

Literature Review of Ebola Virus

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Research Article

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ABSTRACT

Ebola virus is one of the maximum lethal pathogens recognized to contaminate people. The current Ebola outbreak in West Africa is unheard of in magnitude and duration and, as of November 30, 2014, indicates no signs and symptoms of abating. For the primary time, cases of *Ebola virus* ailment have been recognized in the US, originating from sufferers who travelled at some point of the incubation period. The outbreak has generated international problems. Ebola virus ailment is a severe and frequently deadly ailment as a result of the Ebola virus (EBOV). EVD outbreaks normally begin from an unmarried case of in all likelihood zoonotic transmission, observed utilizing human-to-human transmission through direct touch or contact with inflamed physical fluids or contaminated virus. EVD has a high case-fatality fee; it's far characterized through fever, gastrointestinal signs and more than one organ dysfunction syndrome. Diagnosis requires a mixture of case definition and laboratory exams, usually actual-time reverse transcription PCR to stumble on viral RNA or speedy diagnostic checks based totally on immunoassays to hit upon EBOV antigens. The results of a randomized scientific trial of investigational therapeutics for EVD proved survival benefits from two monoclonal antibody merchandise concentrated on the EBOV membrane glycoprotein. EVD is currently one of the international's most feared diseases. In this literature evaluation, we describe the epidemiology, scientific features, diagnosis, and remedy of EVD.

INTRODUCTION

Ebola, also known as *Ebola virus* sickness (EVD) or Ebola hemorrhagic fever (EHF), is a viral hemorrhagic fever in people and different primates, because of ebola viruses. [1] Symptoms typically start everywhere among days and 3 weeks after turning into inflamed with the virus. The first signs and symptoms are commonly fever, sore throat, muscle pain, and complications. These are generally accompanied by using vomiting, diarrhea, rash and reduced liver and kidney features [1] at which point, some people begin to bleed each internally and externally. The disease kills between 25% and ninety% of those infected approximately 50% on common. Death is regularly due to shock from fluid loss and normally occurs between six and sixteen days after the primary signs appear [2]. *Ebola virus* (EBOV) belongs to the circle of relatives Filoviridae, the genus *Ebola virus*, and often reasons deadly infection in people [1]. EBOV disease (EVD) may also display more than one, serial, and nonspecific-ailment signs such as excessive fever, headache, vomiting, anorexia, diarrhoea, and aching muscle groups [3]. Unexplained bleeding within the eyes, nose, gums, and gut occurs in the superior stages [4]. The first outbreak of EVD turned into stated in 1976 in the Democratic Republic of the Congo [5]. Since then, there have been reviews of small EVD outbreaks in some international locations in Central Africa, which includes Sudan and Uganda, with an estimated 2350 cases of EVD taking place between the 1970s [6] (**Figure 1**).

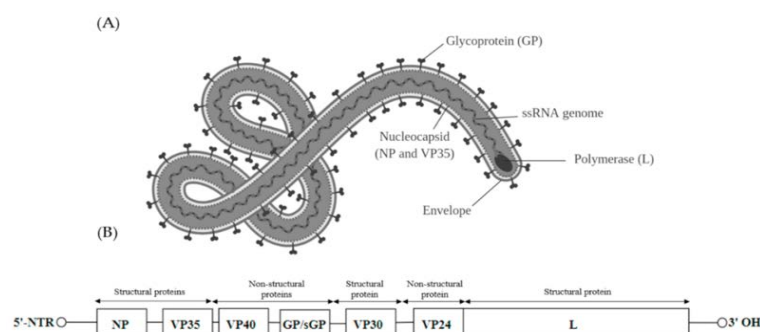


Figure 1. Structure of EVD.

Virus classifications

- (unranked): Virus
- Realm: *Riboviria*
- Kingdom: *Orthornavirae*
- Phylum: *Negarnaviricota*
- Class: *Monjiviricetes*
- Order: *Mononegavirales*
- Family: *Filoviridae*
- Genus: *Ebolavirus*

Species

- *Bombali ebolavirus*
- *Bundibugyo ebolavirus*
- *Reston ebolavirus*
- *Sudan ebolavirus*
- *Tai Forest ebolavirus*
- *Zaire ebolavirus*

The virus and its genome

EBOV belongs to the order *Mononegavirale* (unmarried-stranded, non-segmented, bad-sense RNA virus) of the family *Filoviridae*, genus *Ebolavirus* [7]. Other members of the own family include *Marburgvirus* and *Cuevavirus*, of which *Marburgvirus* has additionally been implicated in inflicting hemorrhagic illnesses similar to EBOV, and both of those are filamentous shape viruses. The genus *Marburgvirus* includes a species. The genus *Cuevavirus* consists of an unmarried species, *Lloviu cuevavirus* with *Lloviu virus* (LLOV). The EBOV became first isolated in 1976 close to Zaire valley inside the Democratic Republic of Congo (Zaire) rooting its call from the area of isolation, initially named as an Ebola-like virus which becomes later changed into EBOV inside the 12 months 2002. The genus *Ebolavirus* consists of 5 species viz., (1) *Zaire ebolavirus* (*Zaire virus* a ZEBOV), (2) *Sudan ebolavirus* (*Sudan virus* a SUDV), (3) *Reston ebolavirus* (*Reston virus* a RESTV), (4) *TaA Forest ebolavirus* (*TaA Forest virus* a TAFV) and (5) *Bundibugyo ebolavirus* (*Bundibugyo virus* a BDBV) and Cote d'Ivoire ebolavirus use Configuration of Ebola virus and its genome. Ebola virus possesses a negative-sense RNA genome with noticeably 14000 nm length with 3' nucleoprotein and 5' RNA polymerase cease.

