booster vaccination	1/3 (33%) survived
VSIVΔG vector (Mire *et al.* 2013)	Blend of EBOV/Yam-May $GP_{1,2}$ + SUDV/Nza-Bon $GP_{1,2}$: IM 1×10^7 PFU of each vector	• 1/3 (33%) survival of crab-eating macaques exposed IM to 1000 PFU BDBV/But-811250 28 days post-vaccination • Symptoms and viremia lower in survivor than non-survivors	• 1/3 (33%) survived • Symptoms lower in survivor than non-survivors
VSIVΔG vector (Mire *et al.* 2013)	BDBV/But-811250 $GP_{1,2}$: IM 2×10^7 PFU given IM	3/3 (100%) survival of crab-eating macaques exposed IM to 1000 PFU of BDBV/But-811250 28 days post-vaccination	• 1/3 (33%) controls survived • Symptoms lower in survivor than non-survivors
VSIVΔG vector (Marzi *et al.* 2011)	BDBV/But-811250 $GP_{1,2}$: IP 2×10^5 PFU in Hartley guinea pigs	1/6 (17%) survival of guinea pigs exposed IP to 1000 LD_{50} guinea pig-adapted EBOV/Yam-May at day 21	0/6 (0%) survived
VSIVΔG vector (Marzi *et al.* 2011)	TAFV/Pau-CI $GP_{1,2}$: IP 10^4 PFU in BALB/c mice	22/22 (100%) survival of mice exposed IP to 1000 LD_{50} of mouse-adapted EBOV/Yam-May on day 21 post-vaccination	4/21 (19%) survived
VSIVΔG vector (Marzi *et al.* 2011)	TAFV/Pau-CI $GP_{1,2}$: IP 2×10^5 PFU in Hartley guinea pigs	1/6 (17%) survival of guinea pigs exposed IP to 1000 LD_{50} guinea pig-adapted EBOV/Yam-May on day 21 post-vaccination	0/6 (0%) survived
VSIVΔG vector (Marzi *et al.* 2011)	RESTV/Phi89-Pen $GP_{1,2}$: IP 10^4 PFU in BALB/c mice	20/20 (100%) survival of mice exposed IP to 1000 LD_{50} mouse-adapted EBOV/Yam-May on day 21 post-vaccination	4/21 (19%) survived
VSIVΔG vector (Marzi *et al.* 2011)	RESTV/Phi89-Pen $GP_{1,2}$: IP 2×10^5 PFU in Hartley guinea pigs	1/6 (17%) survival of guinea pigs exposed IP to 1000 LD_{50} guinea pig-adapted EBOV/Yam-May on day 21 post-vaccination	0/6 (0%) survived
VSIVΔG vector (Falzarano *et al.* 2011)	TAFV/Pau-CI $GP_{1,2}$: IM 2×10^7 PFU in crab-eating macaques	• 1/3 (33%) survival of macaques exposed IM to 10 000 $TCID_{50}$ BDBV/But-811250 on day 28 post-vaccination • Time to clearance of viremia was similar in surviving treated- or control-macaques	• 1/4 (25%) survived • Higher viremia than treated macaques
VSIVΔG vector (Falzarano *et al.* 2011)	EBOV/Yam-May $GP_{1,2}$: IM 2×10^7 PFU in crab-eating macaques	• 3/4 (75%) survival of macaques exposed IM to 10 000 $TCID_{50}$ BDBV/But-811250 on day 28 post-vaccination • Lower viremia than other treated groups	• 1/4 (25%) survived • Higher viremia than treated macaques

Table 7. (*Continued*).

