EBOLA HEMORRHAGIC FEVER: OUTBREAKS, MODELING, AND VACCINE
DEVELOPMENT

by

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Abstract

Between the years 2014 and 2015, the world experienced a catastrophic outbreak of Ebola virus, which killed over 26,000 people. Several authorities and organizations actively participated in fighting the epidemic. Infectious disease modelers proved to be invaluable towards this goal. This report provides a background on the Ebola epidemic in West Africa and reviews the biological features of the Ebola virus. Moreover, this report applies a new model for Ebola propagation using data collected by the World Health Organization during the span of the outbreak. The model estimates the reproduction number and assesses the role of mitigation strategies in slowing down the progress of the disease. The report also concludes a review of recent advancements in vaccine production against Ebola.

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Acknowledgements

Dedication

This report is dedicated to my mother who has always been by my side despite the vast miles between us. I am very grateful for your unconditional love and support.

Preface

This report's subject of interest is the Ebola Hemorrhagic Fever, the disease that killed thousands of people in West Africa since its outbreak in March 2014. I chose to investigate this topic while working with Dr. Scoglio's networks group and finding out how important this research is for the two fields of biological engineering and computational epidemiology.

In this report, I introduce various aspects of the Ebola Virus Disease such as its history, transmission mechanisms, and impact on West Africa. I then focus on two important topics related to the disease. The first being its epidemic modeling using extensive data from the World Health Organization. The second being vaccine development and therapeutic interventions.

Chapter 1 - Introduction

Ebola hemorrhagic fever (EHF), or simply Ebola, is a lethal disease caused by infection with Ebola virus. It is named based on the location of the first discovery of the disease in 1976 in Sudan and Zaire (now, the Democratic Republic of the Congo) near the Ebola River [1]. Since then, Ebola outbreaks have appeared intermittently in several African countries. Its symptoms start with headaches, fever and muscle pain during the first few weeks followed by vomiting, diarrhea and extensive internal and external bleeding. The main identified strains of the Ebola virus are Zaire, Sudan, Bundibugyo and Tai Forest [1].

In this chapter, we review a brief history of Ebola including the spatial and temporal progression of the recent outbreak, mitigation strategies to prevent transmission, and the impact of the epidemic on West Africa.

1.1. Brief History of Ebola

Ebola first started in Sudan and Zaire in 1976 near the Ebola River, which infected over 284 people with a fatality rate of 53%. Within a few months, the second Ebola virus, EBOZ, emerged from Yambuku, Zaire, where it infected 318 people with the very high mortality rate of 88%. The third strain of Ebola, known as Ebola Reston (EBOR), was first introduced into US in 1989 by infected monkeys imported from Mindanao, Philippines. Fortunately, only a few people were infected with EBOR, and those individuals did not develop Ebola hemorrhagic fever (EHF). The last strain of Ebola, Ebola Cote d'Ivoire (EBO-CI) was discovered in 1994 in Tai Forest, when an ethnologist unintentionally infected herself while doing a surgery on a dead chimpanzee [1].

Ebola outbreaks have been intermittently experienced through history. However, all but the last outbreak, i.e., 2014 West Africa outbreak, were limited to a few cases. Figure 1 shows all the recorded Ebola outbreaks in the history. Prior to the 2014 Ebola outbreak, the maximum numbers of cases and deaths were 425 (2000-2001 Uganda outbreak) and 280 (1976 Zaire outbreak), respectively. As shown in Figure 1, the number of cases and deaths during the 2014 Ebola outbreak made an unprecedented jump to 27,000 and 11,000 on July 18, 2015, respectively. Comparing previous experiences of Ebola outbreaks with the 2014 outbreak highlights that sporadic, small-scale occurrences of an infectious disease should not be taken as a guarantee of its little potential threat.

	First outbreak of Ebola in Zaire	Ebola Reston introduced into US quarantine facilities by monkey from the Philippines	Outbreak in DRC Index patient worked in the forest adjoining the city	Outbreak in Uganda associated with funeral attendance and caring for patients with Ebola without proper PPE	Outbreak in DRC Last confirmed case on 10/4 and last death 10/10
Year	1976	1989	1995	2000-2001	2007
Cases	318	0	315	425	264
Deaths	280	0	250	224	187

	Lab infection by accidental stick of contaminated needle	Outbreak in Gabon in gold-mining camps in the rainforest	Lab contamination in Russia	First outbreak reported in the Republic of Congo	Outbreak of a new strain of Ebola in western Uganda	Ebola outbreak in West Africa; largest in history
Year	1976	1976	1996	2001-2002	2007	2014
Cases	1	1	1	57	149	*27,000+
Deaths	0	0	1	43	37	*11,000+

CS 252971   * As of July 18, 2015

Figure 1 Ebola outbreaks in history. *Source: Centers for Disease Control and Prevention:* http://www.cdc.gov/vhf/ebola/outbreaks/history/chronology.html.

The 2014 West African Ebola epidemic has been the largest and deadliest Ebola outbreak in history (Figure 1). The outbreak started in December 2013 and severely affected countries including Sierra Leone, Guinea, and Liberia. Moreover, cases were also reported in Nigeria, Senegal, Mali, United States, United Kingdom, and Spain. Some features made this outbreak different and more intense than the previous ones. For example, Ebola spread into unprepared countries in West Africa, which did not have previous experience with Ebola outbreak and required mitigation measures to control it. Furthermore, instead of the virus being transmitted through isolated, rural areas like previous outbreaks in Central and East Africa, this time, it quickly moved to densely populated urban hot spots. Also, the low levels of literacy, poor access to health information, and delayed public health campaigning made the affected situation worse. These factors contributed to the deadliest Ebola outbreak in history. To better understand the extent of the outbreak spread and its impact on West African countries, we review the mechanisms through which Ebola virus can spread.

1.2. Transmission Mechanisms of Ebola

Ebola disease is a zoonotic disease, i.e., it involves animals and humans. In particular, researchers consider fruit bats as the primary host for the Ebola virus. Figure 2 depicts a schematic of the main transmission mechanisms of the Ebola virus. The transmission of Ebola virus among fruit bats is enzootic, i.e., the virus routinely transmits among them, making fruit bats the primary reservoir for the virus. In the event of a spillover of the virus from the primary reservoir to secondary hosts, Ebola virus can be transmitted among other animals and humans as in epizootic cycle. Initially, Ebola virus entered the human population through direct contacts with infected animals like chimpanzees, gorillas, fruit bats, monkeys, forest antelope, and porcupines found in the rainforest [1]. After animal-to-human transmission, Ebola virus then spread in the human

population through direct contact with the blood or other bodily fluids of infected people. There has been no indication that mosquitos or other insects can be vectors for Ebola virus transmission. Only mammals such as bats, monkeys, apes, and humans can spread the virus and become infected with Ebola virus.
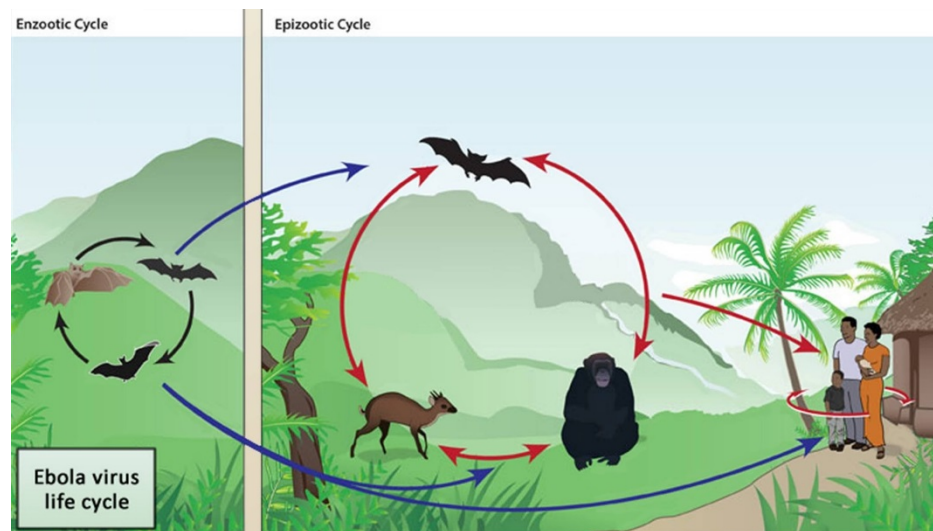