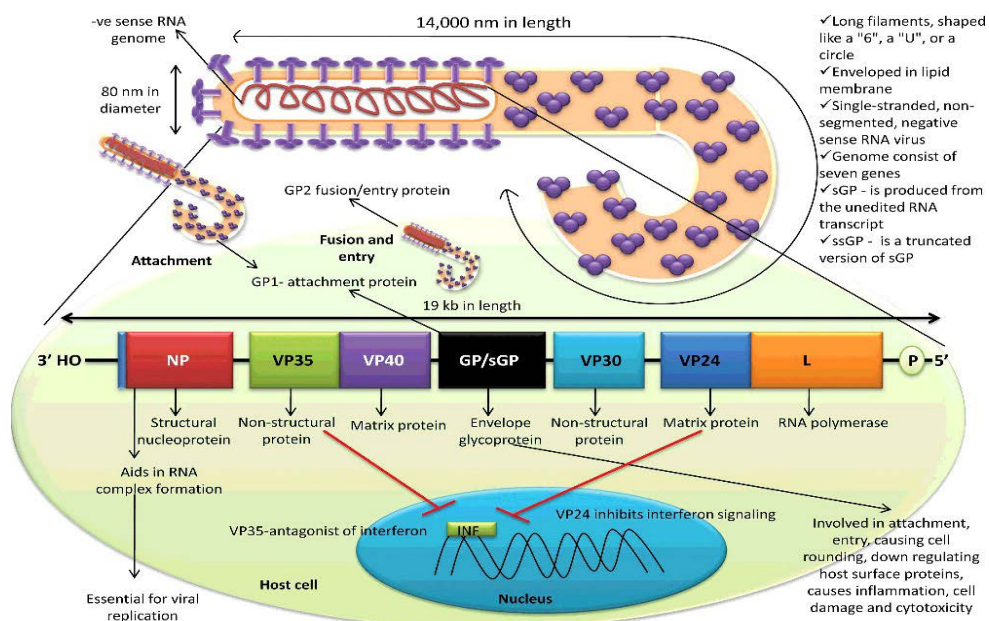


Figure 2. Configuration of Ebola virus and its genome.

EPIDEMIOLOGY

The first cases of *Ebola virus* infection had been mentioned in Zaire (now known as the Democratic Republic of the Congo

(DRC) in 1976. There have been 318 instances and 280 deaths, an 88% case fatality charge. Transmission in this outbreak changed into traced back to using contaminated needles in an outpatient temperament facility at Yambuku Mission Hospital. Since then, frequent outbreaks have come about in Central and Western Africa. The maximum commonplace species of *Ebola virus* accountable for outbreaks is the *Zaire ebolavirus*, the second maximum common species being the *Sudan ebola virus*. The *Zaire ebola virus* becomes liable for the outbreak that commenced in West Africa in 2014 and finished in 2016. It was first started in March 2014 and is the largest outbreak because the virus changed into first located in 1976. Genetic sequencing has proven that the virus isolated from infected sufferers inside the 2014 outbreak is ninety seven% similar to the virus that first emerged in 1976. It is also accountable for smaller outbreaks within the DRC from 2017-2021. The *Zaire ebolavirus* has a remembered case fatality fee of up to ninety% in preceding outbreaks. Direct contrast of case fatality rates among unique Ebola treatment centers and outbreaks need to be interpreted with warning as many variables can introduce bias and skew even massive cohort facts. The case fatality charge at some point of the 2014 outbreak changed into up to sixty-four. Three% in clinic admissions, falling to 31. Five% in some treatment facilities in West Africa and around 20% in sufferers managed outside West Africa.(28) In contrast to this, the *Sudan ebolavirus* has a decrease case fatality charge of 53% to 65% in previous outbreaks, with the biggest outbreak occurring in 2000 [4]. There has only been one outbreak of *Bundibugyo ebolavirus*. In 2007 in western Uganda, and this outbreak had a case fatality rate of 25% [6] (Table 1).

Table 1. IAV, Influenza A viruses - Ebola virus drugs.

