Part I — The Cycle Starts Again

It was a hot day in August in a small village near Kikwit, Zaire, and Dr. Mombutubwa had another patient who was severely ill. With a note of resignation in his voice he told his nurse: “I don’t think she’ll make it past the next couple of days.” The telltale red pinprick spots of blood had started to appear on the patient’s arms, and her eyes were red and blood-filled. Since the outbreak began in early July it was mostly women who came to see him with such an ailment. They generally complained of flu-like symptoms (headache, fever, muscle pain, sore throat) followed closely by stomach pain, vomiting, and diarrhea, which sometimes progressed to the bruising and hemorrhaging that he was seeing in his current patient. He knew there was little that could be done.

Dr. Mombutubwa turned his thoughts to what life in his village was like when it was not burdened by epidemics. Women stayed in the village, tending to the house, preparing food, and caring for the children. If the family had a farm, it was the women’s responsibility to tend to the crops, removing weeds and fending off rodents, birds, and insects that found the crops appealing. Often, the women gathered together to fetch water at a local well. The men headed out every morning into the forest, machete in hand, to hunt wild animals. They returned home in the evening with their quarry slung over their shoulders, often with blood on their hands and body. They would then proceed to carve up the carcass, distributing the meat among them. The men were especially jubilant when they returned with a butchered ape, whose body parts were used in traditional shamanistic practices and fetched high prices at the market. When bats were plentiful, men used shotguns to kill them. Women of child-bearing age were forbidden from eating bats, but they were happy to remove the shotgun bullets by hand to prepare the meat for their husband, children, and post-menopausal kin. The animals were barbecued over a fire and feasted on. Life continued on in this way and the people of Dr. Mombutubwa’s village seemed happy with their daily routines.

In the years that Dr. Mombutubwa had practiced medicine, he had seen a few outbreaks of this disease that once again threatened his village. He now knew that this disease was called Ebola, that it was caused by an RNA virus, and that it killed people quickly and dramatically. Having encountered Ebola several times and having worked with colleagues who came to help stop the spread of the disease during outbreaks, he was now an expert at recognizing its symptoms, diagnosing it despite the similarity of its symptoms to other viral diseases, and acting swiftly to contain the epidemic. He had first seen an outbreak when he was a boy, and he had wondered at the rapidity with which the disease decimated his village and disrupted daily life. So many people died, and the villagers became afraid of one another. They stopped trading, they stayed inside their houses, and village life ground to a halt. Instead of tending to their corn or rice crops and their families, the women were consumed with cleansing preparations for funerals in which the female relatives of the deceased bathed and re-dressed the body of the dead, preparing it for the afterlife. When epidemics ended, there were never any traces of the disease in the village; it was as though the virus had disappeared. People returned to their daily activities, but once large families only had a few remaining members, since so many had succumbed to the disease.
Questions

1. Given that the initial symptoms of Ebola are very similar to influenza, how might Dr. Mombutubwa recognize and successfully diagnose Ebola in his patients? How might the colleagues who assist him during times of epidemic and who have access to more specialized equipment be able to diagnose Ebola?

2. What are the possible points of contact that allow the transmission of Ebola between villagers during the epidemic? You should be able to list and discuss at least five factors.

3. Who is particularly at risk for contracting Ebola? Why might this group be at higher risk of infection? What factors (biological, environmental, and social) might favor this distribution of risk (do not focus on genetic or immunological differences)? Is one factor more likely to be the way that Ebola is spread?

4. Dr. Mombutubwa thinks about the sporadic recurrence of Ebola epidemics in his life time. What kinds of social practices, biological, or environmental factors might cause an infectious agent to disappear and then re-emerge at a later date?
Part II – Consulting the Disease Detectives

Joe Mackey was suddenly woken by the sound of his home phone ringing. “Who could possibly be calling at such an early hour?” he thought to himself. It was the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, where Joe worked for the Epidemiology Intelligence Service (EIS). The EIS was often deployed around the world to help control epidemics.