Candidate vaccine (Reference(s))	Antigen: vaccination regimen	Efficacy in experimental animals	Efficacy in control animals
VSIVΔG vector (Daddario-DiCaprio *et al.* 2006a)	MARV/MtE-Mus $GP_{1,2}$: IM 2×10^7 PFU of MARV/MtE-Mus $GP_{1,2}$ in crab-eating macaques	3/3 (100%) survival of macaques exposed IM to 1000 PFU RAVV/KiC-810040 on day 28 post-vaccination	0/1 (0%) survived
VSIVΔG vector (Daddario-DiCaprio *et al.* 2006a)	EBOV/Yam-May $GP_{1,2}$: IM 2×10^7 PFU in crab-eating macaques	0/1 (0%) survival of macaques exposed IM to 1000 PFU RAVV/KiC-810040 on day 28 post-vaccination	0/1 (0%) survived
VSIVΔG vector (Geisbert *et al.* 2009)	EBOV/Yam-May $GP_{1,2}$ + MARV/MtE-Mus $GP_{1,2}$ + SUDV/Nza-Bon $GP_{1,2}$: IM 1×10^7 PFU of each vector in crab-eating macaques	3/3 (100%) survival of macaques exposed IM to 1000 PFU TAFV/Pau-CI on day 28 post-vaccination	2/5 (40%) survived
	VLP vaccines		
Tissue culture-derived VLP (Swenson *et al.* 2008b)	MARV/MtE-Mus $GP_{1,2}$ + MARV/MtE-Mus VP40: IM 50 μg VLPs for three doses at 21-day intervals in strain 13 guinea pigs	6/6 (100%) survival of guinea pigs exposed SC to 1000 PFU of guinea pig-adapted RAVV/KiC-810040 at day 72 post-vaccination	0/6 (0%) survived
Insect cell-derived VLP (Swenson *et al.* 2008b)	MARV/MtE-Mus $GP_{1,2}$ + MARV/MtE-Mus VP40 + MARV/MtE-Mus NP: IM 1 mg of VLPs for three doses at 42-day intervals in crab-eating macaques	• 3/3 (100%) survival of macaques exposed SC to 1000 PFU of RAVV/KiC-810040 at day 112 post-vaccination • Increased liver enzyme concentrations in 1/3 surviving macaques in absence of viremia	0/3 (0%) survived
Insect-cell-derived VLP (Warfield *et al.* 2015)	EBOV/Kik-9510621/SUDV/Nza-Bon GP + EBOV/Kik-9510621/SUDV/Nza-Bon VP40 + EBOV/Kik-9510621/SUDV/Nza-Bon NP: IM 3 mg total of VLPs in 0.1 mg of QS-21 adjuvant for one to two doses at 42-day interval in crab-eating macaques	5/5 (100%) survival of macaques receiving Ebola VLPs or mix of Ebola and Sudan VLPs and then exposed IM to TAFV/Pau-CI (5275 PFU) on day 70 after first vaccination	3/5 (60%) of controls vaccinated with QS-21 adjuvant succumbed after TAFV/Pau-CI exposure
Insect-cell-derived VLP (Warfield *et al.* 2015)	SUDV/Nza-Bon GP + SUDV/Nza-Bon VP40 + SUDV/Nza-Bon NP: IM 3 mg total of VLPS in 0.1 mg of QS-21 adjuvant for one to two doses at 42-day interval in crab-eating macaques	3/5 (60%) survival of macaques exposed IM to TAFV/Pau-CI (5275 PFU)	2/5 (40%) of such controls developed severe disease but survived after TAFV/Pau-CI exposure
	Inactivated vaccines		
Irradiated virions (Swenson *et al.* 2008b)	MARV/MtE-Mus: IM 50 μg of virions for three doses at days 21-day intervals in strain 13 guinea pigs	6/6 (100%) survival of guinea pigs exposed SC to 1000 PFU guinea pig-adapted RAVV/KiC-810040 on day 72 after first vaccination	0/6 (0%) survived
Irradiated virions (Hevey *et al.* 1997)	MARV/MtE-Mus: SC 100 μg of virions for three doses at 28-day intervals in strain 13 guinea pigs	5/5 (100%) survival of guinea pigs exposed SC to 100–1000 PFU of guinea pig-adapted RAVV/KiC-810040 on day 70 after first vaccination	1/5 (20%) survived
Irradiated virions (Hevey *et al.* 1997)	RAVV/KiC-810040: SC 100 μg of virions for three doses at 28-day intervals in strain 13 guinea pigs	5/5 (100%) survival of guinea pigs exposed SC to 100–1000 PFU guinea pig-adapted RAVV/KiC-810040 on day 70 after first vaccination	0/5 (0%) survived

Table 7. (*Continued*).

Candidate vaccine (Reference(s))	Antigen: vaccination regimen	Efficacy in experimental animals	Efficacy in control animals
Irradiated virions (Hevey *et al.* 1997)	RAVV/KiC-810040: SC 100 μg of virions for three doses at 28-day intervals in strain 13 guinea pigs	5/5 (100%) survival of strain 13 guinea pigs exposed to 100–1000 PFU guinea pig-adapted MARV/MtE-Mus SC on day 70 after first vaccination	1/5 (20%) survived
	Subunit vaccines		
Glycoprotein (Hevey *et al.* 1997)	Baculovirus-expressed MARV/MtE-Mus $GP_{1,2\Delta TM}$: SC 100 μg of vaccine for three doses at 28-day intervals in strain 13 guinea pigs	0/5 (0%) survival of strain 13 guinea pigs exposed SC to 100–1000 PFU of guinea pig-adapted RAVV/KiC-810040 on day 70 after first vaccination	0/5 (0%) survived

LD_{50}, lethal dose 50; ID, intradermally; IM, intramuscularly; IP, intraperitoneally; PFU, plaque-forming units; SC, subcutaneously; $TCID_{50}$, tissue-culture infectious dose 50; VLP, virion-like particle.

assay (ELISA) or western blot, assuming that LLOV $GP_{1,2}$ is presented correctly on the recombinant EBOV/LLOV chimeric virion (Ilinykh *et al.* 2016).