Drug	Status	Feature	Company
Zmapp	Phage I	Three chimeric monoclonal antibodies	LeafBio, Inc.
Favipiravir	Approved for IAV	Inhibition of viral RNA-dependent RNA	Fujifilm
TKM- Ebola	Phage I	Si RNA	Tekmira
Brincidofovir	Phage III	Oral nucleotide analog	Chimerix
BCX4430	Pre-clinical	Inhibition of viral RNA-dependent polymerase	Biocryst
AVI-7537	Phage I	Binding Ebola RNA	Sarepta

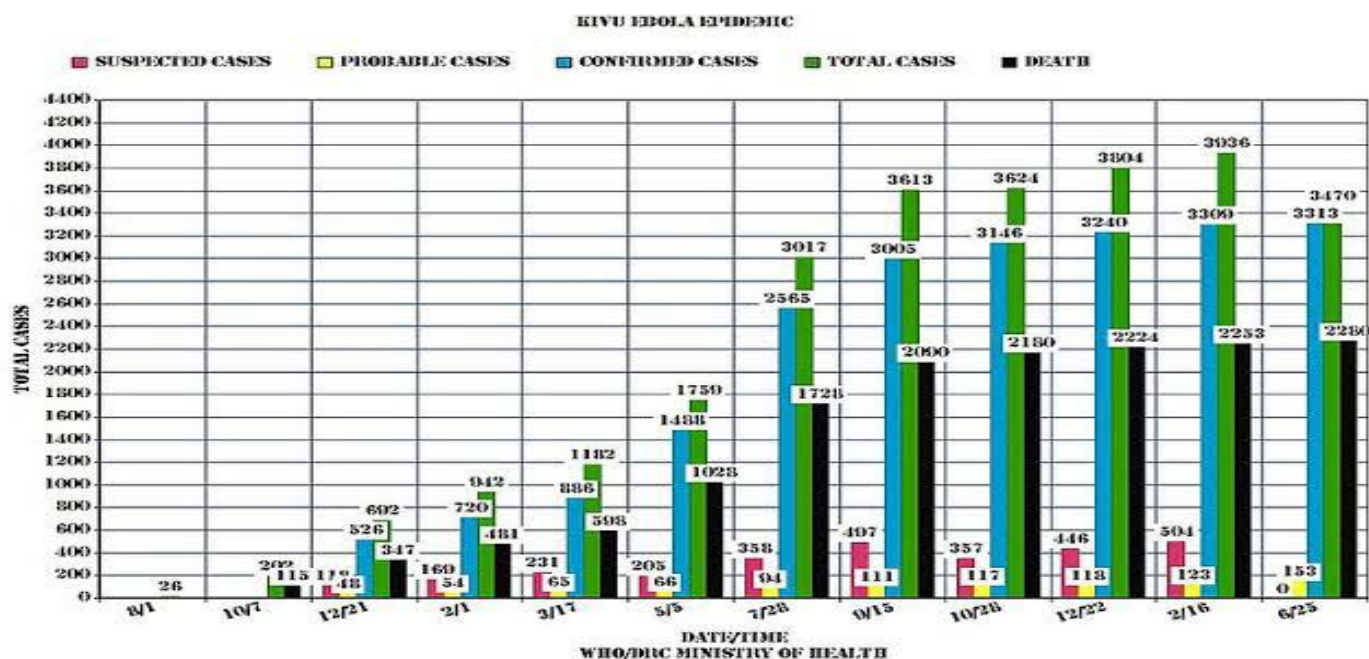


Figure 3. Kivu Ebola Epidemic

Mode of action

Ebola virus takes gain of a non-unique engulfing system called macro pinocytosis, which allows the virus to be “eaten” by using a wave-like gesture of the cell membrane (Figure 4) [2]. Once in the cellular, the virus hijacks the mobile's very own equipment to create extra copies of itself. Ebola virus infects human cells. (A) (B) Ebola has a protein referred to as glycoprotein that stands proud of its membrane and binds to receptors (in red) at the cellular surface. (C) The binding of these receptors triggers a cellular “eating” procedure known as macro pinocytosis, resulting in the virus being engulfed by using a wave-like motion of the cell. (D) Once inside the mobile, the virus' RNA is uncoated, at which factor it hijacks the human cellule's proteins to create greater copies of itself. (E) Once new viral particles had been assembled, they pass to the mobile membrane and “bud off,” at which point they can journey to contaminate new cells (F).

