1 **Modelling Ebola Virus Dynamics: Implications**

2 **for Therapy**

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21 **Abstract (150/150)**

22 Ebola virus (EBOV) causes a severe, often fatal Ebola virus disease (EVD),
23 for which no approved antivirals exist. Recently, some promising anti-EBOV drugs,
24 which are experimentally potent in animal models, have been developed. However,
25 because the quantitative dynamics of EBOV replication in humans is uncertain, it
26 remains unclear how much antiviral suppression of viral replication affects EVD
27 outcome in patients. Here, we developed a novel mathematical model to
28 quantitatively analyse human viral load data obtained during the 2000/01 Uganda
29 EBOV outbreak and evaluated the effects of different antivirals. We found that
30 nucleoside analogue- and siRNA-based therapies are effective if a therapy with a
31 >50% inhibition rate is initiated within a few days post-symptom-onset. In contrast,
32 antibody-based therapy requires not only a higher inhibition rate but also an earlier
33 administration, especially for otherwise fatal cases. Our results demonstrate that an
34 appropriate choice of EBOV-specific drugs is required for effective EVD treatment.

35

36 **Keywords**

37 Ebola virus infection, Virus Dynamics, Mathematical model, Experimental treatment

38

39 Introduction

40 Ebola virus (EBOV), which is the causative agent of Ebola virus disease
41 (EVD) in humans(Chowell and Nishiura, 2015; Feldmann and Geisbert, 2011;
42 Geisbert et al., 2003; Kilgore et al., 2015; Towner et al., 2004), belongs to the family
43 *Filoviridae*, together with Marburg virus and Cueva virus. The non-segmented,
44 single-stranded, negative-sense RNA genome encodes at least seven structural
45 proteins, five of which (nucleoprotein [NP], viral protein [VP]24, VP30, VP35, and
46 RNA-dependent RNA polymerase [L]) constitute the nucleocapsid together with the
47 viral genomic RNA, and the remaining two proteins (surface glycoprotein [GP], and
48 matrix protein [VP40]) form the filamentous EBOV particle.

49 EVD mortality is high, sometimes approaching 90%(Chowell and Nishiura,
50 2015; Feldmann and Geisbert, 2011; Geisbert et al., 2003; Lyon et al., 2014). The
51 largest outbreak on record occurred in West Africa in 2014/15, lasting for more than
52 a year(Bah et al., 2015; Cohen, 2014; Kilgore et al., 2015; Kraft et al., 2015), and, as
53 of 14 February 2016, there have been a total of 28,639 reported cases, which
54 resulted in 11,316 deaths. In spite of the severity of EVD, current medical
55 management is only supportive (or palliative) care(Cohen, 2014; Meyers et al., 2015;
56 Qiu et al., 2014), and there are no licensed vaccines or EBOV-specific therapeutics.
57 For these reasons, EBOV is classified as a biosafety level-4 (BSL-4) agent and a
58 Center for Disease Control and Prevention (CDC) Category A potential bioterrorism
59 agent. Currently, several antivirals experimentally show efficacy against EBOV
60 infection in animal models(Cohen and Kupferschmidt, 2014; Kilgore et al., 2015;
61 Kupferschmidt and Cohen, 2015). For example, ZMapp, which is a combination of
62 three monoclonal antibodies against GP and blocks EBOV entry into host cells,
63 shows strong protection in non-human primates against lethal EBOV

64 challenge(Pettitt et al., 2013; Qiu et al., 2014). TKM-Ebola, which is a combination of
65 small interfering RNAs (siRNA) against EBOV L, VP24, and VP35 genes and
66 suppresses transcription and viral genome replication as well as the functions of
67 VP24 and VP35 as Type I interferon antagonists, protects non-human primates
68 against lethal EBOV infection(Geisbert et al., 2010). Favipiravir, which is a
69 pyrazinecarboxamide derivative and is thought to inhibit the RNA-dependent RNA
70 polymerase function of L, shows efficacy against EBOV infection in mice(Oestereich
71 et al., 2014). In the 2014/15 outbreak in West Africa, these experimental drugs were
72 exceptionally administrated for emergency use (called “experimental
73 treatment”)(Cohen and Kupferschmidt, 2014; Florescu et al., 2015; Kilgore et al.,
74 2015; Kondratowicz and Maury, 2012; Kraft et al., 2015; Kupferschmidt and Cohen,
75 2015; Lyon et al., 2014). However, due to the lack of quantitative studies on these
76 drug treatments in humans, it is still unknown how much they suppress EBOV
77 replication and how much these experimental treatments improve the clinical course
78 of EVD(Bah et al., 2015; Cohen and Kupferschmidt, 2014; Kraft et al., 2015;
79 Kupferschmidt and Cohen, 2015). Thus, the efficacy of these drugs in humans
80 remains to be determined.

81 In a previous study that was conducted during the second largest EBOV
82 outbreak in Uganda in 2000/01, Towner *et al.* examined the viral load (RNA copy
83 number per millilitre of serum) in patients throughout the course of EVD by using
84 quantitative reverse transcription-PCR (qRT-PCR) and found that the average peak
85 number of RNA copies from fatal cases is 3.4×10^9 , while that from nonfatal cases is
86 4.3×10^7 (Feldmann and Geisbert, 2011; Geisbert et al., 2003; Kondratowicz and
87 Maury, 2012; McElroy et al., 2014; Towner et al., 2004). Furthermore, a 2-log_{10}
88 difference in viral load significantly changes the survival rate of infected individuals,

89 suggesting that the RNA copy number in patient sera can be an important index of
90 EVD severity. Therefore, in this study, by applying a novel mathematical model
91 coupled with computer simulations to the viral load data from the EVD patients
92 reported by Towner *et al.* (18 survivors and 27 fatalities)(Towner et al., 2004), we
93 quantitatively investigated the relationship between EBOV replication and EVD
94 severity. We also quantitatively evaluated promising anti-EBOV drugs, such as
95 ZMapp(Cohen and Kupferschmidt, 2014; Kilgore et al., 2015; Kupferschmidt and
96 Cohen, 2015; Lyon et al., 2014; Qiu et al., 2014), favipiravir(Kupferschmidt and
97 Cohen, 2015; Oestereich et al., 2014; Smither et al., 2014), and TKM-Ebola(Cohen
98 and Kupferschmidt, 2014; Kilgore et al., 2015; Kondratowicz and Maury, 2012; Kraft
99 et al., 2015), by utilizing the mathematical model and estimated parameter values.
100 This unique mathematical approach provided us novel and quantitative insights into
101 EBOV pathogenesis, which promotes our understanding of optimal EVD treatment
102 and may improve drug treatment outcomes.