Ravn virus

Ravn virus (RAVV; pronounced rævn vaɪrəs) was discovered in 1987 and has been reencountered in 1999, 2007 and 2009 (Fig. 1). Thus far, only three human infections, two of them lethal, have been recorded. These cases result in a mean CFR of 66.7% (higher than that of any ebolavirus infection), but the low case number results in extremely high confidence intervals (Table 3). Of note, several human RAVV infections may have been overlooked during the relatively extensive MVD outbreak in the Democratic Republic of Congo in 1998–2000 (Bausch *et al.* 2006).

In addition to the scientific literature (Tukei 1988; Johnson *et al.* 1996), the initial 1987 infection of a 15-year-old Danish boy with RAVV has been vividly described in a popular science book using the synonym 'Peter Cardinal' (Preston 1994). The boy had lived with his expatriate sister and parents in Kisumu District of the now dissolved Nyanza Province in Kenya. The boy fell sick on 10 August 1987, with symptoms and clinical signs indistinguishable from MVD. Despite extensive treatment and life-sustaining measures started at Aga Khan Hospital in Mombasa on 13 August and continued at Nairobi Hospital on 18 August, the boy died on 20 August (Tukei 1988; Preston 1994; Johnson *et al.* 1996). Where and under which circumstances the boy became infected with RAVV are unclear. Epizootiological studies focused primarily on Kitum Cave in Kenya's Mt. Elgon National Park, which the family had visited on 2 August, because this cave was also loosely associated with a fatal MARV infection of a French engineer in 1980 (Smith *et al.* 1982; Preston 1994). Being large enough to give shelter to animals including bats, large felids and elephants, Kitum Cave was thought to be a possible hotspot for zoonotic viral spillover. However, even after examination of several thousand collected samples and sentinel animals from the cave, no filovirus was found (Tukei 1988; Preston 1994).

The second case of human RAVV infection was recorded in 1999 in Durba, Haut-Uele District, Province Orientale, Democratic Republic of the Congo, during a much larger outbreak of MVD due to MARV infection (1998–2000) including 153 infections and 128 deaths. All these human MARV infections and the single RAVV infection were traced back to an illegal underground gold mine. The original source of the RAVV and MARV infections is unclear. However, the outbreak came to an abrupt end when the gold mine was flooded, indicating a marburgvirus (MARV and RAVV) host reservoir inside of the cave and, therefore, zoonotic transmission (Bausch *et al.* 2006).

Between June and July 2007, three people developed MVD due to MARV infection in Ibanda District, Western Region, Uganda, after entering and working in the local Kitaka Cave (Adjemian *et al.* 2011). This lead ore mine was closed and secured by a guard who developed MVD in mid-September due to RAVV infection (Towner *et al.* 2009; Adjemian *et al.* 2011).

A total of three human RAVV isolates have been sequenced, one from each outbreak (Table 4). The coding-complete sequence of the isolate obtained from the 1987 case, Ravn virus/H.sapiens-tc/KEN/1987/Kitum Cave-810040 (RAVV/KiC-810040), was designated the type RAVV isolate (Kuhn *et al.* 2014). RAVV/KiC-810040 is also the isolate that has been used for virtually all laboratory studies with RAVV. The RAVV genome is identical in organization to the MARV genome (Fig. 2) (Johnson *et al.* 1996). Molecular-biological characterization studies focusing on RAVV have been extremely rare. In one study, RAVV VP40 was found to be a functional analog to MARV VP40, inhibiting the host cell's interferon type I and II responses (Valmas and Basler 2011).

