Fighting Ebolavirus

Biomedical researchers hope the development of Ebola preventatives and treatments using nonhuman primates lessens the impact of the virus in wild African ape populations.
In the aftermath of the 2014 Ebola virus outbreak, this infamous filovirus has once again taken center stage in the biomedical research and global health communities. For many the mention of Ebola virus brings to mind the 1995 movie classic Outbreak or its source material Richard Preston’s The Hot Zone. However, for those in the laboratory animal medicine community, the critical role our animal models and staff have and continue to play in Ebola discovery research and vaccine development looms foremost in our minds. Here, we remind the reader of the history and basic pathogenesis of ebolaviruses as well as expand upon the important contributions of nonhuman primate models in ebolavirus research and highlight the largely silent impact historical and recent ebolavirus outbreaks have had on the wild African ape populations.

Figure 1A. Imaging room, animal biosafety level 4 side containing pathogens.
Ebola virus (EOBV), one of the five ebolaviruses in the Filoviridae family, causes a rare but deadly disease marked by fever, severe headache, fatigue, weakness, diarrhea, vomiting, rash, and, in some cases, both internal and external hemorrhage. The incubation period can be between 2–21 days post-exposure, but is most commonly 8 days. The case fatality rate ranges from 25–90%, with a typical rate of around 40%.

The natural reservoir host of EBOV has not been proven, but is thought to be fruit bats of the Pteropodidae family. EBOV disease outbreaks can occur from contact with the blood, secretions, organs, or other bodily fluids of infected animals such as African great apes (chimpanzees and gorillas), fruit bats, monkeys, or other animals hunted or found dead in the forests. EBOV then spreads by human-to-human direct contact with body fluids of the infected patient, or materials contaminated by body fluids. Currently, evidence of aerosol transmission is lacking. Broken skin, mucous membrane contact, or iatrogenic exposure because of the use of sharps (needles, scalpels, and glass containers) are the most common routes of exposure.

In 1967, the first filovirus, Marburg virus, was isolated and characterized during an outbreak of hemorrhagic fever in Marburg and Frankfurt, Germany and Belgrade, Yugoslavia. Laboratory workers preparing cell lines derived from African green monkey tissue became infected with this unknown pathogen, while the associated shipment of African green monkeys was normal. The source of initial infection of the nonhuman primates was unknown, but human exposure resulted in 25 primary cases including 7 deaths. Ebola virus disease (EVD) first appeared in 1976 in two simultaneous outbreaks, one in Sudan (caused by Sudan ebolavirus (SUDV), a second member of the ebolavirus genus), and the other in the Democratic Republic of Congo (caused by EBOV). The latter occurred in a village near the Ebola River, from which the virus and disease takes its name. Since that time, sporadic outbreaks occurred in Central Africa. The 2014 outbreak in densely populated Western Africa is the largest and most severe, causing over 11,000 fatalities across 6 countries on 3 continents with a case fatality rate of 39.5%. This outbreak has more cases and deaths than all other previous outbreaks combined. The most severely affected countries, Guinea, Liberia, and Sierra Leone, have weak health systems, lack infrastructural resources, and have experienced long periods of conflict and instability.

Nonhuman Primate Models of Ebolavirus Disease

Animal models of filovirus infection have been developed in mice, guinea pigs, hamsters, and nonhuman primates (NHPs). The wild-type virus replicates to high titers in NHPs and causes symptoms, including hemorrhage and shock, which are similar to those of patients with EVD. Thus, NHP models are commonly used to study candidate vaccines and therapeutics. While hamadryas baboons (Papio hamadryas), African green monkeys (Chlorocebus aethiops), and common marmosets (Callithrix jacchus) have been utilized, the features of disease in cynomolgus (Macaca fascicularis) and rhesus macaques (Macaca mulatta) appears to best reproduce the human condition among the NHP species. Cynomolgus macaques have been the species most often used for vaccine studies, while rhesus macaques have been more frequently studied in evaluating post-exposure treatments. The disease course appears on average slightly shorter in cynomolgus macaques than in rhesus macaques.

EBOV is a Centers for Disease Control and Prevention (CDC) Select Agent and requires an Animal Biosafety Level 4 (ABSL4) laboratory in order to safely handle the agent. The Integrated Research Facility (IRF), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) was designed and constructed for the study of dangerous, exotic pathogens. This facility is unique in that it is the only maximum containment laboratory in the world where conventional and molecular medical imaging equipment (Figure 1A-1B) are incorporated into the design of the facility. This capability provides investigators with unique tools to dissect disease pathogenesis, evaluate the ability of animal models to recapitulate human disease, and test candidate countermeasures.