Results

Monocytes/macrophages and dendritic cells (DCs) are the primary targets of EBOV (Geisbert et al., 2003; Ignatyev, 1999; Ryabchikova et al., 1999) (defined as “susceptible-target” cells), followed by hepatocytes, splenocytes, and endotheliocytes (Feldmann and Geisbert, 2011; Geisbert et al., 2003; Takada, 2012; Zaki and Goldsmith, 1999) (defined as “potential-target” cells). EBOV spreads from the initial infection site to regional lymph nodes via monocytes/macrophages and DCs, then to the liver and spleen through the blood (Geisbert et al., 2003; Ignatyev, 1999; Ryabchikova et al., 1999). As the disease progresses, EBOV-infected monocytes/macrophages release proinflammatory cytokines, such as monocyte chemoattractant protein 1 (MCP-1) and macrophage inflammatory protein 1 (MIP-1) (Baize et al., 2002; Ebihara et al., 2011; Feldmann and Geisbert, 2011; Geisbert et al., 2003; McElroy et al., 2014). These mediators recruit additional monocytes/macrophages to infection sites, which increases the number of susceptible-target cells and thus facilitates virus replication (Escudero-Perez et al., 2014; Feldmann and Geisbert, 2011; Geisbert et al., 2003). Based on EBOV replication *in vivo*, we made a mathematical model of target cell–EBOV interactions during EBOV replication (**Fig. 1**). Our quantitative analysis using the viral load data reported from the 2000/01 outbreak in Uganda revealed that the immune inflammation rate, but not the death rate of infected cells, was significantly different between the fatal and nonfatal cases (**Table 1**). This demonstrates that the higher viral load in non-survivors is maintained by a massive recruitment of potential-target cells (**Fig. 2a,b**) because the potential-target cells in fatal cases frequently turned into susceptible-target cells (**Fig. 2c**), while those in nonfatal cases did so with a much lower frequency (**Fig. 2d**). These findings concur with the massive amount of

monocyte/macrophage infection observed in fatal cases(Baize et al., 2002; Feldmann and Geisbert, 2011).

Characterizing EBOV infection in fatal and nonfatal cases

The fitness (or speed) of EBOV replication in individuals is described by the Malthusian coefficient, M , defined for Eqs. (1–4)(Nowak and May, 2000) (**Methods**). Here, the Malthusian coefficient is $M = p\beta T_1(0)/c - \delta$ in Eqs. (1–4), which corresponds to $M = \gamma f_1(0) - \delta$ in Eqs. (8–10). To quantitatively assess the variability of kinetic parameters, we performed a Bayesian estimation for the whole dataset using Markov Chain Monte Carlo (MCMC) sampling (**SI Text 1**). Using estimated parameter distributions from the mean EBOV viral load (**SI Text 1**), we calculated the distribution of M for the fatal and nonfatal cases (**Fig. 3a**, red: fatal, blue: nonfatal). The mean values of M for the fatal and nonfatal cases are significantly different (**Fig. S1c**; $p = 0.009$ by the repeated bootstrap t -test) at 7.58 (95% confidence interval (CI): 3.48–13.8) and 1.83 (95% CI: 0.76–3.23), respectively (**Table 1**). This finding indicates that the viral load (i.e., $e^{(7.58-1.83)\times 1}$) in fatal cases, for example 1 day post-symptom-onset, is on average 412-fold higher than that in nonfatal cases, where the differences in viral loads between fatal and nonfatal cases increased over time. We also found that the mean value of ratios of M for each fatal case to each nonfatal case is 4.27 (**Fig. S1a**, 95% CI: 4.08–4.75). This 4.27-fold average difference in the value of M between fatal and nonfatal cases explains the earlier and more rapid viral load expansion (i.e., peak viral load) in fatal cases a few days post-symptom-onset(Feldmann and Geisbert, 2011; McElroy et al., 2014; Towner et al., 2004), confirming a strong relationship between EBOV replication and its disease severity.

The other important quantity characterizing virus dynamics is the basic reproduction number, R_0 , which is the average number of newly infected cells produced from any one infected cell, under conditions where the most of the target cells are uninfected (Iwami et al., 2012; Nowak and May, 2000; Perelson, 2002). In Eqs. (1–4), the basic reproductive number is defined as $R_0 = p\beta T_1(0)/\delta c$, which corresponds to $R_0 = \gamma f_1(0)/\delta$ in Eqs. (8–10). Similarly, we calculated the distributions of R_0 for the fatal and nonfatal cases (**Fig. 3b**). The mean value of R_0 for the fatal cases is 5.96 (95% CI: 2.28–11.1), which is significantly higher (**Fig. S1c**; $p = 0.02$ by the repeated bootstrap t -test) than that for the nonfatal cases (2.75; 95% CI: 1.33–5.08) (**Table 1**). The distribution of the ratios of R_0 for each fatal case to each nonfatal case is shown in **Fig. S1b** (the mean value and the 95% CI are 2.21 and 1.72–2.52, respectively). The 2.21-fold average difference in R_0 between fatal cases and nonfatal cases indicates that EBOV more efficiently causes systemic infection in fatal cases, demonstrating that the efficiency of EBOV replication is responsible for the EVD severity. This finding also implies that a 53.9% (i.e., $1 - 2.75/5.96$) reduction of the initial EBOV expansion in a fatal case could lead to an easing of symptoms and hopefully increase patient survival.

A critical inhibition rate to prevent EVD establishment

It is possible that the differences in R_0 between fatal and nonfatal cases affect the effectiveness of antiviral therapies and the clinical outcomes of patients. We next calculated the critical inhibition as $C^* = 1 - 1/R_0$ induced by antivirals to prevent primary virus infection (Iwami et al., 2012; Nowak and May, 2000). The mean values of C^* for the fatal and nonfatal cases are 80.5% (95% CI: 56.2–91.0%) and 58.5% (95% CI: 24.6–80.3%), respectively (**Table 1, Fig. 3c**), meaning that if 80.5% of the

initial virus expansion could somehow be blocked, we could prevent the establishment of EVD in otherwise fatal cases. In contrast, for nonfatal cases, a 58.5% block of the initial virus expansion could prevent EVD establishment. Thus, the effectiveness of a prophylaxis or treatment depends on the severity of disease and on the host immune inflammatory response in any given patient (Baize et al., 2002; Ebihara et al., 2011; Escudero-Perez et al., 2014; Feldmann and Geisbert, 2011; Geisbert et al., 2003; McElroy et al., 2014). The significant difference between C^* in fatal and nonfatal cases (**Fig. S1c**; $p = 0.04$ by the repeated bootstrap t -test) at least partially explains the variable outcomes of treatments (i.e., patient prognoses). These results are consistent with the fact that pre-exposure vaccination and post-exposure (but very early) vaccination or antiviral therapy were able to protect against the establishment of EVD or mitigate its symptoms in nonhuman primates (Feldmann et al., 2007; Marzi et al., 2015; Qiu et al., 2014). Our analysis also suggests that 100% protection by antivirals against EBOV infection is not necessary to prevent EVD (Butler et al., 2015).