Among the neglected filoviruses, RAVV's ecology is the least shrouded in mystery. RAVV (and MARV) genome-related nucleic acids fragments were detected in Egyptian rousettes (*R. aegyptiacus*) sampled in Kitaka Cave in 2007–2008 and 2012. Several replicating isolates of RAVV (and MARV) could be isolated from Kitaka Cave Egyptian rousettes sampled in 2007–2008 and from Egyptian rousettes sampled from 2008 and 2009 in Python Cave, a tourist site in Uganda's Queen Elizabeth National Park less than 30 miles away from Kitaka Cave (Table 4) (Towner *et al.* 2009; Amman *et al.* 2012, 2014). These findings indicate that Egyptian rousettes are reservoir hosts for marburgviruses. The circumstances under which extremely rare marburgvirus transmission occurs to humans has not been defined, and explanations are not available for the lack of marburgviruses in Egyptian rousette populations elsewhere in Africa (Wahl-Jensen *et al.* 2013).

The clinical presentation of human RAVV infection has only been described for the Danish boy who died in 1987 (Preston 1994; Johnson *et al.* 1996). As the source of infection remains

unknown, so is the incubation time. The boy first developed headache and fever, accompanied by malaise, anorexia and vomiting. The disease then worsened, resulting in bloody diarrhea and hypotension, ecchymoses, leukocytosis (20 000/mm^3) and thrombocytopenia (26 000/mm^3). Deterioration was characterized by high fever (40°C), disseminated *Pseudomonas aeruginosa* superinfection, continuously falling blood pressure reaching unrecordable values, delirium, cyanosis, blood coagulation abnormalities resembling DIC, and increases in serum potassium and urea levels. The boy then died because of cardiac arrest due to shock despite all efforts, including administration of antibiotics, steroids, heparin, fresh plasma and blood, and dialysis. Autopsy revealed massive petechial and purpuric hemorrhages in the skin, conjunctivae and gastrointestinal mucosa; hemorrhages in the lungs, tracheobronchal tree, epicardium, renal cortices and bladder (but not in spleen, pancreas, adrenals or in the acutely congested liver); and retroperitoneal edema; and pleural, pericardial and peritoneal effusions (Preston 1994; Johnson *et al.* 1996).

RAVV is the best researched neglected filoviruses in regard to medical countermeasures. Immunocompetent and immunodeficient mouse models and guinea pig models of RAVV infection have been established for high-throughput medical countermeasure evaluation, and immunopathological studies and uniformly lethal crab-eating macaque and rhesus monkey models are available for pathogenesis studies and specific countermeasure evaluation (Table 6). RAVV was long seen as an outlier 'subtype' or 'strain' of MARV. Consequently, many experiments aiming at identification of medical countermeasures against MARV infection included RAVV. This inclusion explains why several promising candidate vaccines for prevention (adenovirus, DNA, virion-like particles [VLP], vesicular stomatitis Indiana virus [VSIV]-based, inactivated and subunit; Table 7) and candidate therapeutic treatments (antisense/interfering RNAs and small molecules; Table 8) of RAVV infection have been studied. However, candidate therapeutic treatments have not been evaluated in non-human primates.

Reston virus

Reston virus (RESTV; pronounced 'rɛstən vaɪrəs) was discovered during a highly lethal VHF epizootic that occurred almost simultaneously in Virgina, Pennsylvania and Texas in the USA in 1989–1990 among captive crab-eating macaques (Geisbert and Jahrling 1990; Jahrling *et al.* 1990; Miranda and Miranda 2011). The first affected US location was Hazleton Research Products' Primate Quarantine Unit in Reston, Virginia, for which RESTV was named, and this virus became a household name through the vivid descriptions of the epizootic in Richard Preston's popular science book *The Hot Zone* (Preston 1994). The affected macaques of all US locations, several of them also co-infected with a yet-to-be-identified simian arterivirus (suspected to be simian hemorrhagic fever virus), had been imported from a Ferlite Scientific Research non-human primate export facility in Calamba, Luzon and Philippines (Fig. 1). Follow-up studies indeed found RESTV circulating in that facility (Hayes *et al.* 1992; Miranda *et al.* 1999), suggesting that RESTV may be an Asian filovirus or that the virus had been imported to the Philippines from another location. Since then, RESTV epizootics were recorded three more times. In 1992 and 1996, RESTV once again killed numerous crab-eating macaques in Hazleton non-human primate facilities in Siena, Italy and Alice, Texas, respectively, after being imported from the same Philippine facility in Calamba that was implicated in the 1989 epizootic (Centers for Disease Control and Prevention 1996; Ciorba *et al.* 1997; Miranda *et al.* 1999, 2002; Rollin *et al.* 1999; Miranda and Miranda 2011). Depopulation of the facility terminated RESTV circulation (Miranda *et al.* 2000). From 2008 to 2009, RESTV was repeatedly isolated in the Philippines from several of hundreds of captive domestic pigs (*Sus scrofa*) dying of a respiratory and abortion disease and co-infected with a porcine arterivirus (porcine reproductive and respiratory disease syndrome virus) and/or circoviruses (Barrette *et al.* 2009). Whether RESTV caused or contributed to the observed clinical signs in the affected pigs remains unclear.