Figure 2 Major transmission mechanisms of Ebola. *Source: Centers for Disease Control and Prevention:* http://www.cdc.gov/vhf/ebola/resources/virus-ecology.html

The infection transmission mechanism from an infected animal or person to a healthy one is mainly through direct contact with blood or other bodily fluids of an infected individual. EHF can develop upon direct contact with blood, secretions, organs, or other bodily fluids of an infected human or person. Bodily fluids include vomit, urine, semen, vaginal fluids, saliva, sweat, tears, mucus, and feces. When blood or bodily fluids of an alive or deceased infected person touch another person's mouth, nose, eyes, wound, or open cut, the contact is considered direct. Ebola, in some rare cases, can be transmitted through sex with an infected or even a recovered male person. In summary, Ebola can be transmitted to humans in two ways. First, the virus can move from one animal to another animal in the wildlife, and then from an infected wild animal to a human. Second,

the virus can be transmitted from an infected human to another human. The latter happened in West Africa during the 2014-2015 Ebola outbreak, the deadliest and the largest outbreak of Ebola experienced so far.

1.3. Ebola Spatial and Temporal Propagation in West Africa

Ebola disease spread in 2014 became a severe global concern when it affected cities in West Africa with major commercial airports such as Conakry, Freetown, Monrovia, and Lagos. Figure 3 shows the spatial distribution of new and total cases in Guinea, Liberia, and Sierra Leone until March 27, 2016.
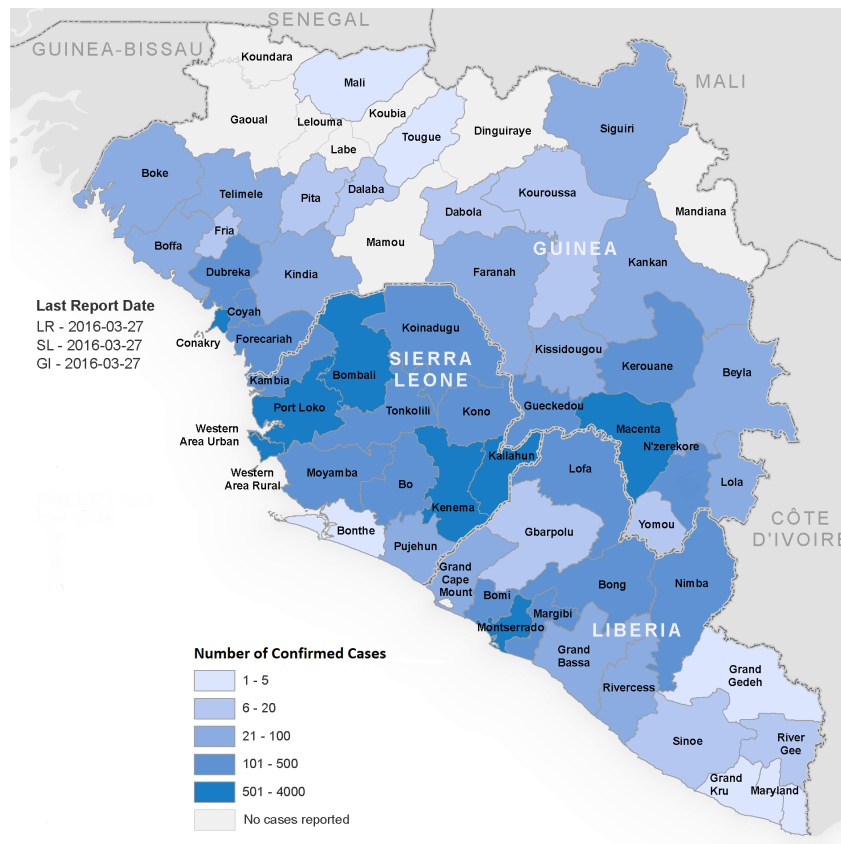

Figure 3 Spatial distribution of Ebola cases in West Africa. *Source: World Health Organization* (*WHO*). http://www.who.int/csr/disease/ebola/maps/en/

As Figure 3 indicates, those regions in Guinea and Libera that are closer to Sierra Leone are more severely affected compared to areas far from Sierra Leone. According to the latest report of CDC [1], as of April 13, 2016, the maximum number of cases and deaths are 14,124 (Sierra Leone) and 4,810 (Liberia), respectively. The spread of Ebola from West Africa to other countries occurred primarily due to international travels of exposed individuals. Even though the total number of external infections was very low, fear of globalization of the epidemics urged governments to impose increased monitoring and restrictions to international flights originating from affected West African countries.

1.4. Mitigation Strategies to Stop and Contain the Ebola Outbreak

Since there is no proven vaccine for Ebola on the market yet, mitigation strategies play a significant role in preventing transmission of the virus. The risk of transmission is particularly high in health care settings. Therefore, health care workers should be mindful of preventive practices such as wearing the protective clothing (e.g., masks, gloves, goggles, and gowns) and use infection-control measures (e.g., complete equipment sterilization and routine use of germicide). In addition, they should isolate patients from exposed people without full protection. All these techniques are used to avoid any contact with the blood or fluids of an infected patient. Moreover, direct contact with the body of a dead patient should be prohibited, because the virus can also spread through contact with a dead person's body fluid.

During the 2014-2015 outbreak, governments of infected nations have taken extraordinary measures under the supervision of the World Health Organization (WHO), and in close collaboration of international public health experts and workers. In Liberia, the government

ordered all schools to remain closed temporarily. Ministry of Health allocated resources for disinfection of the affected areas with chemical disinfectants while health worker would check on people from house to house to observe whether they were infected or not, and isolate the infected people to treatment facilities. The Ministry of Health complemented these efforts by conducting public education and awareness on the dangers of Ebola and the necessary measures to avoid contracting and spreading the virus. Places like churches and mosques, shopping centers, and other public areas were required to provide hand washing facilities and to post notices about the Ebola epidemic for more awareness [1].

Similar to many other outbreaks of infectious diseases among humans, travelers are the most concerning vectors [1]. Travels between cities and countries can allow the virus to spread and as consequence worsen the epidemic outbreak. Therefore, one of the first strategies to stop further propagation of Ebola is to quarantine infected people or affected areas. In the case of the 2014-2015 Ebola outbreak, quarantining was practiced as well. For example, the residents of Port Loko, Bombali, and Moyamba, which were the hotspots for Ebola's propagation, were quarantined with the orders of the Sierra Leonean president, Ernest Bai Koroma. The total population of the quarantined area was about a million people, almost a third of the country's population. Also, People traveling to the quarantined areas or moving to the isolated areas were carefully monitored to ensure that the deadly Ebola disease did not transmit outside of the region.

The CDC issued travelers' alert to all US residents, asking them to avoid travel to Guinea, Liberia, and Sierra Leone. Those traveling to these regions were asked to avoid any contact with the blood and body fluids of people with Ebola. Furthermore, enhanced monitoring was placed on international flights originating from one of the affected countries, and in particular, if the US was the final destination.

1.5. Effects of Ebola on West Africa

Even though mitigation strategies were helpful to slow down outbreak spreading, the Ebola outbreak already imposed severe economic difficulties and food shortage on West African countries. According to the UN Food and Agriculture Organization (FAO) [4], the three affected countries, Guinea, Sierra Leone, and Liberia were at the risk of facing a terrible food shortage due to restrictions on movements and the fear of Ebola spread. The fear of getting infected kept many people away from markets, farms, schools, and other places. Furthermore, since eating bushmeat was banned, hunters had to stay in their homes, and due to restrictions on travels, many farmers were not able to go their fields and farms to harvest the rice and corn. Moreover, limitations in trade relations through the seaports lead to the high prices of cereal, corn, palm oil, meat, and cocoa. The rapid increase in the food prices caused serious issues for many people of affected countries. In response to this crisis, the UN FAO and the World Food Program sent 65,000 tons of food to feed about 1.3 million people in West Africa.