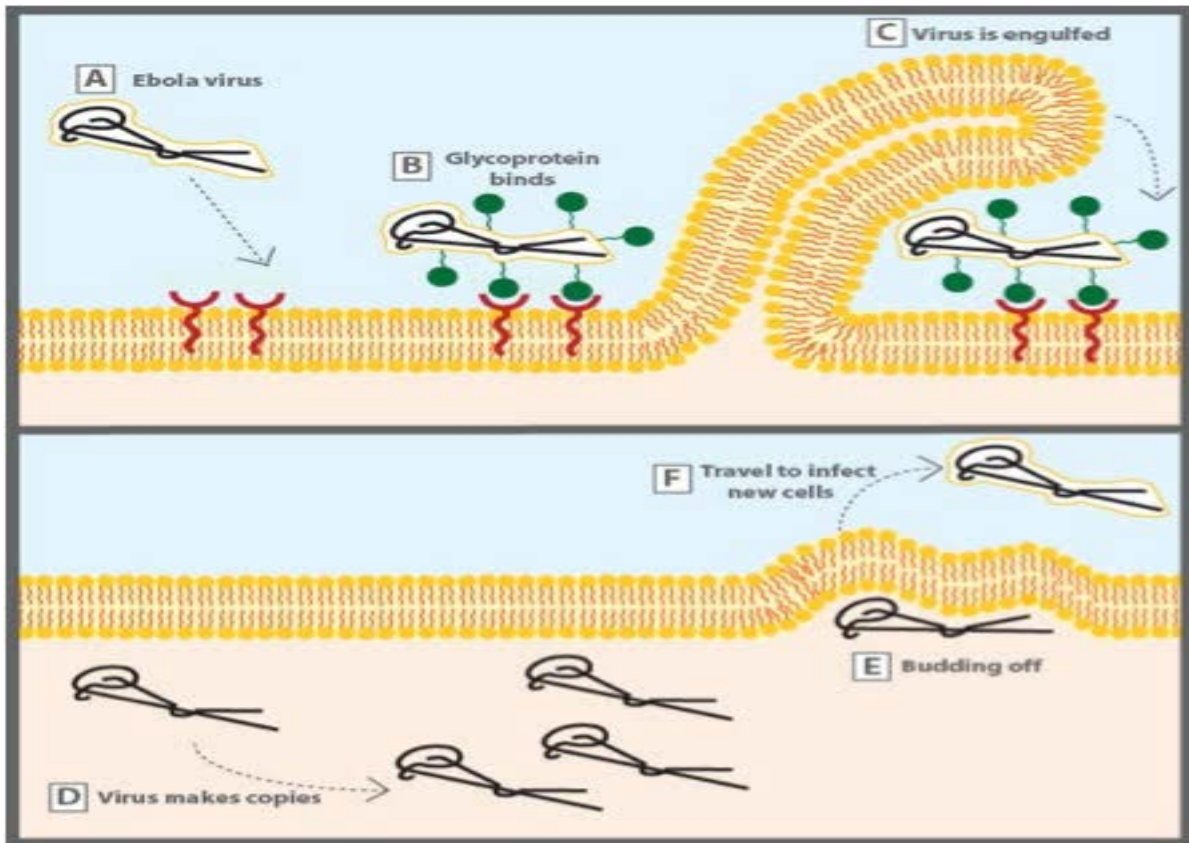


Figure 4. The supply of exposure to virus-sporting frame fluids.

Transmission

For people, the supply of exposure to virus-sporting frame fluids may additionally consist of

- An infected animal
- Another man or woman who has symptoms of the ailment or has died from the disease

Contaminated items, which includes clothing, mattress linens, doorknobs, needles and different clinical device, or different surfaces After people have recovered, the virus can often be detected for plenty months in certain body fluids, which includes semen, breast milk and urine. The virus enters a person through a destroy in the pores and skin or through the mucous membranes, together with the tissues of the eyes, nose, throat or vagina. For example, you could come to be infected in case you touched inflamed frame fluids after which touched your eyes. The time from infection to the arrival of signs (incubation duration) is commonly 8 to 10 days but can vary from 21 days. Body fluids that could transmit Ebola include **(Figure 5)**.

- Blood
- Faeces
- Vomit
- Urine
- Semen
- Saliva
- Breast milk
- Vaginal fluids
- Pregnancy-associated fluids
- Tears
- Sweat

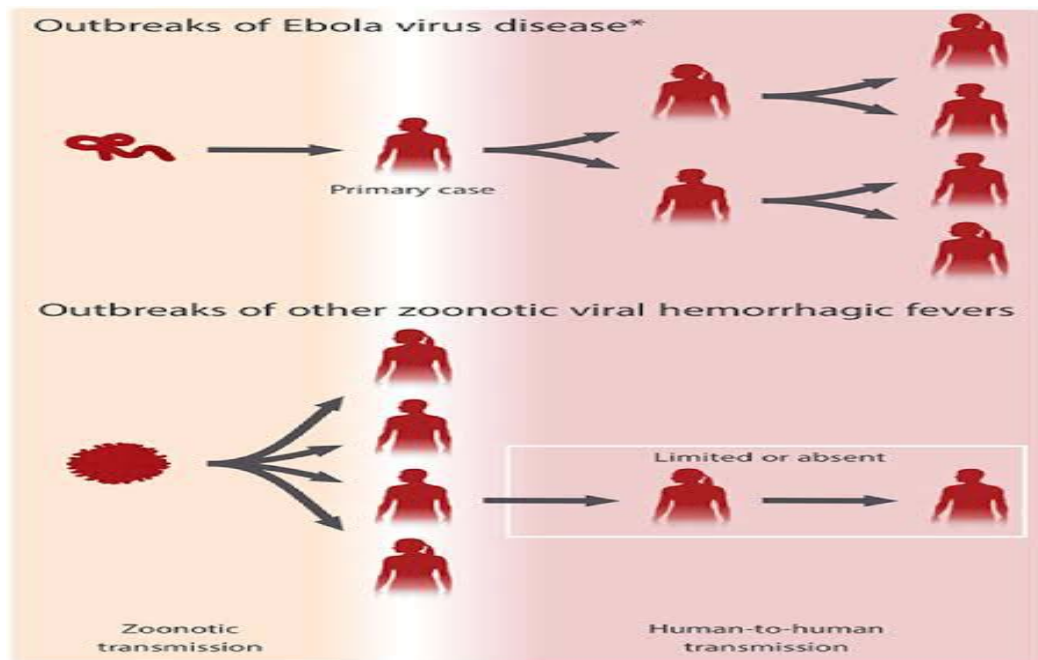


Figure 5. Outbreak of Ebola Virus.