Evaluation of anti-EBOV therapies

Our quantitative information provides novel insights into optimizing EVD prophylaxis or treatment. However, it remains poorly understood how a delay of treatment initiation, or how an incomplete blocking of virus replication affects the severity of EVD in patients (Cohen and Kupferschmidt, 2014; Feldmann and Geisbert, 2011; Kilgore et al., 2015; Kondratowicz and Maury, 2012). Based on our mathematical model (i.e., Eqs. (8–10)) and estimated parameter values (**Table 1**), we investigated the theoretical effects of candidate drugs possessing different antiviral mechanisms (**Fig. 4a**). To simulate possible variations in the viral load and

in the susceptible- and potential-target cell numbers under the above different types of anti-EBOV therapy (**SI Text 1**), sampled parameter sets were used to predict an expected outcome of each therapy (represented by the light regions in **Fig. 4b-g** and **Figs. S3–S5**). In other words, although no actual drug administration trials have been conducted yet, we were able to infer the degree of uncertainty in the efficacy of each drug treatment based on our MCMC computations.

(i) Antibody-based therapy

The most famous drug candidate, ZMapp, is an experimental cocktail of three EBOV GP-specific monoclonal antibodies (Cohen and Kupferschmidt, 2014; Kilgore et al., 2015; Kupferschmidt and Cohen, 2015; Lyon et al., 2014; Qiu et al., 2014) that inhibits *de novo* viral infection by blocking interactions between the GP of EBOV and the target cell (**Fig. 4a**). This immunotherapeutic has been successful in animal studies (Pettitt et al., 2013; Qiu et al., 2014) and was used on several patients in 2014 EVD outbreak in West Africa (Kilgore et al., 2015; Kupferschmidt and Cohen, 2015; Lyon et al., 2014). However, there has not been an exhaustive study to investigate the effectiveness of inhibiting *de novo* EBOV infection in humans.

The mean reductions of cumulative virus production, i.e., the area under the curve of viral load ($\text{AUC: } \int_0^{14} V(s)ds$), induced by this antibody-based therapy for fatal and nonfatal cases are summarized in **Fig. 5a**. Here, the darker green signifies a stronger antiviral effect against EBOV infection. The severity of the systemic infection is categorized by the mean residual fraction of potential-target cells (i.e., $f_2(14)$) in **Fig. 5b**. The darker red indicates a larger reduction in the number of potential-target cells (i.e., a more severe systemic infection). In a nonfatal case, there is little difference in the amount of potential-target cell recovery, regardless of

the timing of therapy initiation or the effectiveness of the treatment. This is because, in a nonfatal case, a large fraction of potential-target cells hardly turn into susceptible-target cells (i.e., $f_2(t)$ does not decrease), and, therefore, the level of potential-target cells remains relatively high, even without any treatments (**Fig. 2d**). Note that the expected values at day 0 post-symptom-onset ($t^* = 0$) correspond to the antiviral effect of pre-exposure vaccines (or post-exposure prophylactic use of vaccines or antiviral drugs), which is consistent with the estimated values of critical inhibition C^* (**Table 1** and **Fig. 3c**). In **Fig. S2a,b**, we show the standard deviation (S.D.) of these antiviral effects.

Interestingly, our results demonstrate that only a high inhibition rate of *de novo* infection (>90%) could reduce the AUC of viral load and recover the potential-target cells more than 50% in a fatal case, regardless of the therapy initiation timing (**Fig. 5a,b**). This suggests that antibody-based treatments, such as ZMapp(Lyon et al., 2014; Pettitt et al., 2013; Qiu et al., 2014), hyperimmune globulin(Jahriling et al., 1999; Kudoyarova-Zubavichene et al., 1999), and convalescent serum(Cohen and Kupferschmidt, 2014; Kilgore et al., 2015; Kupferschmidt and Cohen, 2015) are not likely to be effective for fatal cases unless the intervention blocks *de novo* EBOV infection quite strongly and is initiated before or very soon after the onset of symptoms. In contrast, a relatively low inhibition rate (around 60%) effectively reduces the AUC in nonfatal cases, depending on the timing of therapy administration (**Fig. 5a**, right). For example, in **Fig. 4b,c**, we assumed that antibody-based therapy was started at 4 day post-symptom-onset with 80% inhibition rate (i.e., $\varepsilon_{Ab} = 0.8$ and $t^* = 4$). **Fig. 4b** and **c** show the expected outcomes of the abilities of antiviral therapy for fatal and nonfatal cases, respectively. In fatal case (**Fig. 4b**), the therapy could not change the dynamics of the viral loads (left) and of the potential

(right) target cells at all, but in nonfatal case (**Fig. 4c**), the viral load (left) is dramatically suppressed after the initiation of therapy. Thus, these incomplete or delayed therapies could be still effective for nonfatal cases in terms of reducing the AUC of viral load, which decreases the probability of secondary EVD transmission(Yamin et al., 2015).

(ii) siRNA-based therapy

Therapeutic small interfering RNA (siRNA) effectively suppress viral production from EBOV-infected cells via degradation of viral messenger RNAs (mRNAs) (**Fig. 4a**). One promising anti-EBOV siRNA is TKM-Ebola (TKM-100802), which targets sequences of viral mRNA corresponding to VP24, VP35, and polymerase L(Cohen and Kupferschmidt, 2014; Kilgore et al., 2015; Kondratowicz and Maury, 2012; Kraft et al., 2015). TKM-Ebola has been successful in animal studies(Geisbert et al., 2010) and is also available for emergency use in treating EVD(Kilgore et al., 2015; Kraft et al., 2015; Kupferschmidt and Cohen, 2015). Another type of related small molecule therapeutic is an antisense phosphorodiamidate morpholino oligomer (PMO); these also target polymerase L, VP24, and VP35(Cohen and Kupferschmidt, 2014; Kilgore et al., 2015; Kondratowicz and Maury, 2012; Warren et al., 2010). PMO-based therapy has shown partial protection in nonhuman primates(Warren et al., 2010). Thus, siRNA therapeutics also may provide effective protection against EBOV.