1.6. Conclusion

In this chapter, after a brief history of Ebola and its transmission mechanisms, we reported the 2014 outbreak and its impact on West Africa. Then, we discussed some mitigation strategies. When such outbreak happens, it is very crucial to act immediately and effectively to fight further spread and reduce the toll on people and global safety. For designing successful mitigation policies, particularly under resource limitations, quantitative understanding of the disease outbreak and its time projection is invaluable. Computer models of disease spread played a significant role to this end during the 2014 Ebola outbreak. The following chapter is devoted to modeling Ebola propagation.

Chapter 2 - Modeling Ebola Propagation

Computational epidemic modelers played a valuable role to help health authorities allocate resources to mitigate effectively and efficiently Ebola from spreading. The objective of a computational model is to provide mathematical and simulative tools to describe, understand, and predict the spatial and temporal projection of an outbreak, as well as to evaluate mitigation strategies for informed decisions. An epidemic model uses biological features of the disease as well as recorded incident cases to set up a computational tool. Several modeling approaches have been proposed for the Ebola outbreak [4–11]. In this chapter, we review a background on modeling Ebola spread, propose a simple epidemic model inspired by a recently proposed model [4], state our data collection effort from WHO reports, and finally apply our model to real incidence data in Liberia. We conclude the chapter by discussing our results.

2.1. Background on Epidemic Modeling

Epidemic models are mathematical descriptors of the dynamics of communicable diseases among a group of individuals in society. Upon introduction of infection in a population, several questions become immediately relevant. For example, will the infection be limited to a few cases and die out shortly after introduction? Does it have the potential to become an epidemic? What are possible conditions that can lead to such catastrophic event? What would be the total cost of the outbreaks, say, measured in terms of the total number of individuals who experience the infection? What is the growth rate? When can we expect the maximum peak of the epidemic, after which infections tend to slow down? What are the best mitigation strategies to suppress the epidemics? How should a health authority prioritize strategies, allocate resources to most effectively, and

efficiently eradicate the disease? The objective of epidemic modeling is to help answering the above questions.

Efforts to capture dynamics of communicable diseases date back to pioneering work of Daniel Bernoulli, who used simple differential equations to model the spread of smallpox [12]. However, there has been a substantial amount of work devoted to epidemic modeling during the past decade, particularly due to recent large-scale outbreaks as well as due to advances in computational power.

There are several approaches to epidemic modeling. For example, models can be deterministic or stochastic. The modeling scale can be compartmental — where the whole population is divided into groups depending on their infectious state, meta-population — where geographically distant subpopulations are considered, or individual-level — where disease propagation is studied from on individual to another. From a computational complexity perspective, deterministic compartmental models are the simplest while individual-based models are the most complex.

In this report, we only consider deterministic compartmental models. In such models, a population of interest is divided into different compartments [13]. A typical model considers, at least, a susceptible compartment and an infected compartment. The infected compartment denotes the subset of the population who have received the infection and can potentially infect others. The susceptible compartment denotes those individuals who have not contracted the infections but are at risk of contracting it if they are exposed to an infected individual. Depending on the disease, modelers add more compartments to comply with reality. For example, for a disease such as chicken pox, an infected individual becomes immune to future infections after the infectious period. For such cases, a third "removed" compartment is usually considered. People belonging to

the removed compartment are those who had previously contracted the disease and now are immune; in other words, they are removed from future dynamics of infection growth. We should point out that not all infections lead to immunity. For example, an infected individual with a sexually transmitted disease can potentially get the infection repeatedly. Another very common compartment in epidemic models is the exposed compartment. Exposed are those individuals who have received the infection but are still in the incubation period and cannot pass the virus to another susceptible person. For example, models of Ebola disease commonly include the exposed compartment since an individual typically needs 5-21 days after exposure to the Ebola virus to show symptoms and become infectious [1].

One of the key descriptors of an infectious disease spreading is the *basic reproduction number*, denoted by R_0. This quantity measures how aggressively a virus spreads within a population [13]. The epidemiological definition of R_0 is the expected average number of secondary infections from a single initial infected individual in an entirely susceptible population during its infectious period. Importantly, R_0 provides a criterion for the epidemic outbreak. If $R_0<1$, then initial infections will disappear soon. The reason is that from a single infected individual, the average secondary infected cases are less than one. Therefore, within a short period, all infected individuals are recovered. Contrarily, when $R_0>1$, then initial infections have the chance to grow exponentially in the population. The awareness is that if for example $R_0=2$, the single initial infected individual leads to 2 new cases, who in turn, leads to 4, 8, 16 new cases and so on [13].

The epidemiological definition of R_0 is not very practical when dealing with realistic incidence data because it is not directly measurable. A standard approach is that parameters of an epidemic model are tuned so that model outputs fits observed incidence data. Therefore, since such models are mathematical equations, a theoretical expression for the value of R_0 can be computed.

2.2. An SEIR Model For Ebola

The SEIR model proposed in [4] has the following structure: The population is divided into five compartments, namely, susceptible (S), exposed (E), infected (I), recovered (R), or dead (D). Figure 4 illustrates the transition among these compartments.
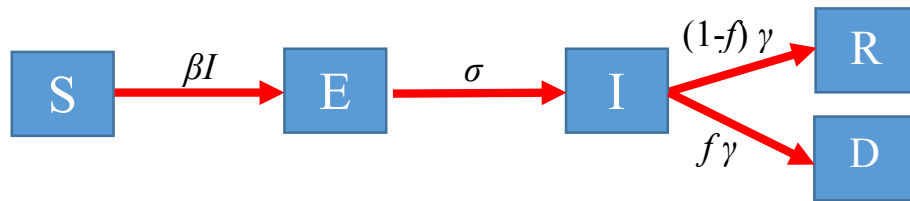

Figure 4 Transition diagram for SEIR Ebola model proposed in [4].

As Figure 4 indicates, in SEIR model susceptible individuals (S) become exposed at rate βI, exposed individuals (E) become infected at rate σ, infected individuals (I) recover or die at rate γ. The mortality rate is f, which is a number between 0 and 1. For example, f=0.4 means 40% of infected individuals will die. In this model, it is assumed that a mitigation effort is going on which is reducing the infection rate β at a reduction rate k.

According to these transition rules, we can derive the differential equations governing the evolution of fraction of each compartment (see [4]).

$$\frac{dS}{dt} = -\beta SI$$

$$\frac{dE}{dt} = \beta SI - \sigma E$$

$$\frac{dI}{dt} = \sigma E - \gamma I$$

$$\frac{dR}{dt} = (1 - f)\gamma I$$

$$\frac{dD}{dt} = f\gamma I$$

$$\frac{d\beta}{dt} = -k\beta$$

(Eq.1)

In the above set of differential equations $S + E + I + R + D = 1$, the initial condition is $(S, E, I, R, D)|_{t=0} = (1 - \frac{1}{N}, 0, \frac{1}{N}, 0, 0)$ and $\beta|_{t=0} = R_0\gamma$, assuming that at the initial time there is only one infected individual in the population and the rest are susceptible, while the basic reproductive number is R_0 to be estimated from data. Stability analysis of Eq.1 reveals that for SEIR model $R_0 = \beta/\gamma$.