Only a few RESTV isolates have been obtained (Table 4). Reston virus/M.fascicularis-tc/USA/1989/Philippines89-Pennsylvania (RESTV/Phi89-Pen) was designated the type RESTV isolate (Kuhn *et al.* 2014).

Infection of domestic piglets with RESTV in the laboratory only resulted in viremia in the absence of disease signs, indicating that pigs or other suids may be able to maintain subclinical infections and, therefore, serve as RESTV reservoir hosts (Marsh *et al.* 2011). However, no evidence has been obtained thus far supporting RESTV infection in wild suids. RESTV is widely assumed to be apathogenic for humans. This assumption is based on the absence of recorded clinically overt human infections despite numerous possibilities for non-human primate-to-human or pig-to-human transmission during the various epizootics and on the detection of anti-RESTV antibodies in a few clinically healthy individuals that were exposed to RESTV (Center for Disease Control 1990a,b; Miranda *et al.* 1991; World Health Organization 2009; Miranda and Miranda 2011). Consequently, ecological studies focusing on RESTV have been sparse as the virus is frequently not considered as an immediate threat to humans as are most other filoviruses. Anti-RESTV antibodies were detected in pteropodid bats sampled in Africa (Ogawa *et al.* 2015), Bangladesh (Olival *et al.* 2013), China (Yuan *et al.* 2012) and the Philippines (Taniguchi *et al.* 2011; Jayme *et al.* 2015), and in orangutans in Indonesia (Niikura *et al.* 2001). Short RESTV NP gene-like fragments (519 bp) were amplified from samples taken from bats trapped in the Philippines (Jayme *et al.* 2015), and short RESTV L gene-like fragments could be detected by PCR in samples taken from domestic pigs in China (Pan *et al.* 2014). However, neither replicating RESTV isolates could be isolated from any sample nor could complete or coding-complete genomes be assembled to prove actual infection of these animals. Injection of RESTV into Egyptian rousettes did not result in virus replication (Jones *et al.* 2015). Consequently, Asian endemicity of RESTV remains a hypothesis at this time, and epizootics due to RESTV infection can neither be geographically nor temporally anticipated.

Clinical descriptions of RESTV infections are limited. Due to the co-infection with simian arteriviruses (non-human primate epizootics) or porcine arteriviruses and circoviruses (pig epizootic), clinical descriptions of the disease outbreaks (Dalgard *et al.* 1992; Geisbert *et al.* 1992; Hutchinson *et al.* 2001; Ikegami *et al.* 2002a) have to be viewed with extreme caution. Which virus caused which clinical sign and how the various co-infecting viruses interfered with each other remain unclear. Therefore, our understanding of the clinical presentation of RESTV infection relies on results of very few published experimental laboratory infections (Table 6). Initial experiments suggested that RESTV infection in crab-eating macaques presents clinically and pathologically similar to EBOV and SUDV infections, but progresses more slowly (death within 8–14 days after infection compared to 8 days, respectively), is less lethal (<100%), and results in lower viremia. Interestingly, whereas EBOV is

Table 8. Overview of candidate peri-exposure treatment against infections with neglected filoviruses.