The mean antiviral effects of siRNA-based therapy on fatal and nonfatal cases are summarized in **Fig. 5c,d** (see **Fig. S2c,d** for the corresponding S.D.). Interestingly, our results suggest that, even if therapy is not initiated very soon after the onset of symptoms (even it is delayed more than two days), the AUC of viral load

is substantially reduced in fatal cases if the inhibition rate is around 80 % (**Fig. 5c**). In nonfatal cases, the AUC of viral load is effectively reduced compared with that following antibody-based therapy. However, siRNA-based therapy is not able to protect against the massive reduction in the amount of potential-target cells in fatal cases unless the inhibition rate is quite high (>90%), which is similar to the outcome of antibody-based therapy (**Fig. 5b,d**). As an example, in **Fig. 4d,e**, we assumed that siRNA-based therapy was started at 4 day post-symptom-onset with 80% inhibition rate, which is a relatively delayed therapy (i.e., $\varepsilon_{si} = 0.8$ and $t^* = 4$). In both fatal (**Fig. 4d**) and nonfatal (**Fig. 4e**) cases, the viral load (left) is still quickly suppressed after the initiation of therapy. However, the therapy fails to mitigate the massive reduction of the potential target cells in fatal case (right panel in **Fig. 4d**). Thus, siRNA therapeutics, such as TKM-Ebola (Cohen and Kupferschmidt, 2014; Kilgore et al., 2015; Kondratowicz and Maury, 2012; Kraft et al., 2015) and PMOs (Cohen and Kupferschmidt, 2014; Kilgore et al., 2015; Kondratowicz and Maury, 2012; Warren et al., 2010), may also be a good candidate for preventing person-to-person transmission by reducing the viral load in patients.

(iii) Nucleoside-based therapy

Favipiravir (T-705), which was originally developed as an influenza drug and targets the RNA polymerase enzyme (**Fig. 4a**), has undergone animal trials to evaluate its efficacy against EBOV (Oestereich et al., 2014; Smither et al., 2014) and is part of an ongoing evaluation in Guinea (Kupferschmidt and Cohen, 2015). Another important anti-EBOV candidate is brincidofovir, which targets the DNA polymerase enzyme but surprisingly also shows antiviral activity against EBOV (Cohen and Kupferschmidt, 2014; Florescu et al., 2015; Kilgore et al., 2015; Kupferschmidt and

Cohen, 2015). Brincidofovir is available for compassionate use through an emergency investigational new drug application(Florescu et al., 2015). Novel viral nucleoside analogues, such as JK-05(Kilgore et al., 2015) and BCX4430(Wong et al., 2014), are also under development.

The mean antiviral effects of nucleoside analogue-based therapy for fatal and nonfatal cases are summarized in **Fig. 5e,f** (see **Fig. S2e,f** for the corresponding S.D.). Our results show that, for both fatal and nonfatal cases, nucleoside analogue-based therapy is as effective as siRNA-based therapy at exerting an antiviral effect by reducing the AUC of viral load. Furthermore, with appropriate therapy initiation, the potential-target cells are largely recovered in the fatal cases (**Fig. 5f**), suggesting that nucleoside-based therapy can not only suppress the viral load but can also reduce systemic infection and EVD severity. In **Fig. 4f,g**, we assumed that nucleoside analogue-based therapy was started at 4 day post-symptom-onset with 80% inhibition rate (i.e., $\varepsilon_{NA} = 0.8$ and $t^* = 4$). The potent antiviral effects are observed on the dynamics of the viral load and the potential-target cells in both fatal and nonfatal cases. Therefore, the nucleoside analogues, such as favipiravir(Kupferschmidt and Cohen, 2015; Oestereich et al., 2014; Smither et al., 2014), brincidofovir(Cohen and Kupferschmidt, 2014; Florescu et al., 2015; Kilgore et al., 2015; Kupferschmidt and Cohen, 2015), JK-05(Kilgore et al., 2015), and BCX4430(Wong et al., 2014), might provide favourable options for post-exposure prophylaxis or treatment against EBOV, even for otherwise fatal cases, unless their inhibition rate is too low and their initiation timing is too late.

Discussion

Using our novel mathematical model, Eqs. (1–4), we quantitatively analysed the mean number of EBOV RNA copies/ml in sera from the survivors and fatalities during the Uganda outbreak (Towner et al., 2004). Our Bayesian estimation successfully extracted novel model parameter values from the datasets (**Table 1**) that are consistent with current knowledge on EVD clinical evidence and EBOV pathogenesis. They also reproduce the time-course progression of EBOV load in patients (**Fig. 2a,b**). Furthermore, our simulation based on this model suggests that the higher viral load in non-survivors is maintained by a massive infection in the potential-target cells (**Fig. 2c,d**). Massive recruitment of the target cells such as monocytes and macrophages are induced by hypersecretion of numerous proinflammatory cytokines, chemokines, and growth factors including MIP-1 and MCP-1, which is characteristic of non-survivors (Wauquier et al., 2010). Increased number of the potential target cells observed in non-survivor would theoretically cause increased virus infection and burden. Actually, using the estimated parameter distributions, we found a 4.27-fold difference in the Malthusian coefficient, which indicates a fitness (or speed) of virus infection, and a 2.21-fold difference in the basic reproduction number, which indicates the potential of virus infection, between fatal and nonfatal cases (**Fig. S1a,b**). Based on previous quantitative information that 10^8 RNA copies/ml is an approximate threshold for fatal outcomes (Feldmann and Geisbert, 2011; Geisbert et al., 2003; Kondratowicz and Maury, 2012; McElroy et al., 2014; Towner et al., 2004), we also provide novel insights into the establishment of an effective EVD treatment. For example, we calculated the critical inhibition rate, which revealed that blocking at least 54.8% of the initial virus expansion is required to decrease the mortality of the non-survivors (**Fig. 3c**). Conversely, we found that

blocking 80.5% is enough to prevent EVD for even otherwise fatal cases. These types of information help us determine how to improve the effectiveness of EBOV vaccination and prophylaxis.