The MATLAB code for the SEIR model in Eq. 1. is as follows:

```matlab
% Population
N=1000000; I0=1;

% SEIR model paramters
gamma =1/5.61; sigma=1/5.3; f=0.71; R0=1.59; beta = gamma*R0; k=0;
SEIRfun=@(t,x) SEIR(x,k,gamma,sigma,f);

T=8*30;    % Simulation Time
[t,x] = ode45(SEIRfun, [0:T], [1-I0/N 0 I0/N 0 beta]);

% Computing Populations
S=x(:,1)*N; E=x(:,2)*N; I=x(:,3)*N; R=x(:,4)*N; D=N-S-E-I-R;

% Plotting
figure(1);
plot(t,I+D+R,'r', 'LineWidth', 3)
hold on;
plot(t,D,'k', 'LineWidth', 3)

legend({'Cases', 'Dead'})
ylim([0,10000]);
xlabel('Time (day)','fontsize',14);
ylabel('Population','fontsize',14);
ttl=['Liberia R0=',num2str(R0)];
```

```
title(ttl,'fontsize',14);
```

```matlab
% Script for function SEIR

function dx = SEIR(x,k,gamma,sigma,f)
dx = [0; 0; 0; 0; 0];

beta=x(5);

dx(1) =-beta* x(1) * x(3);
dx(2) = beta * x(1) * x(3)-sigma*x(2);
dx(3) =sigma*x(2)-gamma*x(3);
dx(4) =(1-f)*gamma*x(3);
dx(5)=-k*x(5);

end
```

2.3. Exploratory Simulations of the SEIR Model

The SEIR model of Ebola explained in the previous section can show the evolution of the size of each subpopulation through time. The model has several parameters that need to be estimated either from prior knowledge about the virology of the disease or should be estimated from data. Before using any parameter estimation, we first simulate the model with synthetic values for R_0 to have a primary understanding of the model. Here, we have chosen a population of N=1,000,000 individuals with I_0=10 initial infected cases. According to recorded clinical data, we choose the time to recover or die as 5.3 days (γ=1/5.3), and the time that an exposed individual becomes infected as 5.61 days (σ=1/5.61). Furthermore, we assume a 40% morbidity rate (f=0.4).

Our numerical exploration of the model, as depicted in Figure 5, shows that R_0 has a significant role in the number of infected: the higher the R_0 value, the greater the number of infected cases. For example, in Figure 5a for R_0=1.56, the number of dead cases is about 22,000, whereas the death counts jump to 35,000 for R_0=2.53 in Fig. 5c. Furthermore, we see that peak infection

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size occurs earlier for larger infection rates. For example, in Figure 5a when R_0=1.56, the peak of epidemics happens around day 175, while for R_0=2.53 in Figure 5c, the peak of epidemics around day 85. A long time to the peak of infection allows more time for mitigation efforts and hence makes eradication of the disease more feasible.
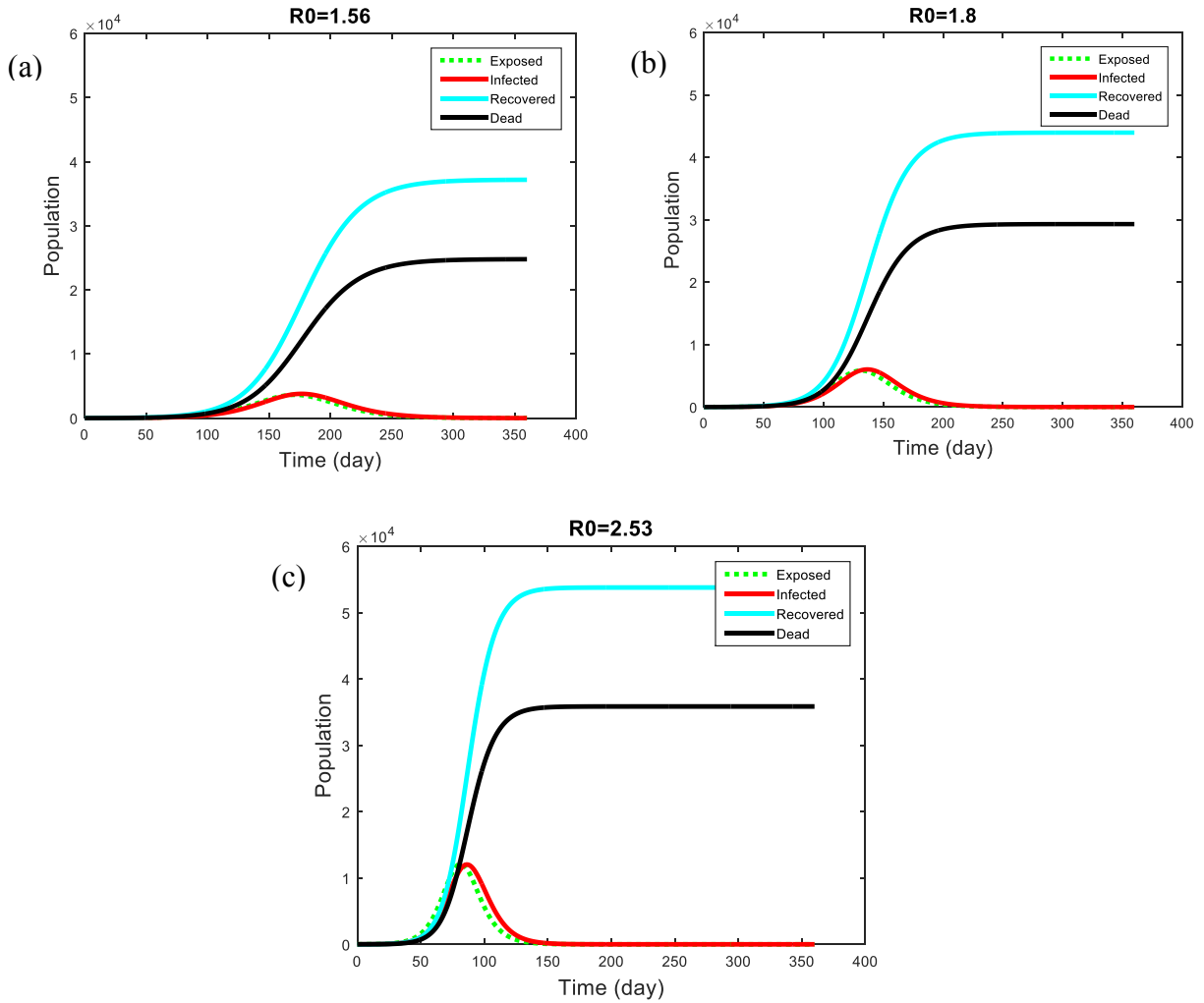

Figure 5 Simulation results for three scenarios with different reproduction numbers: (a) R_0=1.56, (b) R_0=1.80, and (c) R_0=2.53, shows its impact on the projected number of exposed, infected, recovered, and dead cases.

2.4. Real-time Data Collection of Ebola Cases

We have regularly collected data from Health Map [2] which is an accessible, automated electronic information system for monitoring and visualizing reports of global disease outbreaks. Health Map provides a comprehensive view of the current global infectious diseases according to geography, time, and infectious disease agent. In March 2014, the Healthmap software tracked reports of Ebola in West Africa, and they were updating daily data about Ebola outbreak cases and death. The HealthMap team created a dedicated HealthMap visualization [3]. We have collected all the reported data from HealthMap in an Excel file. Figures 6-8 show the number of total cases and the number of death cases in Guinea, Liberia, and Siera Leone, respectively. A total number of cases at a given date includes all the cases that have experienced the infection and has been detected by that date. Death numbers at a specified time show the total number of individuals who have died as the result of Ebola disease.