Symptoms of *Ebola*

The time c programming language from infection with *Ebola* to the onset of signs and symptoms is two-21 days, even though eight-10 days is most common. Signs and signs consist of

- fever
- headache
- joint and muscle aches
- weak point
- diarrhea
- vomiting
- belly pain
- lack of appetite

MATERIALS AND METHODS

Cells and viruses, Vero E6 (monkey kidney), 293T (human embryonic kidney), and U937 (human monocytic) cells were grown and maintained at 37 °C in Dulbecco's MEM (DMEM) or RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum, 2 mmol/L L-glutamine, one hundred U/mL penicillin, and one hundred µg/mL streptomycin. Primary human macrophages derived from blood samples from wholesome donors had been remoted with the aid of the use of Ficoll gradient (Amersham) and had been incubated at 37 °C in RPMI 1640 supplemented with five% human type AB serum . The Virus infectivity titers (in focus-forming units) were acquired by counting the wide variety of infected cell foci, by way of the use of an oblique IFA the use of rabbit polyclonal anti-ZEBOV/VP40 or murine monoclonal anti-ZEBOV/glycoprotein (GP) antibodies for floor staining underneath non permeable conditions and a goat anti-rabbit IgG-fluorescein isothiocyanate conjugate or a goat anti-mouse IgG-Cy3 conjugate, as defined someplace else (17, 18). All paintings with infectious ZEBOV become performed inside the biosafety degree (BSL) four laboratories at the National Microbiology Laboratory of the Public Health Agency of Canada in Winnipeg, Manitoba (tissue lifestyle and mouse experiments), and the USA Army Medical Research Institute for Infectious Diseases at Fort Detrick, MD (nonhuman primates).

Generation of infectious cDNA clones of ZEBOV that include an eGFP gene. Two infectious cDNA clones of ZEBOV that specific the eGFP reporter protein from an extra transcription unit as an 8th gene was built with the aid of the use of traditional molecular cloning techniques. To create the primary eGFP-ZEBOV clone, targeted "NP/35-eGFP," the eGFP open analyzing body (ORF) changed into engineered with flanking ZEBOV NP transcription start and termination alerts and become inserted between the NP and VP35 genes via the use of newly created restrict-enzyme sites in the NP/VP35 intergenic vicinity (IGR; discern 1A). The 2d eGFP-ZEBOV clone, distinct "VP30/24-eGFP," changed into built via insertion of the eGFP ORF among the VP30 and VP24 genes as an extra transcription unit composed of the five' noncoding place (NCR). The eGFP expression cassette of VP30/24-eGFP possesses a replica of the VP30/VP24 IGR, to imitate the mechanism of VP24 gene transcription (discern 1A).