Furthermore, the predicted dynamics information, based on our mathematical model and estimated parameter values, explains how anti-EBOV drugs transiently suppress the viral load and induce a massive reduction in the number of potential-target cells (i.e., progression of viral infections) in fatal and nonfatal cases. It is important for us to understand the useful window of unlicensed therapeutics, especially for experimental treatment, because some of these unapproved anti-EBOV drugs are available for use during EVD outbreaks(Cohen and Kupferschmidt, 2014; Florescu et al., 2015; Kilgore et al., 2015; Kondratowicz and Maury, 2012; Kraft et al., 2015; Kupferschmidt and Cohen, 2015; Lyon et al., 2014). Our exhaustive simulations found that different drugs with different mechanisms of action have different curative effect depending on whether the case would otherwise be fatal or nonfatal (**Fig. 5**). One of our important findings is that nucleoside analogue-based therapy is effective for both otherwise fatal and nonfatal cases, unless the inhibition rate is too low and/or the initiation timing is too late (**Fig. 5e,f**). A recent study(Madelain et al., 2015) used a mathematical model to analyse time-course viral load data from EBOV-infected mice treated with or without favipiravir and found that the antiviral effectiveness of favipiravir could reach a maximum of 99.6%. Although mouse models of EBOV infection do not completely reproduce EVD progression in humans(Rasmussen et al., 2014), such a high antiviral rate is expected to efficiently reduce the viral load; our analyses suggesting nucleoside analogue-based therapy protects patients by greatly reducing the number of target cells is consistent with their conclusion(Madelain et al., 2015).

Notably, effective treatment of EBOV infection requires an appropriate choice of class-specific drugs; otherwise, the antivirals do not alter the clinical outcome of EVD and are wasted. Identifying non-survivor-specific clinical symptoms is needed to design optimal treatments and to effectively use the limited amount of anti-EBOV drugs (i.e., saving a dose)(Cohen and Kupferschmidt, 2014; Kraft et al., 2015; Lyon et al., 2014). Future studies could use our mathematical model to analyse the effect of hypothetical drugs, such as immunosuppressive drugs, on EBOV infection(Daubeuf et al., 2007; Escudero-Perez et al., 2014). Additionally, effective combinations of anti-EBOV drugs and vaccines might enhance the antiviral effects and/or reduce the individual drug dose and/or the side effects, all of which are highly desirable. In **Fig. S6**, we simulated the antiviral effects of three combination therapies (i.e., **(a)** antibody- and siRNA-based therapy, **(b)** antibody- and nucleoside analogue-based therapy, **(c)** nucleoside analogue- and siRNA-based therapy) under the assumption that all therapies were started at 4 day post-symptom-onset with 80% inhibition rate (same conditions in **Fig. 4b-g**). As summarized in **SI Table 1**, the combination of nucleoside analogue- and siRNA-based therapy shows the most potent antiviral effect on the reduction of AUC of viral load in fatal cases (**Fig. S6c**). However, we found that adding antibody-based therapy does not enhance the antiviral effect of nucleoside analogue- or siRNA-based mono therapy in these conditions (**Fig. S6a,b**). In contrast, for nonfatal cases, mono therapies in themselves show sufficient strong antiviral effects (see **SI Table 1**). Although we need more knowledge on the antiviral effects of these candidate drugs (i.e., the values of ε_{Ab} , ε_{si} and ε_{NA}), our theoretical approach could complement the difficult experimental investigations of EBOV infection in BSL-4 laboratories and help establish a basis for EVD treatment.

399 Early diagnosis of EVD is challenging due to its non-specific symptoms, such
400 as high fever, headache, vomiting, and diarrhoea(Feldmann and Geisbert, 2011;
401 Fhogartaigh and Aarons, 2015; Kilgore et al., 2015; Meyers et al., 2015). Therefore,
402 the careful follow-up of “highly possible EVD cases” and the identification of these
403 cases are important, and treatment should be initiated as soon after the diagnosis as
404 possible. Our analyses showed that both siRNA-based and nucleoside analogue-
405 based therapies effectively reduce the AUC of EBOV load, even if the therapy is
406 initiated after a few days post-symptom-onset (**Figs. S4 and S5**). Because person-
407 to-person EVD transmission occurs through mucous membrane or percutaneous
408 contact with body fluids(Chowell and Nishiura, 2015; Feldmann and Geisbert, 2011;
409 Fhogartaigh and Aarons, 2015; Kilgore et al., 2015; Meyers et al., 2015; Yamin et al.,
410 2015) and the transmissibility is dependent on the patients’ viral load(Feldmann and
411 Geisbert, 2011; Fhogartaigh and Aarons, 2015; Yamin et al., 2015), treatments
412 reducing the viral load are important for the prevention of secondary transmission
413 and outbreak control. Using the expected time-course progression of EBOV load,
414 which is affected by treatments (**Figs. S3–S5**), we are able to calculate the
415 probability of secondary cases from a patient(Yamin et al., 2015). Evaluating the risk
416 reduction of secondary transmission to healthcare workers of a treatment is an
417 important and interesting proposition, to be investigated in future work. Thus,
418 effective interventions block EBOV spread both within a host and between hosts. To
419 our knowledge, no previous studies have quantified the EBOV dynamics in humans,
420 and a data analysis approach based on our mathematical model will improve
421 understanding of EBOV infection and aid in the establishment of optimal treatments
422 for EVD.

Conclusion

We developed a novel mathematical model and used it to quantitatively analyse the viral load data from 18 survivors and 27 fatalities of the EBOV outbreak that occurred in Uganda in 2000/01. We also used our model to evaluate the effects of three different classes of antivirals which experimentally show efficacy against EBOV infection in animal models: antibody-, siRNA-, or nucleoside analogue-based therapies. We found that if a nucleoside analogue- or siRNA-based therapy with a >50% inhibition rate is initiated within a few days following the onset of symptoms then it is likely to be effective. However, for antibody-based therapies, both a higher inhibition rate and an earlier administration are required.

Methods

Study data

The data examined here came from the EVD outbreak in Uganda from August 2000 to January 2001 and were previously reported by Towner *et al.* (Towner et al., 2004). Mean numbers of post-symptom-onset viral RNA copies/ml in serum from 18 survivors (nonfatal cases) and 27 fatalities (fatal cases) were quantitatively analysed by a mathematical model in this study. Note that, at each time point, the number of data-points (i.e., N : the number of cases) was different ($1 < N < 11$ for fatal cases and $1 < N < 8$ for nonfatal cases) (Towner et al., 2004).