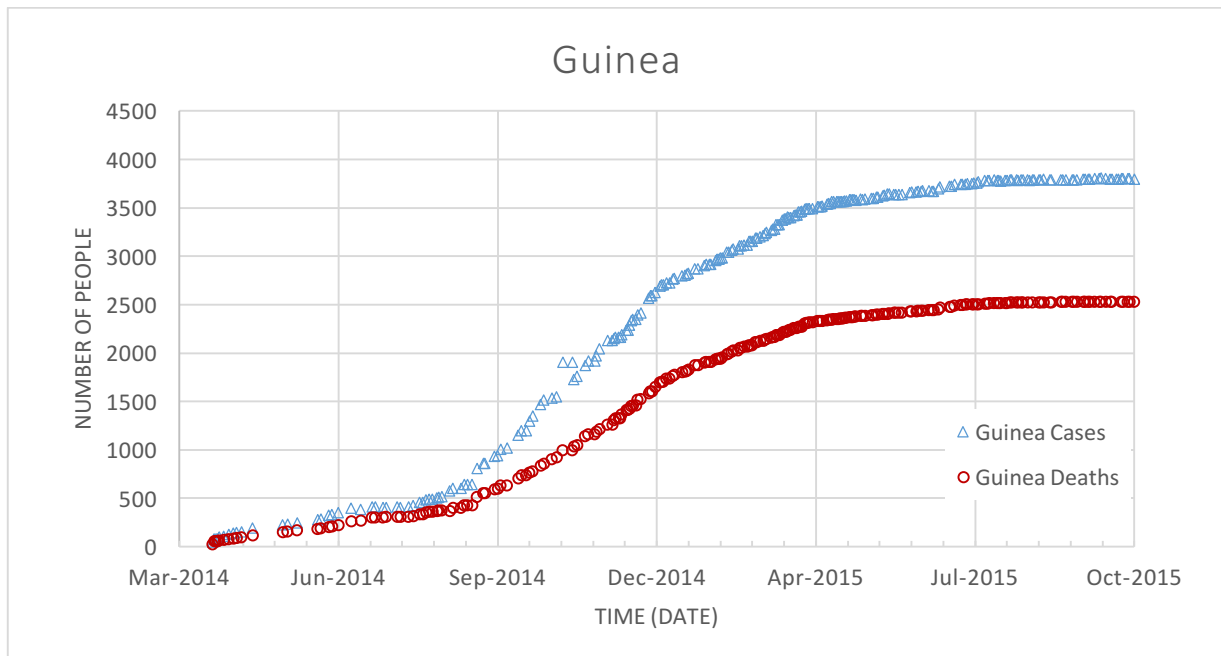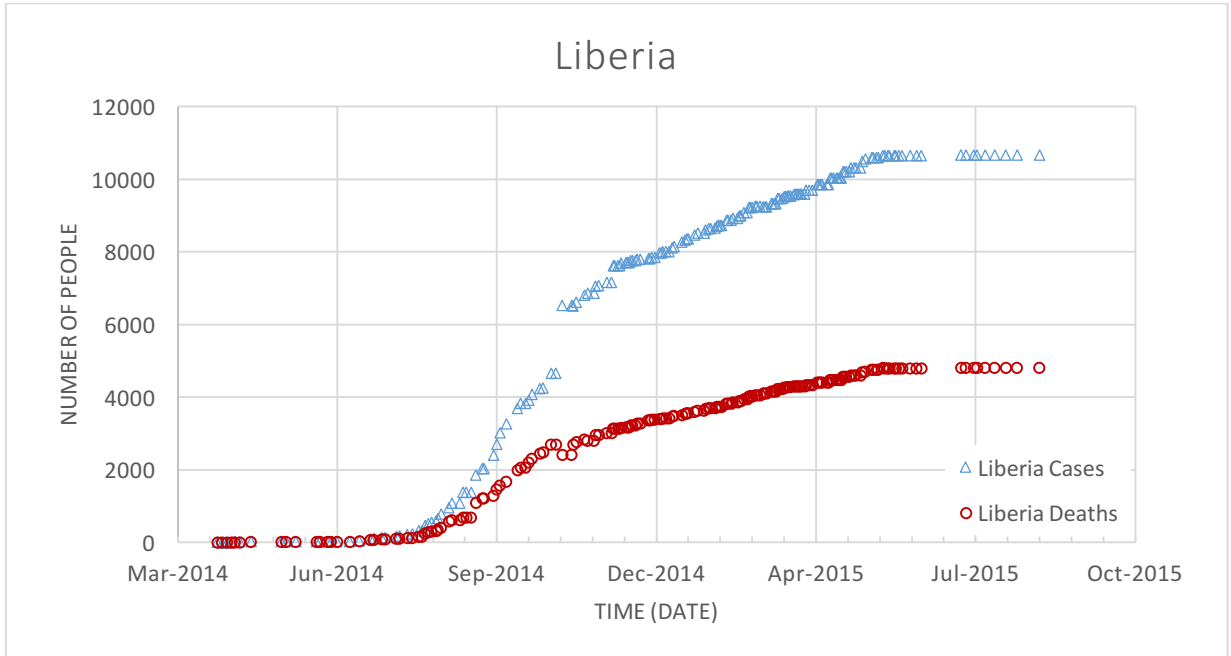**Figure 6** Ebola cases and death numbers in Guinea. Data collected from [2].

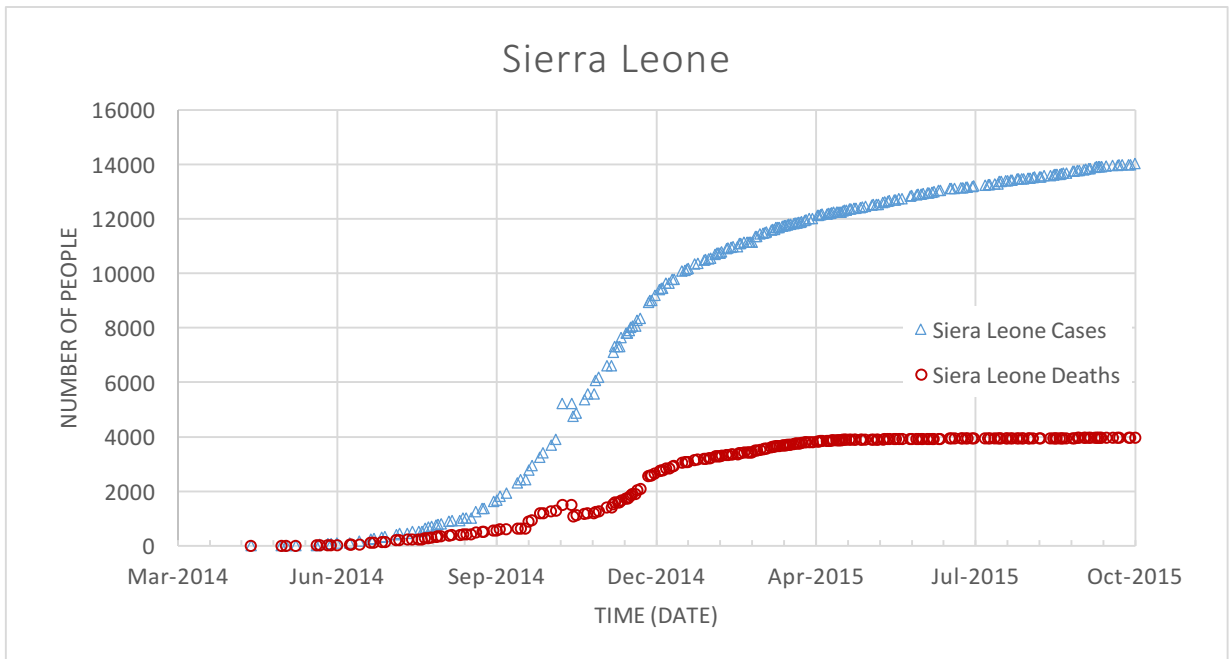**Figure 7** Ebola cases and death numbers in Liberia. Data collected from [2].

Figure 8 Ebola cases and death numbers in Sierra Leone. Data collected from [2].

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2.5. SEIR Model Fitting to Actual Ebola Data

The SEIR model (Eq. 1) for Ebola can be applied to real data of Ebola cases and death numbers collected in Section 2.4. For this end, we need to estimate the parameters involved in the model, namely σ, γ, R_0, k, f. Following steps explain our approach in this report for parameter estimation:

1. Choose σ=1/5.61 day^{-1} and γ=1/5.3 day^{-1}, which are obtained according to clinical record of the Ebola virus.

2. Change the reproductive number R_0 and the infection reduction rate k so that the output of the SEIR model in Eq.1. obtained using the MATLAB codes in section 2.2 fit the number of Ebola cases collected in section 2.4. This step is trial-and-error, i.e., values of the two parameters R_0 and k are tweaked until a reasonable fit is obtained. More advanced parameter estimation methods could improve the model fitting, which is out of the scope of the current report.

3. Select f such that death numbers fit the reported values. This step is a simple linear regression on the total number of cases as the x-axis and the total number of deaths as the y-axis.