Joe was on a plane the next morning to join Dr. Mombutubwa in his efforts to stop the spread of Ebola. The trip took over 30 hours, giving him plenty of time to organize his thoughts about this situation:

*Humans must not be the normal host for the Ebola virus. The virus is too aggressive, killing all potential hosts. There must be an animal or plant host where the virus can proliferate between human epidemics. There must be a “reservoir” of the virus. Since epidemics of Ebola sporadically re-emerge, either the villagers infrequently come in contact with this animal or plant reservoir, or else this reservoir species is not able to transmit the disease at all times. Let’s hypothesize that the initial transmission of Ebola into a human is from an animal. In other words, Ebola is a zoonotic disease (it normally has an animal host). Men spend their days in the forest. They catch bush meat and are also in contact with snakes, spiders, and insects. They also handle the bats for the communal dinners. Women tend to the fields, farming crops such as corn, millet, or rice. They probably have frequent contact with insects while working outside, and also with animals that are attracted to the crops, such as rodents and birds. They also help to prepare the bats for the communal feast. I need to know what animal is acting as a host so that we can try and prevent continued contact with this species and stop Ebola’s occasional re-emergence.*

Joe then thought about the gender bias in Dr. Mombutubwa’s clinic:

*Women seem more vulnerable to Ebola than men in this village. Perhaps this is an indication that they are exposed to the reservoir more often than men. However, women are more prone to catch Ebola during an existing outbreak. They are not more prone to be the first person in the village to catch the disease. This suggests that their vulnerability stems from social behaviors and practices once the epidemic has started, not exposure to infected animals. The funeral practices, where women prepare the body of dead relatives, are a noteworthy risk factor that could create a gender bias in infection.*

Questions

1. Ebola epidemics recur sporadically.
   a. How is this statement evidence that the virus can live in species other than humans? If the virus could only live in humans, what would happen at the end of each epidemic? How might an epidemic recur?
   b. In between epidemics, what is the virus “doing”? How is it replicating and being maintained? How does this knowledge help you predict the likely effect of the virus on other species?

2. Joe begins his investigation by creating a set of criteria to evaluate the suitability of an animal as the reservoir host. Help him establish these criteria.
   a. What sorts of contact/interaction between a reservoir species and humans would increase the likelihood of viral transmission?
   b. Is direct contact between a reservoir species and humans necessary? Why? Could a species serve as a reservoir species if it never interacts with humans? If so, how?
   c. How might the virus be transmitted between the reservoir animal and humans?
   d. Predict the effects that the virus will have on the health of its reservoir host.

3. Make a list of all the animals that the villagers are known to interact with. Consider the list of characteristics of a reservoir host species that you created in Question 2. Which species seems most suspect?

4. How might you determine that your suspected species is the reservoir for Ebola?
Part III – News Spreads Quickly

Joe receives a letter from his supervisor in Atlanta at the CDC. The letter includes a copy of an article on great apes and Ebola that has recently been published in *The New York Times*. Joe’s supervisor has highlighted a passage from the article:

*In 2002, following human disease outbreaks caused by the Zaire strain of the Ebola virus, the researchers began finding dead gorillas. Over a number of months they found 33, tested 12 for Ebola and found that 9 were infected. And from October 2002 to January 2003, 130 of the 143 gorillas they had been studying — 91 percent — simply disappeared. The losses continued: 91 of 95 gorillas the researchers were watching died from October 2003 to January 2004.*


Questions

1. Assess whether great apes could be a reservoir species for Ebola. In order to do this, review each of the criteria for a reservoir species that you established in Part II.