A mathematical model for EBOV infection *in vivo*

To describe EBOV dissemination from initial susceptible-target cells (e.g., monocytes/macrophages and DCs) to existing and recruited potential-target cells (e.g., recruited monocytes/macrophages, hepatocytes, splenocytes, and endotheliocytes), we wrote the following mathematical model:

$$\frac{dT_1(t)}{dt} = aT_2(t)I(t) - \beta T_1(t)V(t), \quad (1)$$

$$\frac{dT_2(t)}{dt} = -aT_2(t)I(t), \quad (2)$$

$$\frac{dI(t)}{dt} = \beta T_1(t)V(t) - \delta I(t), \quad (3)$$

$$\frac{dV(t)}{dt} = pI(t) - cV(t), \quad (4)$$

where $T_1(t)$, $T_2(t)$, and $I(t)$ are the number of susceptible-target, potential-target, and infected (virus-producing) cells, respectively. The variable $V(t)$ is EBOV RNA copies/ml in patient sera. The parameters β , δ , p , and c represent the infection rate, infected cell death rate, virus production rate, and virion clearance rate, respectively. Because EBOV-infected cells release proinflammatory cytokines (Baize et al., 2002;

Ebihara et al., 2011; Escudero-Perez et al., 2014; Feldmann and Geisbert, 2011; Geisbert et al., 2003; McElroy et al., 2014), we assumed that the potential-target cells are recruited at a constant rate, a , that is proportional to the number of infected cells, and that the recruited cells subsequently become susceptible-target cells for EBOV (**Fig. 1**). In the case of EBOV acute infection, the loss of target cells by physiological turnover could be ignored, considering the long life-span of the target cells (Davenport et al., 2006; Petravic et al., 2008; Wilson et al., 2007). Eqs. (1–4) are consistent with the idea of systemic inflammation contributing to EBOV pathogenesis (Baize et al., 2002; Ebihara et al., 2011; Escudero-Perez et al., 2014; Feldmann and Geisbert, 2011; Geisbert et al., 2003; McElroy et al., 2014), suggesting that the mathematical model developed here is theoretically reasonable.

To accurately extract information about EBOV replication dynamics from the mean number of EBOV RNA copies/ml in sera, we further simplified Eqs. (1–4) by using a quasi-steady state (QSS) approximation for the viral particles (Ikeda et al., 2014). Since the clearance rate of viral particles, c , is typically much larger than the death rate, δ , of the infected cells *in vivo* (Madelain et al., 2015; Nowak and May, 2000; Perelson, 2002; Stafford et al., 2000), we made a QSS assumption, $dV(t)/dt = 0$, and replaced Eq.(4) with $V(t) = pI(t)/c$. Because EBOV RNA copies, $V(t)$, rather than the number of infected cells, $I(t)$, were fitted, $I(t) = cV(t)/p$ was substituted into Eqs. (1–3) to obtain

$$\frac{dT_1(t)}{dt} = \frac{ca}{p}T_2(t)V(t) - \beta T_1(t)V(t), \quad (5)$$

$$\frac{dT_2(t)}{dt} = -\frac{ca}{p}T_2(t)V(t), \quad (6)$$

$$\frac{dV(t)}{dt} = \frac{p\beta}{c}T_1(t)V(t) - \delta V(t). \quad (7)$$

Furthermore, we defined the fractions of the susceptible- and potential-target cells as the total pool of target cells ($T_1(0) + T_2(0)$) as $f_1(t) = T_1(t)/(T_1(0) + T_2(0))$ and $f_2(t) = T_2(t)/(T_1(0) + T_2(0))$, respectively). Accordingly, we derived the following simple model, which we employed for data analysis in this study:

$$\frac{df_1(t)}{dt} = \alpha f_2(t)V(t) - \beta f_1(t)V(t), \quad (8)$$

$$\frac{df_2(t)}{dt} = -\alpha f_2(t)V(t), \quad (9)$$

$$\frac{dV(t)}{dt} = \gamma f_1(t)V(t) - \delta V(t), \quad (10)$$

where the composite parameters of $\alpha = ca/p$ and $r = p\beta(T_1(0) + T_2(0))/c$ are defined as the immune inflammation rate and the maximum viral replication rate in EBOV infection, respectively. Both fractions $f_1(t)$ and $f_2(t)$ are less than 1, and we used the value of $f_2(t)$ as an index of severity of EVD; small $f_2(t)$ due to inflammation represents a systemic progression of EBOV infection.

Quantification of EBOV dynamics in fatal and nonfatal cases

Previous reports (Baize et al., 2002; Ebiyara et al., 2011; Feldmann and Geisbert, 2011; McElroy et al., 2014) have suggested that the host inflammatory response is strongly associated with EBOV pathogenesis (e.g., death rate and severity). Therefore, when fitting Eqs. (8–10) to our datasets, we assumed that the host-immunological parameters α and δ are different between fatal and nonfatal cases, but that the other virological parameters, β and γ , are similar. The joint posterior predictive distributions of the model parameters (the posterior distribution) were inferred by fitting Eqs. (8–10) to the serum viral load data. The viral load dynamics for fatal and nonfatal cases that were produced with the best fit parameter values are shown in **Fig. 2a** and **2b**, respectively.

A model prediction is derived from the computation of Eqs. (8–10) with a sampled parameter set from the posterior distribution. Note that a model prediction represents an expected outcome (e.g., time-course progression of viral load and susceptible- and potential-target cell numbers) at each day post-symptom-onset. To account for the uncertainty of model predictions in response to possible parameter changes, a global sensitivity analysis was performed for viral load and numbers of susceptible- and potential-target cells. After sampling 100 parameter sets, we calculated the possible variation, which is defined as a two-sided 95% CI of model prediction for viral load and susceptible- and potential-target cell numbers. The coloured regions correspond to the 95% posterior predictive intervals, the solid lines indicate the best-fit solution (mean) for Eqs. (8–10), and the red and blue dots show the mean viral load data. Few data points deviate from the coloured region, suggesting that the possible variation of viral load is narrow for both fatal and nonfatal cases. Thus, our mathematical model, Eqs. (8–10), accurately reproduces the mean numbers of EBOV RNA copies/ml for both fatal and nonfatal cases. A summary of the kinetic parameters estimated by Bayesian inference is shown in **Table 1**.

We next calculated the dynamics for the susceptible- and potential-target cell fractions ($f_1(t)$ and $f_2(t)$) for fatal and nonfatal cases (**Fig. 2c** and **2d**, respectively) using the estimated parameter values (**Table 1**). The estimated initial values of the fractions ($f_1(0) = 0.83\%$ and $f_2(0) = 99.2\%$ for fatal cases and $f_1(0) = 0.29\%$ and $f_2(0) = 99.7\%$ for nonfatal cases) are consistent with a fact that the amount of susceptible-target cells, such as monocytes/macrophages and DCs, is much smaller than that of potential-target cells, such as hepatocytes, splenocytes, and endotheliocytes (Haller Hasskamp et al., 2005; Seidler et al., 2010). Furthermore, in

fatal cases, the potential-target cells are expected to turn into susceptible-target cells (Fig. 2c). This also occurs in nonfatal case, but with much lower frequency (Fig. 2d), implying that the higher viral load in non-survivors is maintained by additional potential-target cell recruitment. Therefore, the viral load is a good predictor of clinical outcomes in humans and nonhuman primates (Feldmann and Geisbert, 2011; Feldmann et al., 2007; Geisbert et al., 2003; McElroy et al., 2014; Towner et al., 2004). Our prediction based on Eqs. (8–10) concurs with the finding of massive monocyte/macrophage activation in fatal cases (Baize et al., 2002; Feldmann and Geisbert, 2011). Although it is difficult to actually measure these target cell fractions in patients, our results indicate that the dynamics of $f_2(t)$, which is obtained via the above EBOV load analysis by Eqs. (8–10), could be an index for EVD prognosis prediction.