4. Change the values of the basic reproduction number (R_0) and mitigation rate k, so that predicted values fit well to the actual recorded data.

5. As a goodness of fit measure, plot the predicted against actual values.

Figures 9, 10, and 11 show the model fitting to the actual data in Liberia, Guinea, and Sierra Leone, respectively. The best parameters that we found were R_0=1.85 and k=0.0027 (1/day) for Liberia, R_0=1.52 and k=0.0023 (1/day) for Guinea, and R_0=1.8 and k=0.0028 (1/day) for Sierra Leone.

Figure 9 Model fitting to the real Ebola data in Liberia. The small circles show the actual recorded data, and the lines are the prediction of the epidemic model [2].
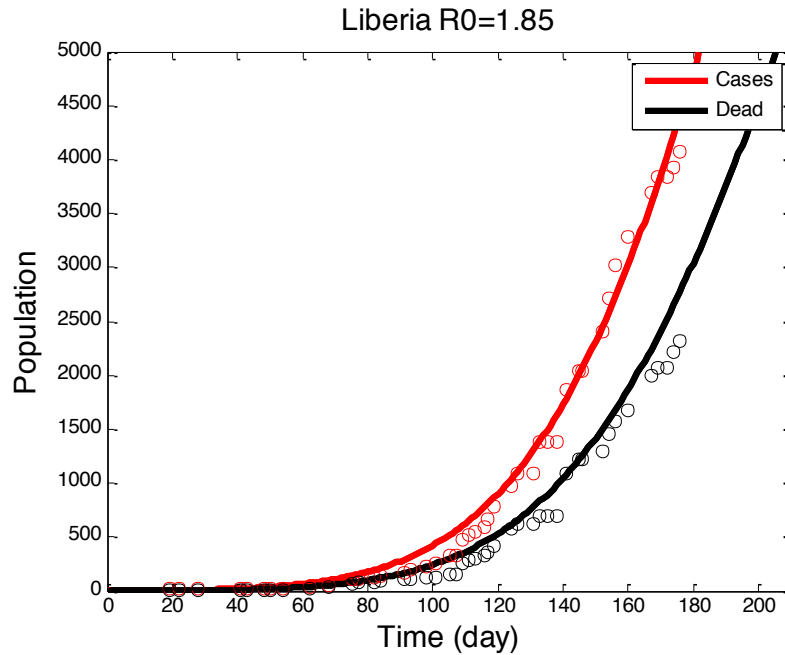

Figure 10 Model fitting to the real Ebola data in Guinea. The small circles show the actual recorded data, and the lines are the prediction of the epidemic model [2].

Figure 11 Model fitting to the real Ebola data in Sierra Leone. The small circles show the actual recorded data, and the lines are the prediction of the epidemic model [2].

As can be seen in Figures 9-11, there is a good agreement between the model output and actual recorded numbers, despite our rudimentary approach to parameter estimation. To further illustrate the goodness of fit in an example, we have plotted the predicted values for the number of cases against actual numbers for the case of Sierra Leone in Figure 12. The slope of the linear fit is very close to 1 with, R^2=0.99, indicating that actual and predicted values are reasonably close.

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Figure 12 Predicted vs. the actual number of Ebola cases in Sierra Leone. Actual data from [2]

2.6. Conclusion

We computed the basic reproductive number according to a SEIR model for Ebola in Eq. 1. For this end, we only used the incidence data from March 2014 to September 2014. The reason we did not include the complete data up to the end of 2015 is that $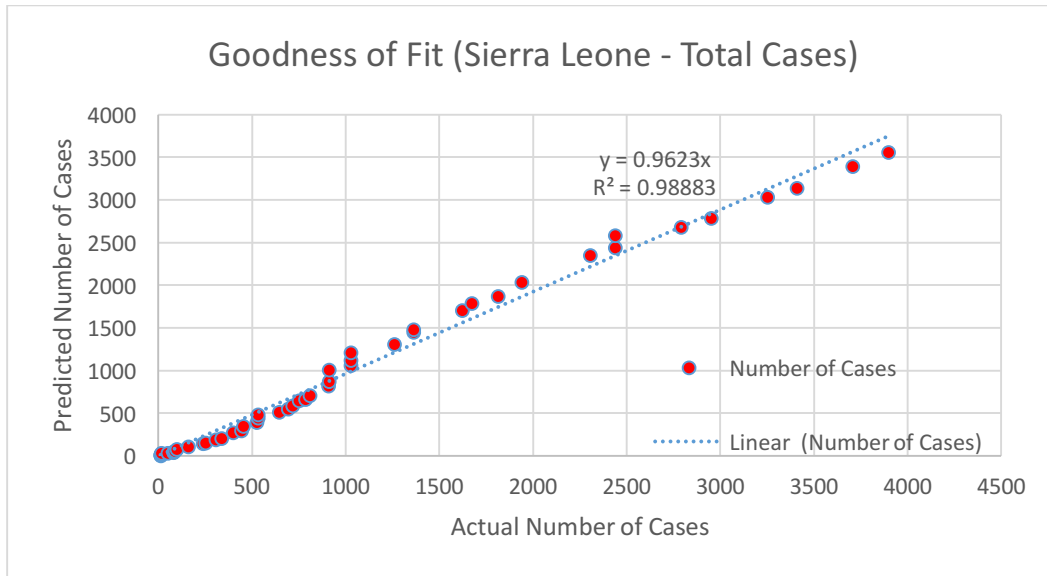R_0$ is a quantity related to the early growth of the epidemic. Since, as recorded data indicates, the epidemic started to slow down in 2015, incorporating the full data would bias the estimation towards lower values. The computed value for R_0 for Liberia (1.85), Guinea (1.52), and Sierra Leone (1.8) in our simulation is compatible with other estimations. For example, Althaus [4] found R_0=1.59 (Liberia), R_0=1.51 (Guinea), and R_0=2.53 (Sierra Leone), which is comparable to our results except in the case of Sierra Leone, which we believe is due to their very large mitigation rate in their calculations.

The values R_0 (in the range of 1.52–1.85) for the basic reproductive number indicates that Ebola is not indeed an aggressively spreading virus. Compared to typical values of R_0 for other

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infectious diseases such as measles 12–18, smallpox 5–7, SARS 2–5, and influenza 2–3, value R_0=1.85 places Ebola among slow viruses.

The analysis in this sections found an estimation for the mitigation rate for Liberia as k=0.0027 (1/day), which means mitigation efforts were indeed slowing down the infection. According to the last equation of Eq.1 for β, this value means it would take about $T = \frac{\ln(R_0)}{k} \sim 227\ days \sim 7.6\ months$ to bring the reproduction number below 1, which in this case would correspond to late 2014.

Obviously, the real scenario of Ebola propagation is very complex that are not considered in the SEIR model. Yet, the simple deterministic SEIR model provides some insights into the dynamics of the disease which might not be intuitive.

Chapter 3 - Vaccines and Therapeutics Development for Ebola

This chapter provides a background on vaccine development against Ebola virus, reviews some of the primary vaccine and therapeutic products, and discusses possible plant-based production procedures.

3.1. Brief history of Ebola vaccines and therapeutics

Vaccines and therapeutics are crucial in the management of infectious disease. Vaccines are intended to immunize susceptible people against possible, future infections while therapeutics refer to medical treatments given to an infected individual to help them recover from the disease or at least alleviate the symptoms. Unfortunately, during the recent Ebola outbreak, there were no vaccines against Ebola for humans. Even if a clinical trial proved to be effective in humans, producing large quantities of vaccines would not be feasible for months and years. Moreover, the virology of Ebola virus has not been described enough due to difficulties in getting samples and laboratory studies and analysis, and high biohazard containment requirements. Plus, previous outbreaks were primarily in remote areas which were not populated.