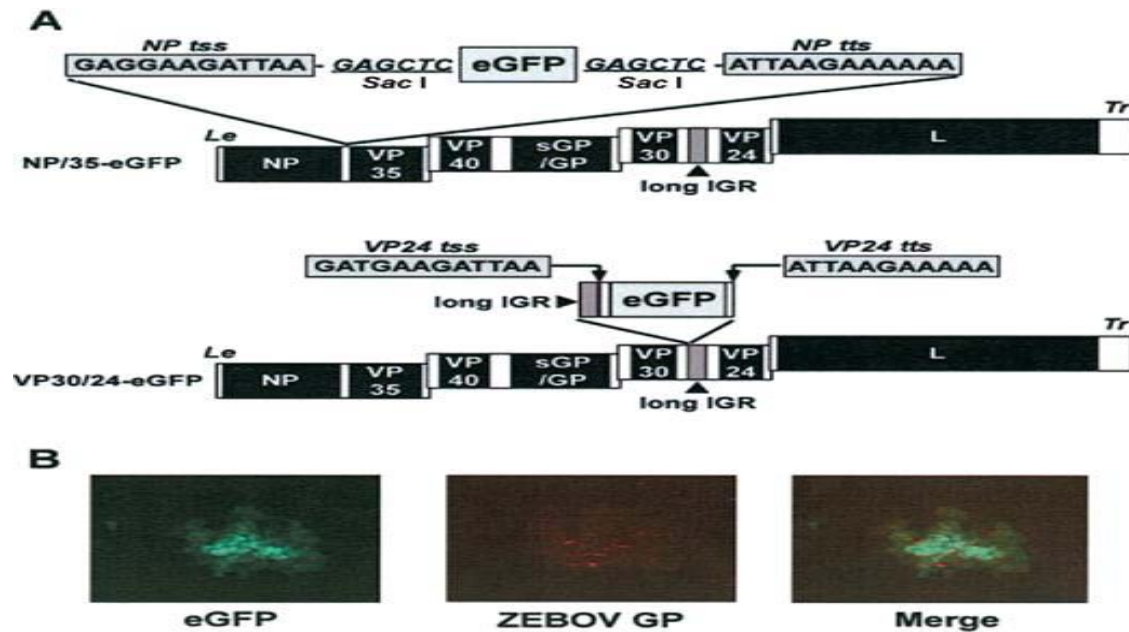


Figure 6. Ebola ailment in EBOV-inflamed guinea pigs

Chloroquine did not guard against Ebola ailment in EBOV-inflamed guinea pigs. Twelve guinea pigs have been challenged with EBOV, and six afterwards a collection of six have been administered 33.75 mg kg^{-1} chloroquine orally. Treatment persevered on a twice day by day schedule and survival of chloroquine-treated animals turned into compared with that of six untreated controls. The dose used become primarily based on 90 mg kg^{-1} is proven to be efficacious in opposition to EBOV sickness in mice and using a conversion element of zero.375 for guinea pigs from steerage provided via America Food and Drug Administration based on frame floor area (Center for Drug Evaluation and Research, 2005). An extensively poorer final results for dealt with animals turned into found ($P=0.001$, log-rank survival evaluation) (**Figure 6a**). The chloroquine treatment also led to a more rapid loss in frame weight than for untreated manage animals (**Figure 6b**). Increases in frame temperature took place to a comparable extent in each chloroquine-handled and untreated animals today five; but, thereafter frame temperatures commenced to decrease lower back to baseline degrees inside the dealt with animals (**Figure 6c**). Clinical signs and symptoms were measured twice a day and a numerical price was turned into assigned to each sign and recorded for useful resource analysis. Chloroquine led to the rapid development of severity of illness after 5 days put up-task (**Figure 6d**).

Survival and clinical signs and symptoms of EBOV-challenged guinea pigs handled with oral chloroquine compared with untreated animals. (a) Survival evaluation between chloroquine-handled animals and untreated animals. (b) Weight adjustments displaying percentage distinction.

In a separate examination, guinea pigs were scheduled to be dealt with chloroquine at 33.75 mg kg^{-1} via the intravenous course, given that oral management changed into no longer well tolerated inside the guinea pigs, with a few animals regurgitating the compound again and again before swallowing. However, the first animals that obtained intravenous chloroquine died within 30s of management, so the take a look at turned into aborted.

Chloroquine remedy did not affect viraemia in EBOV-infected guinea pigs On day 8 submit-challenge, a sample of blood was taken from animals final inside the look at to assess viraemia. Only two animals from the chloroquine-handled organization remained alive, together with all six untreated animals. There turned into no statistically sizable distinction in viral RNA ranges between the chloroquine-dealt with and untreated groups ($P=0.0668$, Mann-Whitney statistical check), although the information indicated higher ranges of viral RNA within the chloroquine-dealt with the organization (**Figure 2a**). Blood became additionally sampled at necropsy, whilst animals met humane clinical give up factors or were culled at the scheduled stop the examine. Viral RNA degrees at the time of euthanasia were no longer considered exceptional among the two corporations ($P=0.0656$, Mann-Whitney statistical test) (**Figure 2b**).

In vitro virus assay

Chloroquine phosphate (Selleckchem) changed into diluted to 5mm with sterile water before similar dilution to the desired concentration with Eagle's minimal essential medium (Sigma). Concentrations have been made at double the final dilution to take into account an identical quantity of virus suspension to be delivered. MRC-5 cells (acquired from the European Collection of Cell Cultures, UK) were seeded into 96-properly plates. Within the CL-four laboratory, the medium becomes eliminated from the internal wells of the 96-properly plates. Owing to facet results, the outer wells were left with medium delivered. Five replicates were used in line with dilution (**Table 2**).

For the first *in vitro* display, EBOV suspension (strain ME718, recently renamed 1976/Yambuku-Ecran changed into brought at an awareness of approximately 500TCID₅₀ according to nicely to triplicate wells in line with chloroquine dilution, with the remaining

Table 2. Effect of Chloroquine phosphate at different dilutions.

Species	Animal number	Virus	Day after exposure	TLM	ALM
A.green	920112	EBOZ	6	16.0 (5.0)	1.8(1.3)
A.green	920113*	EBOZ	6	14.1(3.8)	0.5(0.7)
A.green	920114*	EBOZ	6	13.9(3.7)	0.8(0.8)
A.green	920115*	EBOZ	7	15.0(3.9)	1.5(1.3)
A.green	990020	Control	-	20.3(3.7)	0
Cyno	950076	EBOZ	6	16.7(4.8)	0.2(0.4)
Cyno	950079	EBOZ	7	18.3(6.6)	1.5(2.5)
Cyno	960026	EBOZ	7	15.6(5.0)	0.3(0.7)
Cyno	970016*	EBOZ	6	10.9(2.3)	0.5 (0.7)
Cyno	970017*	EBOZ	6	13.1(3.6)	0.2(0.4)
Cyno	980024*	MBG	9	13.6(3.5)	0.4(0.7)
Cyno	980025*	MBG	9	12.3(3.9)	0.8(0.5)
Cyno	980026*	MBG	10	11.5(2.9)	1.8(2.0)
Cyno	980067*	MBG	10	12.8(5.5)	0.8(0.8)
Cyno	900137*	EBOR	11	13.7(4.6)	2.3(1.6)
Cyno	960167*	EBOR	15	12.1(3.2)	1.0(1.5)
Cyno	990022	Control	-	20.4(3.5)	0
Rhesus	950051	EBOZ	8	18.4(3.2)	0.7(0.5)
Rhesus	950064*	EBOZ	7	16.6(3.4)	0.1(0.3)
Rhesus	950065*	EBOZ	8	14.2(2.9)	0.9(1.0)
Rhesus	990021	Control	-	23.5(5.7)	0