Modelling anti-EBOV therapies based on their mechanism of action

By utilizing our novel mathematical model and the estimated parameter values, we exhaustively investigated the antiviral effects of unlicensed but promising drugs with different mechanisms of action, depending on inhibition rates and therapy initiation timings: (i) antibody-based therapy, such as ZMapp (Cohen and Kupferschmidt, 2014; Kilgore et al., 2015; Kupferschmidt and Cohen, 2015; Lyon et al., 2014; Qiu et al., 2014), hyperimmune globulin (Jahrling et al., 1999; Kudoyarova-Zubavichene et al., 1999), convalescent serum (Cohen and Kupferschmidt, 2014; Kilgore et al., 2015; Kupferschmidt and Cohen, 2015), and vaccinations (Feldmann et al., 2007; Marzi et al., 2015); (ii) nucleoside analogue-based therapy, such as favipiravir (Kupferschmidt and Cohen, 2015; Oestereich et al., 2014; Smither et al., 2014), brincidofovir (Cohen and Kupferschmidt, 2014; Florescu et al., 2015; Kilgore et

al., 2015; Kupferschmidt and Cohen, 2015), JK-05(Kilgore et al., 2015), and BCX4430(Wong et al., 2014); and (iii) siRNA-based therapy, such as TKM-Ebola(Cohen and Kupferschmidt, 2014; Kilgore et al., 2015; Kondratowicz and Maury, 2012; Kraft et al., 2015) and antisense PMOs(Cohen and Kupferschmidt, 2014; Kilgore et al., 2015; Kondratowicz and Maury, 2012; Warren et al., 2010).

For the antibody-based therapy, we assumed an inhibition rate of *de novo* infection by antibody responses of $0 < \varepsilon_{Ab} \leq 1$ and a therapy initiated at t^* days post-symptom-onset. The antiviral effect of an antibody-based therapy initiated at t^* days post-symptom-onset was then considered as:

$$\frac{dT_1(t)}{dt} = aT_2(t)I(t) - (1 - \varepsilon_{Ab} \times H(t))\beta T_1(t)V(t), \quad (11)$$

$$\frac{dI(t)}{dt} = (1 - \varepsilon_{Ab} \times H(t))\beta T_1(t)V(t) - \delta I(t), \quad (12)$$

where $H(t)$ is a Heaviside step function defined as $H(t) = 0$ if $t < t^*$: otherwise $H(t) = 1$. We derived a simplified model from Eqs. (2, 4, 11, and 12) (not shown) and evaluated an expected antiviral effect of the therapy under the different inhibition rates (ε_{Ab}) and initiation timings (t^*) using our MCMC estimated parameter values. The dynamics of how viral load is affected by antibody-based therapy (left, $\varepsilon_{Ab} = 1$ and $t^* = 1$; middle, $\varepsilon_{Ab} = 0.9$ and $t^* = 1$; right, $\varepsilon_{Ab} = 0.9$ and $t^* = 3$) were calculated for fatal and nonfatal cases (**Fig. S3a,d**, respectively: cf. **Fig. 4b,c**). We also calculated the corresponding behaviour for the fractions of susceptible- and potential-target cells (**Figs. S3b,e** and **S3c,f**, respectively). Here, the coloured regions correspond to theoretically predicted possible variation (95% posterior predictive intervals), and the blue and black solid lines indicate the mean solution of mathematical models with and without anti-EBOV therapy, respectively.

Alternately, for siRNA-based therapy, we assumed an inhibition rate of virus production of $0 < \varepsilon_{\text{si}} \leq 1$. The antiviral effect by siRNA-based therapy is described by:

$$\frac{dV(t)}{dt} = (1 - \varepsilon_{\text{si}} \times H(t))pI(t) - cV(t). \quad (13)$$

The dynamics of how the viral load and the fractions of susceptible- and potential-target cells are affected by this therapy (left, $\varepsilon_{\text{si}} = 1$ and $t^* = 1$; middle, $\varepsilon_{\text{si}} = 0.9$ and $t^* = 1$; right, $\varepsilon_{\text{si}} = 0.9$ and $t^* = 3$) were calculated for both fatal and nonfatal cases by Eqs. (1, 2, 3, and 13) (**Fig. S4**: cf. **Fig. 4d,e**).

For nucleoside analogue-based therapy, we assumed an inhibition rate of viral transcription and replication of $0 < \varepsilon_{\text{NA}} \leq 1$. The antiviral effect of nucleoside analogue-based therapy was subsequently calculated as follows:

$$\frac{dI(t)}{dt} = (1 - \varepsilon_{\text{NA}} \times H(t))\beta T_1(t)V(t) - \delta I(t). \quad (14)$$

Similarly, we evaluated an expected antiviral effect of this therapy using Eqs. (1, 2, 4, and 14) (**Fig. S5**: cf. **Fig. 4f,g**).

For combination therapies, we assumed two of inhibition rates among ε_{Ab} , ε_{si} , and ε_{NA} are positive but less than 1. The antiviral effect of the combination therapies is described by:

$$\frac{dT_1(t)}{dt} = aT_2(t)I(t) - (1 - \varepsilon_{\text{Ab}} \times H(t))\beta T_1(t)V(t), \quad (15)$$

$$\frac{dI(t)}{dt} = (1 - \varepsilon_{\text{Ab}} \times H(t))(1 - \varepsilon_{\text{NA}} \times H(t))\beta T_1(t)V(t) - \delta I(t), \quad (16)$$

$$\frac{dV(t)}{dt} = (1 - \varepsilon_{\text{si}} \times H(t))pI(t) - cV(t), \quad (17)$$

and, we similarly evaluated an expected antiviral effect of three different combination therapies (i.e., antibody- and siRNA-based therapy ($0 < \varepsilon_{\text{Ab}}, \varepsilon_{\text{si}} \leq 1$), antibody- and nucleoside analogue-based therapy ($0 < \varepsilon_{\text{Ab}}, \varepsilon_{\text{NA}} \leq 1$), nucleoside analogue- and siRNA-based therapy ($0 < \varepsilon_{\text{NA}}, \varepsilon_{\text{si}} \leq 1$)) using Eqs. (2, 15, 16 and 17) (**Fig. S6**).