Since the first detection of Ebola in 1978, researchers have been trying to develop vaccines against this virus. A significant amount of this effort has focused on vesicular stomatitis virus (VSV) expressing the Ebola virus glycoprotein (VSV-EBOV), which showed good efficacy in protecting rodents and nonhuman primates against Ebola virus. Unfortunately, VSV-EBOV did not progress to clinical trials in humans mainly because, before 2014, the appearance of Ebola was rare and could not justify support from private pharmaceutical companies. Figure 13 depicts the historical timeline of vaccine development against Ebola virus. As can be seen in Figure 13, long before the 2014 Ebola outbreak, Ebola vaccines were developed and successfully tested in rodents

and non-human primates (DNA 2000 and VSV 2005). Although, human vaccines were never developed and tested until 2015, after the outbreak. Perhaps thousands of lives could have been saved during the 2014 West Africa outbreak if the potential threat of Ebola had never been overlooked and research for the development of a human vaccine against Ebola had continued.
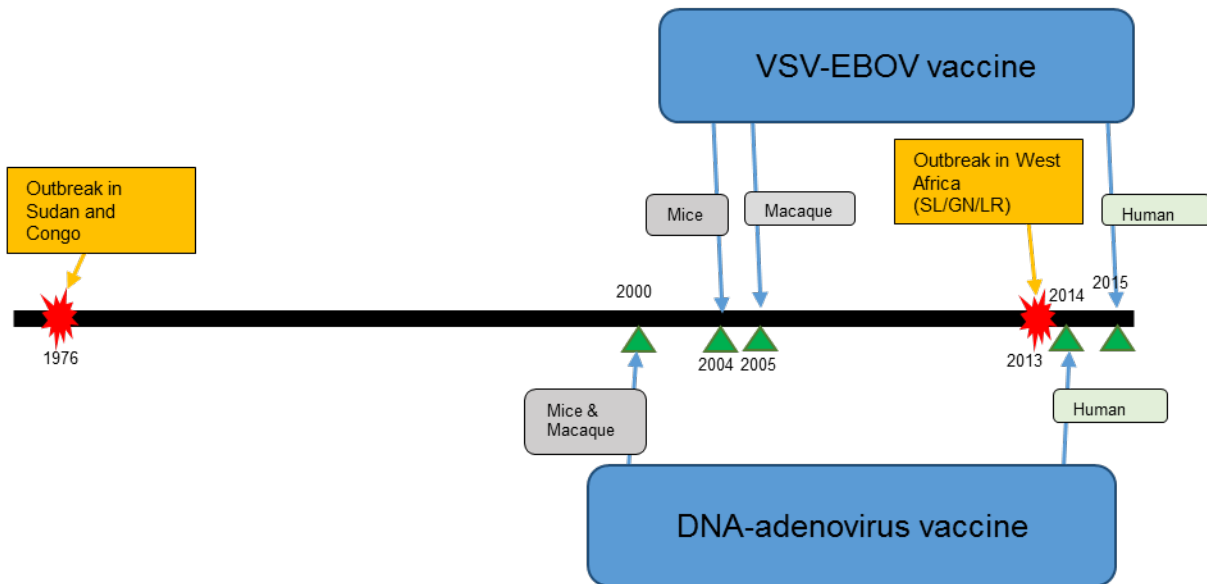

Figure 13 Ebola virus vaccines development timeline [14].

In addition to vaccines, drug development also began in 2002. The efforts were supported by governmental institutes such as National Institute of Health (NIH), United States and Public Health Agency of Canada. In 2004, an antibody therapeutics named ZMapp was developed by the Leaf Biopharmaceutical Inc. (San Diego, CA, USA) [17].

3.2. Ebola Diagnosis, Therapeutics, and Vaccines

The first step to fight Ebola virus disease is finding a diagnostic way to detect it. Enzyme-linked immunosorbent assay (ELISA) and real-time reverse transcription-polymerase chain reaction (rRT-PCR) are the common methods for Ebola virus diagnosis. ELISA is a standard method for detection of antigen or antibody. However, antibody-capture ELISA is not the best method to be applied for early stage diagnosis since it usually takes 1 or 2 weeks for antibodies to become evident. Virus isolation is also limited to laboratories with a biosafety level 4. After virus isolation, rRt-PCR is the most useful diagnostic method to recognize the presence of Ebola virus in specific genes. The next step after diagnosing EDV is treating the infected patient by an advanced form of therapeutics and providing susceptible people at risk with proper vaccination. Unfortunately, bio-processing of medications is very slow as any research associated with EVD is very risky and requires specific facilities comparable to biosafety level 4 laboratories.

Several vaccines and therapeutics have been proposed for Ebola. The most common therapeutics are ZMapp, Favipiravir, and TKM-Ebola and the most common vaccines are VSV-EBOV, cAd3-EBO Z. Table 1 shows a list of anti-Ebola therapeutics and vaccines, along with their manufacturer, production format, and their latest clinical trial phase. Unfortunately, most of the vaccines and therapeutics have only been tested in small rodents, pigs, or nonhuman primates. Unfortunately, only until several months after the outbreak, no phase II clinical and field trials was ever carried out on any of the vaccines and therapeutics.

Table 1 Drug and vaccine candidates for clinical trials. *Adapted from* [17].

Candidate		Manufacturer	Format	Clinical trial
Drugs	ZMapp	Mapp Biopharmaceutical, USA	Plant-derived antibody cocktail	Not yet
	TKM-Ebola	Tekmira, Canada	RNAi	Phase I
	Favipiravir	Toyama Pharmaceutical Co., Japan	RNA polymerase inhibitor	Phase I
Vaccines	cAd3	GSK, UK (developed by NIH, USA)	Chimpanzee adenovirus-based DNA vaccine	Phase I
	VSVΔG-ZEBOV	NewLink Genetics Co., Canada (developed by Public Health Agency of Canada)	Recombinant vesicular stomatitis virus-based VLP vaccine	Phase I

3.2.1. Therapeutics Development

The most common therapeutics for Ebola virus disease treatment are ZMapp, Favipiravir, and TKM-Ebola. ZMapp is a mixture, or cocktail, of three monoclonal antibodies (mAbs) against Ebola virus. These antibodies target the glycoprotein of the Ebola virus, preventing virus replications and neutralizing them. Antibody therapeutic ZMapp—which was under development from 2004 by Leaf Biopharmacutical Inc.—had proven helpful to protect chimpanzees from Ebola virus earlier, however, it was still under study before the West Africa outbreak. In April 2014, ZMapp was tested on two American medical staffs who had contracted the Ebola virus in Liberia, and for the first time, ZMapp showed therapeutic effectiveness against Ebola in humans. [21]. Even though ZMapp seems to have effective ingredients to save EVD patients, it still has some limitation to become the common Ebola therapeutics in clinical fields [17].

The intriguing aspect of ZMapp is its possibility of plant-based production. Production of proteins or antibodies from transgenic plants, genetically modified plants that can express a particular protein of interest, is very time consuming and not very suitable for responding to sudden threats like the Ebola outbreak. Alternatively, Mapp was able to produce the Ebola antibodies on tobacco (specifically, *Nicotiana benthamiana*) leaves as transient expression. The plant has been able to produce pharmaceutical proteins under highly controlled conditions. The main advantage of this plant-made antibody is being a quick process and being scalable to mass production [21]. ZMapp production can be explained in the following simple steps [17]:

1. Tobacco is planted under controlled conditions for six weeks. The tobacco leaves will serve as the transient expression platform.

2. Mice are incubated with a recombinant vesicular stomatitis virus, where its glycoprotein (GP) has been replaced by GP of the Ebola virus. In this way, the immunological response of the mouse would reveal the important neutralizing antibodies against the Ebola virus.

3. These antibodies are cloned for human IgG and coded on bacterial carriers to be transferred to tobacco leaves.

4. The bacterial carriers are then transformed to six-week-old tobacco leaves using methods of agroinfiltration, enabling the tobacco plants to express human anti-Ebola mAbs. After this step, it typically takes about four days for the tobacco leaves to express the desired amount of mAbs.

5. Finally, antibodies are purified after harvest. A typical expression yield for ZMapp is 50 μg of IgG per 1 gram of leaves.