2. Could the villagers be catching Ebola from great apes? Does your answer depend on whether great apes are the reservoir host? Justify your answer.
Part IV – Help from the Lab

Joe wants to identify which animal (or indeed, plant) living near the village might be the reservoir species for Ebola. This requires doing laboratory work with specimens collected around the village. Finding a lab where this work can be done is difficult, since Ebola is a disease with a high human mortality rate for which there is no vaccine. When researchers work with these kinds of infectious agents, they must take extra precautions, such as wearing airtight suits and carrying their own air supply. These precautions are part of a protocol called level 4 biocontainment, and there are very few such facilities in the world. However Joe knows of one person, his colleague Brian Manzunzu, who works at the National Institute for Virology in South Africa. Brian has access to a level 4 facility, so Joe sends his collection of plant and animal specimens to Brian's lab.

Brian receives the samples and considers how to test each species for its ability to support the replication of Ebola. As with any virus, Ebola cannot replicate its genome on its own. First, it must find a suitable host cell in which to replicate. This requires matching the proteins on its surface with the proteins on the surface of the host cell, like two puzzle pieces coming together with complementary shapes. Once the virus has found a matching cell type, it enters the cell, and hijacks the cell's machinery to replicate more viral genome. In essence, the infected cell becomes a virus-producing factory. It is in this way that the virus is able to make many copies of itself, which can then leave the host cell to infect many others.

Viruses are specialized, and their surface proteins can only attach to the proteins of specific host cells. Because of this, each viral species typically has the ability to infect only a specific type of cells (in one species of host). Brian considers this and reasons that Ebola should only have the ability to enter and replicate inside the cells of a very select number of species, one of which should be the reservoir species. Therefore, his plan is to subject the cells of the plant and animal samples collected by Joe to Ebola virus and observe which can sustain the Ebola virus's replication.

Brian proceeds by inoculating the plants and animals that Joe sent him with the Ebola virus. He waits several days, and then determines the concentration of Ebola virus in each plant or animal tissue. The organisms that the virus can use as a host should permit the replication of the virus, and should therefore contain more virus particles at the end of the test period.

Brian injects a known dose of Ebola on the first day (invertebrates were inoculated with a dose of $10^{3.6}$ FFU and vertebrates with a dose of $10^{4.6}$ FFU—these units are explained below). At several days post-infection, Brian obtains a tissue sample from the organism and affixes it on a microscope slide. To determine the concentration of virus in the tissue, Brian uses a technique called immunohistochemistry. This technique is described below and is diagrammed in Figure 1.

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**Figure 1.** Immunohistochemistry. A tissue or fluid sample (pink) is fixed onto a microscope slide. The tissue may contain some virus (blue rectangles). The sample is then incubated with an antibody that binds to Ebola (light blue). Bound antibodies will remain on the slide during the wash which clears away any unbound antibody. A fluorescently-labeled secondary antibody (green circle) that recognizes and binds to all antibodies is then incubated with the sample. Unbound secondary antibodies are washed away. The amount of virus present in the sample can be assessed by measuring the intensity of the fluorescence in the sample.

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“Hunting the Ebola Reservoir Host” by Black and Prud’homme-Généreux
Brian wants to determine the quantity of Ebola virus present in each tissue sample. He affixes the sample to a slide, and then incubates it with antibodies to the Ebola virus. These antibodies bind very specifically to certain proteins on the surface of the Ebola virus—they don’t bind to anything else. Unbound antibodies are washed off the slide. These antibodies are a way to tag the Ebola viruses in the sample so that it can later be detected and their quantities measured. The amount of antibodies remaining on the slide after the wash is proportional to the amount of virus in the sample. However, antibodies are too small to see with the naked eye; so how is the amount of antibody on the slide determined?

Samples are incubated with a second antibody to which a fluorescent particle has been attached (it can be seen in a microscope illuminated with a fluorescent light). These secondary antibodies bind to all other antibodies (i.e., the Ebola antibodies that were incubated with the sample in the first step) and make it visible in fluorescence microscopy. Any unbound secondary-antibodies are then also washed away. By this protocol, it is now possible to quantify the amount of Ebola virus particles on the slide.