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Authors' contributions

Conceived and designed the study: AM SN KS TN SI. Analysed the data: AM SN SI. Wrote the paper: AM SN KS TN SI. All authors read and approved the final manuscript.

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Figure legends

Figure 1 | Mathematical model of EBOV replication: $T_1(t)$, $T_2(t)$, $I(t)$, and $V(t)$

are the number of susceptible- and potential-target cells, virus-producing cells, and amount of EBOV RNA copies/ml in sera in patients, respectively. The parameters a , β , δ , p , and c represent the recruitment/activation rate, infection rate, death rate of infected cells, virus production rate, and clearance rate of virions, respectively.

Figure 2 | Variability of EBOV dynamics in fatal and nonfatal cases: (a–b)

The predicted variability of the EBOV load dynamics for fatal **(a)** and nonfatal **(b)** cases are shown based on a Bayesian estimation for the previously reported (Towner et al., 2004) arithmetic mean values of the viral load (RNA copies/ml serum) using MCMC sampling. The coloured solid lines give the best-fit solution for Eqs. (8–10), and regions correspond to the 95% posterior predictive intervals. The dots show the mean values of EBOV loads, and the horizontal dotted line marks the limit of detection for viral RNA (6,200 RNA copies per ml of serum). **(c–d)** The susceptible- (upper) and potential- (lower) target cell dynamics predicted by Eqs. (8–10) are shown for fatal **(c)** and nonfatal cases **(d)**.

Figure 3 | Distribution of the Malthusian coefficients, the basic reproduction numbers, and the critical inhibition rates for fatal and nonfatal cases:

The distributions of the Malthusian coefficients, $M = p\beta T_1(0)/c - \delta = \gamma f_1(0) - \delta$, the basic reproduction numbers, $R_0 = p\beta T_1(0)/\delta c = \gamma f_1(0)/\delta$, and the critical inhibition rate of EBOV infection, $C^* = 1 - 1/R_0$, that were calculated from the estimated parameter distributions are shown in **(a)**, **(b)**, and **(c)**, respectively, for fatal (red) and

nonfatal (blue) cases. All indexes of M , R_0 , and C^* for fatal cases are significantly different from those for nonfatal cases, as assessed by the repeated bootstrap t -test.

Figure 4 | Anti-EBOV drug target and simulating antiviral effects of current

drug candidates: (a) A summary of EBOV replication in cells is depicted: EBOV enters target cells via interactions between GP and its receptor molecules. After fusion of the EBOV envelope with host endosomal membranes, the EBOV genome in the form of nucleocapsid is released into the cytoplasm, where transcription and replication of the EBOV genome subsequently occurs. Newly-synthesized EBOV genome and proteins are then assembled at the plasma membrane and form progeny virus particles, which bud from the plasma membrane. Anti-EBOV drugs that target each step of viral life-cycle have been developed or are currently being investigated for use in EVD therapy. **(b–g)** Using our estimated parameters, the expected outcomes of antiviral therapies against EBOV infection were simulated. We assumed that the therapy was started at 4 day post-symptom-onset with 80% inhibition rate, indicated by the vertical dotted lines. The expected dynamics, for fatal **(b,d,f)** and nonfatal **(c,e,g)** cases, of the viral loads (left) and of the potential (right) target cells under the antibody-based (e.g., ZMapp) **(b,c)**, siRNA-based (e.g., TKMEbola) **(d,e)**, and nucleoside analogue-based (e.g., T-705) **(f,g)** therapies are shown. The coloured solid lines and regions correspond to the mean predicted value and the 95% posterior predictive intervals affected by the therapy, respectively. The thin black lines show the original best fitted solutions of Eqs. (8–10) from **Fig. 2**. The dots show the mean values of EBOV loads, and the horizontal line marks the limit of detection for viral RNA.

Figure 5 | Exhaustive simulation for antiviral effects of current drug candidates: Using our estimated parameters, the expected outcomes of antiviral therapies against EBOV infection were simulated. **(a–f)** We calculated the effect of the antibody-based **(a–b)**, siRNA-based **(c–d)**, and nucleoside analogue-based **(e–f)** therapies on the mean reduction of AUC of viral load **(a, c, and e)** and on the mean residual fraction of potential-target cells **(b, d, and f)** for the fatal (left) and nonfatal (right) cases. The darker green signifies a stronger antiviral effect against EBOV infection, and the darker red indicates a larger reduction in the number of potential-target cells. The expected values at 0 day post-symptom-onset indicate the antiviral effect of pre-exposure vaccines (or post-exposure prophylactic use of antivirals) in **(a)** and **(b)**.

Table 1. Estimated parameters in the mathematical model for fatal and nonfatal cases

Parameter Name	Symbol	Unit	Fatal case	Nonfatal case
Maximum rate constant for viral replication	γ	$10^3 \times \text{day}^{-1}$	2.22 (0.33 – 10.51) ^a	
Rate constant for virus infection	β	$10^{-7} \times (\text{RNA copies/ml})^{-1} \cdot \text{day}^{-1}$	7.32 (1.95 – 19.60)	
Rate constant for immune inflammation	α	$10^{-9} \times (\text{RNA copies/ml})^{-1} \cdot \text{day}^{-1}$	1.85 (0.43 – 5.03)	0.25 (0.05 – 1.18)
Death rate of infected cells	δ	day^{-1}	1.81 (0.85 – 4.77)	1.32 (0.58 – 3.63)
Initial fraction of susceptible target cells	$f_1(0)$	%	0.83 (0.12 – 2.41)	0.29 (0.03 – 0.90)
Initial fraction of potential-target cells	$f_2(0)$	%	99.17 (97.59 – 99.88)	99.71 (99.10 – 99.97)
Initial value of virions	$V(0)$	$10^4 \times \text{RNA copies/ml}$	5.43 (0.59 – 21.32)	9.43 (1.21 – 35.00)
Malthusian coefficient	M	—	7.56 (3.48 – 13.81)	1.83 (0.80 – 3.23)
Basic reproduction number	R_0	—	5.96 (2.28 – 11.12)	2.75 (1.33 – 5.08)
Critical inhibition rate	C^*	%	80.46 (56.16 – 91.01)	58.49 (24.63 – 80.32)

^a Mean value (95% credible interval)

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5