The use of tobacco to produce ZMapp attracted an unprecedented global attention regarding potential of plant-based pharmaceutics (PMPs) in saving lives. Mapp substantiated that plants are a promising medium for the rapid production of antibodies to face bioterrorism or possible natural infection disease outbreaks [18]. Not only plants are very efficient in producing complex protein, they can be easily planted and sclaed up. In addition, plant-based pharmaceuticals require significantly lower facility and production costs compared to traditional cell culture methods. EVD outbreak in West Africa has highlighted an excellent opportunity for the use of plant-based pharmaceutical [20] as well as their limitations.

In addition to ZMapp, Favipiravir and TKM-Ebola are other successful therapeutics against Ebola [17]. Favipiravir is an RNA polymerase inhibitor that can be synthesized by chemical reactions. This drug is an effective treatment for both influenza and Ebola hemorrhagic fever (EHF) since these two diseases have a similar mechanism. RNA polymerase plays a critical role in the replication of Ebola virus similar to influenza. Favipiravir is under the clinical phase 3 as an influenza treatment. Studies show this antiviral is successful in curing patients with early phase of EVD. Another EVD theraputic drug is TKM-Ebola which include a combination of three small interfering RNAs. TKM-Ebola prevents the reproduction of Ebola Virus by blocking three out of seven effective proteins in Zaire Ebola virus, namely, L polymerase, membrane-associated protein (VP24), and polymerase complex protein (VP35). It was successful in animal tests and also was applied in phase 2 clinical trials. Due to the ability to produce a large amount of RNAi within a short period, TKM-Ebola is one of the important therapeutic treatment in West Africa outbreak [17].

3.2.2. Vaccine Development

Researchers have been studying vaccine candidates since Ebola was identified in 1976. Since then, vaccine candidates have only been tested on animals in preclinical stages and acceptable results have not been achieved for nonhuman primates. Some challenges can be linked to obligations for high level safety facilities as well as lack of enough financial incentive for continued research. Studies on the use of an EVD prevention started during early 2000s, but only recently have achieved promising results in animals like guinea pigs and chimpanzees. For production of DNA vaccinces for Ebola, nucleoprotein or glycoprotein (GP) of Ebola virus is cloned into a plasmid backbone containing the host T-cells. The process for producing the DNA plasmid involved bacterial cell cultures from *Escherichia coli* resulting in intramuscular injectable liquid after fermentation and purification [22]. It makes cells to produce anibody against inserted antigen-delivering DNA and it leads to a response of host immune system by increasing the immunization and preventing replication of EVD virus. Injecting the plasmid cloned by GP into animals resulted in stable production of anti-Ebola antigens. Still, the ability of humans immune system was less efficient in comparison to rodents. In order to boost antigen production in human, several research efforts looked for combining the plasmid with appropriate viral vectors [17].

Recombinant vesicular stomatitis virus Ebola vaccine (rVSV-EBOV)

Recombinant vesicular stomatitis virus known as rVSV-EBOV is a bivalent vaccine against Ebola virus, designed by National Microbiology Laboratory in Canda and the Public Health Agency of Canada and New Link Genetics. It contains a genetically engineered vesicular stomatitis virus (VSV), which can express the Ebola glycoprotein (GP). Viral particles replicate like VSV but express the ZEBOV glycoprotein which is responsible for receptor binding between ZEBOV and host target cell [19]. Various methods of rVSV-EBOV vaccine delivery like oral,

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intranasal, and intramuscular injections have shown a general immune reaction to this virus protecting animal models completely from the Ebola virus

Vesicular stomatitis virus is considered to be appropriate choice as a recombinant DNA vaccine platform. The advantage of such DNA vaccine is that it can protect hosts against not only Ebola but also many other filoviruses. VSV virus is an animal pathogen which does not show any harmful symptoms in human [19]. As a result, preclinical testing shows rVSV-ZEBOV is not only safe for medical use in the human population, but it also provokes a rapid and durable response in the human immune system. Moreover, rVSV vaccine can be a good candidate to support many filoviruses like ZEBOV, Sudan Ebola virus, and Marburg virus [19].

The most advanced form of rVSV Ebola vaccine, VSVΔG-ZEBOV vaccine, was recently developed by the Public Health Agency of Canada and NewLink Generics Corporation. The VSVΔG-ZEBOV vaccine contains diluted recombinant VSV concentration whose surface glycoprotein gene has been replaced with Ebola virus Zaire GP. This vaccine was highly effective in animals showing 100% protection on them. Moreover, it can also be rapidly cultured, and it is fast growing.

cAd3 Ebola Vaccine (cAd3-ZEBOV)

Scientists at GlaxoSmithKline (GSK) and National Institute of Health (NIH) developed a vaccine from a chimpanzee adenovirus named Chimp Adenovirus type 3 (CAd3). This vaccine was designed to express glycoproteins (GP) which were taken from Ebola Virus. It expressed Ebola GP gene in hosts after vaccination and will produce an anti-cAd3 antibody which can cause an immune response against Ebola Virus Disease. Two types of cAd3 Ebola vaccines are a monovalent vaccine, which protects animals from Zaire strain and a bivalent vaccine which is

against both Zaire strain and Sudan strain [17]. Adenovirus vector has been studied as a successful platform for the DNA vaccine. Plus, it can be helpful for other infectious pathogens like HIV and tuberculosis. The main advantage of using Chimpanzee adenovirus vector is its safety since the animal vector cannot be replicated in human hosts. The cAd3 vaccine was tested in 16 animals and produced an excellent result defending animals from Ebola.

3.3. Further Discussion

The vaccine and therapeutic development process is an ongoing process because genes in the virus are changing as the disease spreads. Changes may occur when the virus lives in animals between outbreaks in people. The other possibility is that the virus mutates when it moves through a human population. These changes are imperative because if theraputics or vaccines are developed to treat earlier forms of the virus, they might not work effectively on future ones. Additionally, patient's immune system plays a significant role in recovery. It's not proven that people who survive from Ebola will become immune for lifetime. They might still get the different kind of Ebola virus. Some of the therapeutics and vaccines have already been used on patients within the United States, and several which showed efficacy in animal tests, now are in the clinical trials in West Africa.

Chapter 4 - Conclusion

In this report, we discussed introductory information about Ebola virus and its transmission methods, followed by reviewing the 2014 Ebola outbreak in West Africa, its severity and impact, and mitigation strategies to manage the outbreak. We discussed how infectious disease modelers help design these strategies.

In Chapter 2, we provided a basic epidemic model for Ebola and applied it to real incidence data in Liberia. Through simple trial-and-error approach, we obtained a reproductive number of R_0=1.85 for the Ebola outbreak in Liberia, which is consistent with reported values in the literature. Furthermore, we found that mitigation efforts, simply reflected in our model as infection reduction rate k—in the case of Liberia k=0.0027—was significant to contain the disease spreading, and potentially saving thousands of lives. Even though our model did not incorporate numerous factors affecting the propagation of the disease, it was able to provide a time projection of the virus propagation. This simple modeling effort signifies the importance of epidemic modeling for mitigation and policy making.

In Chapter 3, we reviewed proposed therapeutics and vaccines for Ebola. Luckily, a modest neutralizing antibody response is enough to induce protection against the Ebola virus [16]. The challenge, however, is the timely production of vaccines and therapeutics with success record in actual human trials. A plant-made pharmaceutical (PMP) therapeutics, ZMapp, developed by the Mapp Pharm. Inc. successfully cured the two American Ebola patients and trigerred a global attention to PMP. Plant-made pharmaceuticals are particularly beneficial for timely production of recombinant pharmaceutical proteins in large scales, attracting a substantial attention from the pharmaceutical industry [20].

Finally, despite the proven success of the mitigation efforts and therapeutics and vaccines in development, we are still far from total eradication of the disease. In this regard, it is of utmost importance that allocation of resources are not interrupted even if the public attention to Ebola news might weaken. Indeed, investments in proactive measures through health education and public awareness are a must after the "reactive phase" has successfully passed. The 2014 Ebola outbreak reminded us how much we are not prepared for a new infectious disease outbreak. In this regard, engagment of local communities in their own health, building reliable health infrastructures, and encouraging pharmaceutical companies to refocus their research in developing countries seem to be most effective in preparing the world to fight future infectious disease outbreaks.

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