two wells having medium by myself brought to evaluate for cytotoxicity without the presence of EBOV. Based on consequences from the boom of EBOV in distinctive mobile traces, the supernatants from MRC-5 cells had been harvested on day 3 put up-infection. One hundred and 40 micro liters from each well turned into delivered to 560 µL AVL buffer (Qiagen) for RNA extraction and PCR assay.

To verify pastime of chloroquine towards EBOV, screening assays were repeated for outcomes in opposition to EBOV using MRC-5 cells. However, modifications to the previous approach comprised of a ten-fold higher viral inoculum being used (approx. 5000TCID50 consistent with well) and samples have been harvested after 2 days.

Statistical analysis

All virus titers in the boom-kinetics experiments and the percentages of eGFP-fantastic cells are shown as. Mean SE P values for implying time to demise in the mouse experiments had been calculated through the use of Student's t check (2-tailed distribution and a couple of of-sample unequal variance). P<.05 become taken into consideration to be statistically considerable.

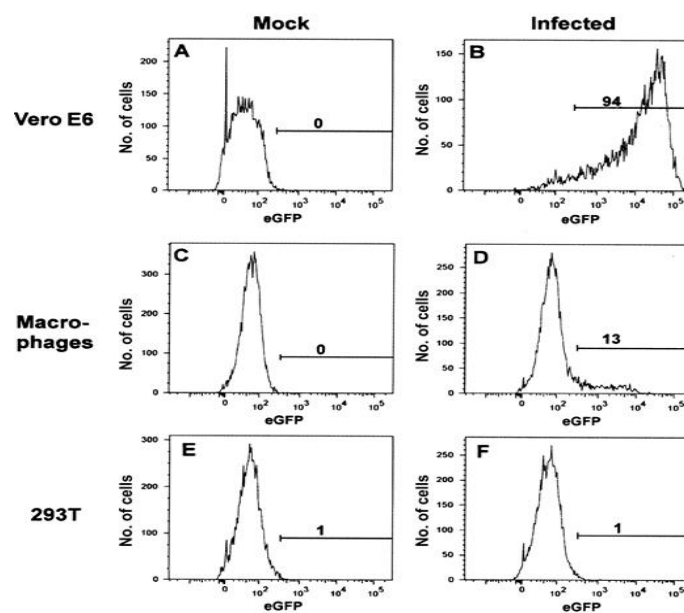


Figure 7. Virus titers in the boom-kinetics experiments and the percentages of eGFP-fantastic cells.

In vitro characterization of eGFP-ZEBOV. We first examined the stability of the eGFP transcription units with the aid of passaging each eGFP-ZEBOV 10 instances in Vero E6 cells. EGFP expression remained solid in all passages, confirming the steadiness of the extra transcription unit *in vitro* (records no longer shown). To show the application of eGFP-ZEBOV, we examined cell tropism

in vitro by the usage of 1 of the two recombinant viruses (NP/35-eGFP). Vero E6 cells and number one human macrophages, which might be permissive for ZEBOV contamination, and 293T cells, which can be nonpermissive for ZEBOV infection and served as a negative manage, have been infected with NP/35-eGFP at an MOI of zero.05, and eGFP-advantageous cells have been quantified by drift cytometry at day four after infection. Infected Vero E6 and macrophage cultures confirmed eGFP expression degrees of ninety four% and thirteen%, respectively, indicating exceptional tiers of susceptibility in these target cell sorts (**Figure 7B and 7D**). In comparison, 293T cells were proof against NP/35-eGFP infection (**Figure 6E and 6F**). This information corresponds with the tropism of wt-ZEBOV (data no longer shown) (**Figure 8**).

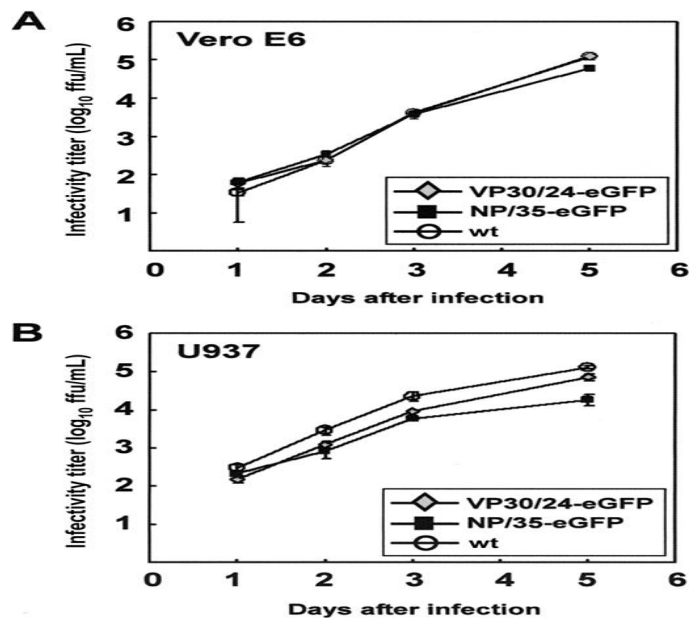


Figure 8. Identification of Ebola virus before and after infection.