Candidate treatment (Reference)	Treatment and exposure regimens	Percent protection in rodents and controls
	Antisense strategies	
Antisense phosphorodiamidate morpholino oligomers (PMOs) • RAVV/KiC-810040 NP • RAVV/KiC-810040 VP24 • RAVV/KiC-810040 VP35 (Iversen *et al.* 2012)	Treatment: IP 20 mg/kg of each PMO at –1 h Exposure: IP 1000 PFU of mouse-adapted RAVV/KiC-810040	• 95% with PMOs targeting RAVV/KiC-810040 VP24 and NP • 80% with PMOs targeting RAVV/KiC-810040 VP24 and 35 and exposed to MARV/Hes-Ci67 • 70% with PMOs targeting RAVV/KiC-810040 NP, VP24 and VP35 • >60% protection with PMOs targeting RAVV/KiC-810040 NP • <10% in untreated control animals
Small interfering RNAs (siRNAs) Anti-MARV/Ang 2′-*O*-methylated siRNAs each targeting *NP* mRNA in lipid nanoparticles (Ursic-Bedoya *et al.* 2014)	Treatment: NP-RNA(s) 0.5 mg/kg at +1 h, then daily for 7 days Exposure: IP 1000 PFU of guinea pig-adapted RAVV/KiC-810040	• 100% cross-protection with NP cocktail of two siRNAs • 60%–75% cross-protection with each siRNA • 0% untreated control
	Small molecules	
Antioxidant NSC 62914 (Panchal *et al.* 2012)	Treatment: IP 2 mg/kg at –1 h Exposure: IP 1000 PFU of mouse-adapted RAVV/KiC-810040 at days 0, +2 and +5	• 90% with NSC 62914 • >25% with vehicle control
Nucleoside analog BCX4430 (Warren *et al.* 2014)	Treatment: IM twice daily with 0.12–30 mg/kg for 8 days initiated 4 h prior to exposure Exposure: IP 1000 PFU mouse-adapted RAVV/KiC-810040	100% protection with BCX4430 at 10 and 30 mg/kg
Nucleoside analog BCX4430 (Warren *et al.* 2014)	Treatment: IM twice daily with 150 mg/kg initiated 4 h prior to exposure and 24–120 h post-exposure Exposure: IP 1000 PFU mouse-adapted RAVV/KiC-810040	100% protection with BCX4430 when administered at 24, 72 or 96 h after exposure
FGI-103 (Warren *et al.* 2010)	Treatment: 5 or 10 mg/kg at day +1 Exposure: IP 1000 PFU of mouse-adapted RAVV/KiC-810040 at day 0	• 100% protection with 10 mg/kg of FGI-103 • 40% protection with 5 mg/kg of FGI-103 • 0% with vehicle control

known to also cause lethal infection in grivets (*Chlorocebus aethiops*), RESTV and SUDV do not (Fisher-Hoch *et al.* 1992; Jahrling *et al.* 1996b).

On the molecular-biological level, RESTV is arguably the best characterized of the neglected filoviruses, but very few studies have been published overall. The genome structure of RESTV is highly similar to other ebolaviruses (Ikegami *et al.* 2001; Groseth *et al.* 2002) (Fig. 2). Crystal structures have been determined for parts or the entire polypeptide chain of RESTV NP (Baker *et al.* 2016), VP35 (Leung *et al.* 2010), VP30 (Preston 1994) and VP24 (Reid *et al.* 2007; Zhang *et al.* 2012). In comparison to EBOV and MARV, RESTV was found to be less able to inhibit cellular type I IFN responses (Kash *et al.* 2006; Zhang *et al.* 2012). Just like EBOV, RESTV suppresses the type I interferon response through VP35 and VP24, and the type II interferon response through VP24 (Kash *et al.* 2006; Zhang *et al.* 2012). RESTV uses NPC1 for cell entry (Ng *et al.* 2015) and is dependent on cathepsin B (but not cathepsin L) for cell entry *in vitro* (Misasi *et al.* 2012). Akin to LLOV, RESTV Δ-peptide has little effect on MARV replication (Radoshitzky *et al.* 2011).

A RESTV minigenome system and RESTV reverse genetics have been used to study the RESTV lifecycle (Boehmann *et al.* 2005; Groseth *et al.* 2005). No reports on medical countermeasures against RESTV infection are available, most likely due to the perception that RESTV is not an imminent threat to humans. Diagnosis of RESTV infection is possible via pan-filovirus RT-PCR or qRT-PCR protocols (Lu *et al.* 2015) and RESTV-specific sequence capture probes for next-generation sequencing (Koehler *et al.* 2014). Several serological or nucleic-acid based RESTV-specific diagnostic systems have been described (Kalter *et al.* 1995; Ksiazek *et al.* 1999; Niikura *et al.* 2001; Ikegami *et al.* 2002b, 2003a,b; Ou *et al.* 2011). RESTV-specific monoclonal or polyclonal murine, rabbit or crab-eating macaque antibodies (Ou *et al.* 2011; Holtsberg *et al.* 2015; Keck *et al.* 2015; Wang *et al.* 2015), RESTV cross-reactive murine antibodies (Fusco *et al.* 2015; Wang *et al.* 2015; Furuyama *et al.* 2016), RESTV cross-reactive murine scFvs

and IgNAR Vs raised against inactivated EBOV particles (Goodchild *et al.* 2011) are available.