The more viral particles present in the sample, the more Ebola antibodies bound to them, and the more fluorescently-labeled secondary antibody bound to the sample as well, and therefore the greater the intensity of the fluorescence. The number of Ebola viruses in the sample therefore correlates with the intensity of the fluorescence. The concentration of virus is reported as “Fluorescent Focus-forming Units per milliliters” or FFU/ml.

Brian found that Ebola virus was not able to replicate in any of the plants that he tested. He also found that none of the tested animals exhibited obvious symptoms of Ebola infection, nor did any die due to Ebola infection. Here are some of his data.

<table>
<thead>
<tr>
<th>Species</th>
<th>Was Any Virus Detected at Any Point in the 28 Days After Inoculation?</th>
<th>Virus Quantity Present (FFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domestic pigeon</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Painted reed frog</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Common toad</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Grey tree frog</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Tropical house gecko</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Brown house snake</td>
<td>Yes</td>
<td>$10^{1.0}$</td>
</tr>
<tr>
<td>Leopard tortoise</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Hinged-back tortoise</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Angola free-tailed bat</td>
<td>Yes</td>
<td>N/A</td>
</tr>
<tr>
<td>Little free-tailed bat</td>
<td>Yes</td>
<td>N/A</td>
</tr>
<tr>
<td>Wahlberg’s epauletted fruit bat</td>
<td>Yes</td>
<td>$10^{4.6}$ – $10^{7.0}$ Virus also recovered from feces</td>
</tr>
<tr>
<td>Multimammate mouse</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>NIH mouse</td>
<td>Yes</td>
<td>$10^{1.0}$</td>
</tr>
<tr>
<td>American cockroach</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Leafhopper</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Myrmicine ant</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Social spider</td>
<td>Yes</td>
<td>$10^{1.0}$</td>
</tr>
<tr>
<td>Millipede</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>African landsnail</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

Questions

1. Why can viruses typically infect only one host species? Hypothesize why and how certain viruses are able to “jump the species barrier” and infect different species of organisms.

2. List the species containing Ebola virus in their body at some point post-inoculation.

3. These species vary in the concentration of virus detected in their tissues. What could account for the relatively small titers (low concentration of the virus) detected in some species?

4. The data show that certain animals could replicate the virus without succumbing to it. What other capacity must your suspected host species possess to truly act as the Ebola reservoir host?

5. Based on these data, which species do you suspect of being the reservoir host, and why?

6. In this experiment, the animals were monitored for the presence of virus in their body every day after inoculation for 28 days. What do you think of this study period? Is this period sufficient to determine that the infected animals can survive long enough to pass the virus to another? Is this period long enough to show that a species is a potential reservoir species?

(Note: In humans, the mean time between exposure to Ebola and the appearance of the first signs and symptoms—i.e., the incubation period—is 12.7 days) (Eichner et al., 2011).
Part V – Further Discoveries

Upon hearing of Brian’s findings, Dr. Mombutubwa shares a few interesting anecdotes with Joe. First, there is a massive annual bat migration that takes place in April. The bats arrive in thousands or tens of thousands and settle briefly in a nearby area. This usually causes large feasts, as the bats are plentiful in the months during their stay. While there, the bats eat large amounts of fruits, on bushes and trees that are also frequented by apes as a food source. The bats are typically gone by mid-May\(^2\).

Questions

1. The maximum incubation period for Ebola in humans is 21 days (CDC, 2012)\(^3\). Develop a plausible timeline and chain of events for the spread of Ebola in this outbreak. Start when the bats arrive near the village and make a schematic diagram showing your hypothesis regarding the various ways that Ebola is transmitted to the villagers and is spreading between villagers.

2. Does this present conclusive proof that bats were the Ebola reservoir species in this epidemic? If not, what other support would you need?

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