Indiana vesiculovirus, formerly named *Vesicular stomatitis Indiana virus* or *Vesicular stomatitis virus* (VSV or VSV, and following indicated as VSV in this review) is a member of the *Rhabdoviridae* circle of relatives, genus *Vesiculovirus*. VSV is a pathogenic virus for livestock even as the human infection is an extraordinary occasion associated with an influenza-like infection. VSV may be handled in the laboratory with BSL-2 containment and, therefore, it has been used as a model to have a look at many elements of negative-strand RNA viral entry and replication. VSV meeting takes place on the plasma membrane and is followed through the budding of virions with a bullet shape of a hundred and eighty nm in step with seventy-five nm from the cellular surface. During budding, VSV acquires an envelope including a lipid bilayer derived from the plasma membrane and spike proteins consisting of trimers of the VSV glycoprotein G (VSV-G) (36). One of the extremely good residences of VSV is that its virions are not mainly selective with admire to the sort of membrane proteins that can be incorporated into the viral envelope. Such capability coupled to that of budding within the absence of the glycoprotein G brought about the development of recombinant viruses in which the VSV-G-encoding gene turned into deleted (rVSV-delta) and changed with a gene encoding for an unrelated envelope protein (replication ready rVSV-) (37). A distinct method is based on the replacement of the VSV-G-encoding gene with reporter genes, including genes.

In vitro cell tropism of *Zaire ebolavirus* (ZEBOV) expressing more desirable inexperienced fluorescent protein (eGFP). Vero E6 cells, primary human macrophages, and 293T cells had been infected with the NP/35-eGFP variation at an MOI of 0.05. On day 4 after contamination, the variety of eGFP-fine cells was quantified via float cytometry. The horizontal line in each panel denotes the eGFP-nice gate used for the evaluation; the no. Above the horizontal line is the percentage of eGFP-fine cells.

We subsequent in comparison the growth traits of NP/35-eGFP, VP30/24-eGFP, and wt-ZEBOV in Vero E6 and U937 cells. The growth kinetics for all three viruses in Vero E6 cells were indistinguishable (determine 3A), that's regular with outcomes said previously (4). In human monocytic U937 cells, which might be capable of inducing a robust antiviral state, we located a minor however statistically large attenuation with the eGFP-expressing viruses, especially NP/35-eGFP (1-log difference on day five after contamination), as compared with wt-ZEBOV (parent 3B). Thus, all of sudden, the localization of the eGFP-expression cassette inside the ZEBOV genome did now not have a dramatic impact on viral replication *In Vitro*.

DISCUSSION

In this have a look, we generated 2 eGFP-ZEBOV variations expressing GFP from a separate transcription unit located near both the three cease (between NP and VP35) or the 5 quits (among VP30 and VP24) of the genome (parent 1A). Similar to different non segmented negative-stranded (NNS) RNA viruses, *In Vitro* characterization established that the two eGFP-ZEBOV editions were solid and confirmed the equal *In Vitro* cell tropism as wtZEBOV (discern 2). Both viruses had been handiest slightly attenuated *In Vitro* but showed a more obvious attenuation *In vivo*, in comparison with wt-ZEBOV. NP/35-eGFP, which carries the extra transcription unit closer to the 3 give up of the genome, showed the least virulent phenotype, which can be defined by altered

transcription of the 6 downstream viral genes (VP35, VP40, GP, VP30, VP24, and L), rather than the altered transcription of only 2 genes (VP24 and L) in VP30/ 24-eGFP. A comparable genome function-dependent impact on gene transcription, from the advent of a foreign gene, has been suggested for different NNS RNA viruses and is steady with the idea of a transcriptional gradient alongside the single-stranded NNS RNA genome. In comparison to the slight attenuation of the eGFP-ZEBO.

CONCLUSION

This study provides the engineering, rescue, and *In Vitro* and *In vivo* characterization of two eGFP-expressing-ZEBOV-based viruses. We ought to exhibit the usefulness of these viruses for *In vivo* studies in a small animal model based on immune deficient STAT1/ mice, even though intense attenuation of virulence became located in nonhuman primates. Plans contain virus tracking of virulent eGFP-ZEBOV in large animals, which include rhesus macaques, and the improvement of eGFP-ZEBOV for use with immunocompetent mice and guinea pigs, for which adapted ZEBOV strains exist. The use of virus mutants expressing reporter genes will greatly facilitate future pathogenesis.

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