Taï Forest virus

Like LLOV, Taï Forest virus (TAFV; pronounced tɑːˈiː ˈfɔːrɪst vʌɪrəs) is a filovirus that thus far has only been encountered once (Table 3). TAFV was discovered in 1994 through the infection of a Swiss ethologist during a necropsy she performed with two colleagues on a western chimpanzee (*Pan troglodytes verus*) in Taï National Park, western Côte d'Ivoire (Fig. 1) (Le Guenno *et al.* 1995; le Guenno, Formenty and Boesch 1999). The ape belonged to a troop under observation since 1979 whose numbers had been reduced by at least two episodes of epizootic hemorrhagic fever in November 1992 (8 deaths) and November 1994 (12 deaths). The 34-year-old ethologist, who performed the necropsy on 16 November 1994 to shed light on the etiological cause of these episodes, developed a febrile disease 8 days later. On 26 November, she was hospitalized in Abidjan, and on 1 December she was transported to a hospital in Basel, Switzerland, where she recovered. Filovirus infection was confirmed by electron-microscopic and serological methods (ELISA and IFA) and by virus isolation in tissue culture (Le Guenno *et al.* 1995). Molecular characterization identified TAFV as a distinct filovirus most closely related to BDBV with a genome organization similar to all ebolaviruses (Fig. 2), an ebolavirus that only was encountered in Eastern Africa ≈4200 km away (Towner *et al.* 2008). Taï Forest virus/H.sapiens-tc/CIV/1994/Pauléoula-CI (TAFV/Pau-CI) was designated the type (and only) isolate of TAFV (Table 4) (Kuhn *et al.* 2014).

Histopathological examination of tissues from the western chimpanzee necropsied by the ethologist strongly indicated that the ethologist acquired TAFV from this animal. Lesions resembled those found in macaques experimentally infected with EBOV: multifocal necroses infiltrated with inflammatory cells, Kupffer cell hyperplasia in the liver, diffuse fibrinoid and hemorrhagic necrosis in the splenic red pulp and lymphoid depletion in all lymphatic tissues. More importantly, macrophages in various affected tissues reacted with TAFV-specific and TAFV-cross-reactive antibodies. However, typical hemorrhagic, thrombotic or vascular lesions of EBOV infection were not present in the ape (Wyers *et al.* 1999), and ultimate confirmation of TAFV infection in chimpanzees by either virus isolation or next-generation sequencing is lacking. Likewise, how the chimpanzees may have become infected is unclear. Whereas MARV and RAVV subclinically infect bats in nature, and data for EBOV–bat associations are suggestive (Wahl-Jensen *et al.* 2013), TAFV does not replicate in Egyptian rousette bats (Jones *et al.* 2015). No clues have been found in regard to TAFV ecology other than possibly TAFV-specific antibodies in Indonesian orangutans (Nidom *et al.* 2012).

Because of the recording of only a single case, the clinical presentation of EVD due to TAFV infection is unclear. The TAFV-infected ethologist developed fever, headaches, myalgia, chills, cough, abdominal pain and nausea accompanied with acute non-bloody diarrhea and vomiting, a generalized maculopapular rash and hematuria. Clinical tests revealed proteinuria, marked liver enzymes (aspartate aminotransferase, alanine aminotransferase) and lactate dehydrogenase elevations, thrombocytopenia, lymphopenia and neutrophilia, and suggested DIC. The woman was released on day 15 after disease onset and recovered without sequelae other than temporary hair loss (Le Guenno *et al.* 1995; le Guenno, Formenty and Boesch 1999; Formenty *et al.* 1999). Thus, the disease resembled non-lethal EVD caused by BDBV (Table 5) and other ebolaviruses and MVD. The CFR of TAFV infection cannot be determined from a single survivor. The molecular and immunological responses to human TAFV infection have not been studied in detail. Consequently, neither cytokine response nor other biomarker data are available. Since the patient fortunately survived and no biopsies had been performed, there are no human pathological data.

Establishment of experimental disease caused by TAFV is currently only possible using a partially lethal crab-eating macaque model (Table 6), thereby development of medical countermeasures is challenging. Consequently, results from only a single study on a candidate vaccine for the prevention of TAFV are ambiguous (Table 7), and no TAFV-specific antivirals have been brought forward. Molecular-biological studies on TAFV are close to absent with the exception of the determination of the crystal structure of the TAFV NP C-terminal domain (Baker *et al.* 2016) and the characterization of TAFV $GP_{1,2}$-mediated cell-entry (NPC1 and cathepsin B) requirements (Misasi *et al.* 2012; Ng *et al.* 2015). Minigenomes and reverse genetics systems have yet to be established for TAFV. TAFV detection is possible via pan-filovirus RT-PCR (Lu *et al.* 2015) and TAFV-specific sequence capture probes for next-generation sequencing (Koehler *et al.* 2014). Very few TAFV-specific antibodies have been described (Ou *et al.* 2011; Furuyama *et al.* 2016), including ebolavirus cross-reactive llama single-domain antibodies (Sherwood and Hayhurst 2013). TAFV-specific serological diagnostic assays have not yet been described.

Other filoviruses

The geographically broad distribution of filoviruses (western to eastern Equatorial Africa, Philippines and Spain) suggests that many filoviruses remain to be discovered. Supporting this hypothesis, He *et al.* (2015) recently described the amplification of filovirus NP-, VP35- and L-like nucleic acids from (frugivorous) Leschenault's rousette bats (*Rousettus leschenaultii*) captured in Yúnnán Province, China. Whereas most fragments were too short (129–354 bp) to unambiguously assign them to the family *Filoviridae*, two fragments from the same bat ('Bt-DH04') could be extended to 2750 and 2682-bp length, respectively. The first fragment aligned with the 3′ end of a filoviral NP and almost an entire filoviral VP35-like gene; the second covered a large portion of a filoviral L-like gene. Phylogenetically, these fragments represent a novel clade of filoviruses basal to ebolaviruses and in between ebolaviruses and marburgviruses/cuevaviruses.

CONCLUSIONS

The ecology of filoviruses needs to be defined, and the natural host reservoirs of cuevaviruses and ebolaviruses are still unclear (Wahl-Jensen *et al.* 2013). The discovery of Egyptian rousettes as natural host reservoirs of marburgviruses in Ugandan caves (Towner *et al.* 2009) was a major step forward in filovirology, but questions remain as to why MARV and RAVV cannot be found in other Egyptian rousette populations in and outside of Africa. Because of these uncertainties, how and under which circumstances filovirus host-human transmission occurs are unclear. The geographic distribution of filoviruses (i.e. their endemicity) remains undefined despite several ecological filovirus niche modeling studies (Peterson, Bauer and Mills 2004; Peterson *et al.* 2006; Pigott *et al.* 2014, 2015). Finally, the discovery of LLOV in Spain (Negredo *et al.* 2011) and the detection of filovirus-like entities in Chinese bat populations (He *et al.* 2015) indicate that

the family *Filoviridae* is undersampled. Its members are probably much more diverse and distributed than previously thought.

Because of this lack of ecological knowledge, prediction of when and where human and/or animal filovirus disease outbreaks may occur is impossible. Since knowledge on neglected filoviruses (BDBV, LLOV, RAVV, RESTV and TAFV) is extremely limited, one cannot exclude the possibility of future large EVD or MVD outbreaks caused by these viruses. The recent EVD/EBOV outbreak in Western Africa, involving 28 646 cases and 11 323 deaths, demonstrates for the first time that EVD outbreaks do not necessarily remain geographically confined or involve only dozens to several hundreds of cases (Table 3). Yet, a disease outbreak of the magnitude of that EVD outbreak caused by a neglected filovirus may prove even more disastrous to Africa or the world. Among the already very low global number of research institutes that are permitted to perform biosafety level 4 research on filoviruses, few even have access to neglected filoviruses. Reagents and assays for neglected filoviruses are not available or extremely limited, especially for commercial products, which primarily fulfill the need for research on EBOV, SUDV and MARV research. The only commerical reagents for neglected filoviruses include: irradiated BDBV, RAVV, RESTV and TAFV or isolated genomic RNA; recombinant complete, partial or tagged $GP_{1,2}$ (BDBV, RESTV, TAFV), VP40 (TAFV), VP24 (RESTV); polyclonal rabbit anti-$GP_{1,2}$ antibodies (BDBV, RESTV, TAFV); and monoclonal antibodies against VP40 (BDBV); commercial ELISA systems for the detection of circulating guinea pig, human, non-human primate and pig antibodies (RESTV) and circulating $GP_{1,2}$ antigen (BDBV, RESTV, TAFV); and qPCR systems for RESTV and TAFV (all based on catalog searches of Alpha Diagnostics, BEI Resources, Integrated BioTherapeutics, Genesig and Sino Biological; Anthony and Bradfute 2015). Animal models for neglected filovirus infections are either absent, not 100% lethal, or not established in non-human primates (Table 6). Consequently, there are few specific candidate vaccines and almost no specific therapeutics in the pipeline to prevent or treat neglected filovirus infections (Tables 7 and 8).

Therefore, we appeal to the international filovirus research community, and even more so to the funders of filovirus (currently almost exclusively EBOV) research and development activities, to create and maintain a global, coordinated and highly collaborative program to prospectively create basic reagents, assays, methodologies, databases, animal models and medical countermeasure platforms that include neglected filoviruses on a routine basis.

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