All comparative medicine staff members at the IRF require a Select Agent security clearance before accessing the vivarium. A formal 3-week course with hands-on experience in a mock ABSL4 facility, followed by 100 hours of mentoring in the vivarium must be completed before unescorted access is permitted. Forty entrances into and out of ABSL4 labs wearing positive-pressure suits are also required for all new employees. Adequate time must be allotted to enter and exit the suit lab. To enter the outer change rooms, a card reader and iris scan is performed. Individuals change into dedicated scrubs prior to passing through the water shower into the suit room. Each positive-pressure suit must be carefully checked prior to donning, and the vivarium is then accessed through the chemical shower. The suit has integrated high efficiency particulate air (HEPA) filters, and air is supplied through air hoses which are located throughout the vivarium and laboratory space (Figure 2). When leaving the vivarium, each person goes through a 7-minute chemical shower while in the suit, followed by a body shower for a minimum of 5 minutes prior...
to returning to the outer change room. All scrubs are auto-
claved out of the lab at the end of each day. Unlike a tradition-
al vivarium, staff members cannot work an 8-hour day inside
the ABSL4 facility. The constant airflow in the suit can lead to
dehydration, and there is no mechanism for restroom breaks
while in a suit. In addition, working with NHPs requires two
people to be present at all times, so fully staffing the ABSL4
necessitates approximately twice the number of workers as
compared to an ABSL2 facility.

The IRF is typically studying multiple pathogens at once,
thus requiring stringent infection control procedures. The
vivarium provides a procedure/support room for each of the
animal rooms to limit transport of animals and the possibility
of cross-contamination. When transporting infected animals
to the imaging suites or aerobiology, they are anesthetized
and placed in a sealed container (Figure 3). Laboratory staff
attend to animals at all times, and animals are never left in a
container for longer than a few minutes. The containers are
useful in the containment of radioactive materials used in
Positron Emission Tomography and Single Photon Emission
Computed Tomography. Whenever possible, comparative
medicine staff are assigned to duties based on pathogen, but
the veterinarians and technicians must take chemical showers
when moving between rooms of different pathogen status.
Taken together, these factors must be considered and ad-
dressed for successful operation of an ABSL4 facility working
on dangerous, exotic pathogens.

Ebolaviruses in Wild African Ape Populations
African great apes include chimpanzees (Pan troglodytes),
bonobos (Pan paniscus), and two species of gorillas (Gorilla
gorilla and G. beringei). Like humans, African apes are highly
susceptible to ebolaviruses although only chimpanzees and
western gorillas are known to have died from natural out-
breaks. Along with poaching and habitat loss, EVD is current-
ly considered a major threat to African ape populations.

The threat of EVD to great apes was not recognized until
after the first known human EVD outbreaks in the late 1970s.
Broad wildlife surveys during and after those first outbreaks
found no evidence of ebolaviruses in any wildlife. Shortly after
the 1989 Reston virus (Reston ebolavirus) outbreak in U.S.
laboratory macaques (Macaca fasicularis) connected nonhuman
primates to that species of ebolavirus, the great ape risk in
Africa also became apparent. Multiple 1992-94 chimpanzee
die-offs in Cote d’Ivoire were eventually recognized as EVD,
caused by another new ebolavirus species– Tai Forest ebolavirus,
after a field researcher was infected and eventually recovered
from EVD. In addition, a series of human EVD outbreaks
from 1994-2004 in Gabon and Republic of Congo were
known or suspected of being initiated by human contact with
dead gorillas and chimpanzees.

These human EVD outbreaks in early 2000s were concur-
rent with massive great ape die-offs in central Africa. The scale
of these ape die-offs is difficult to calculate but most experts
agree that about 10-20 thousand western gorillas, including
some entire local populations, and probably a smaller number
of chimpanzees have died from EVD across Africa in the past
few decades. While this death toll is comparable to the human
death toll from the EVD outbreak in West Africa, it should be
noted that it represents ~10-20% of the total population esti-
mate of western gorillas. There have not been any known large
die-offs of apes since the early 2000s although monitoring
these wild populations is very limited. The 2014 human EBOV
outbreak in West Africa is not known to have affected any
great apes nor in fact had any observed impact on any wildlife.

Protecting great apes from EVD is problematic but would
have the dual benefit of both helping to conserve these endan-
gered species and also lowering the risk of human outbreaks,
which are often started by contact with apes that have died from
EVD. The efficacy of a variety of ebolavirus vaccines shows some
promise for helping to protect apes, but the issue remains vaccine
delivery. While some individual apes such as those habituated
to human contact can be directly darted (Figure 4) or even fed
cricket, which might protect small groups or populations, the

Figure 2. Animal biosafety level 4 vivarium corridor at the Inte-
grated Research Facility, NIAID, NIH.

Figure 3. Prevention of cross contamination when transporting
animals through the corridor.
scale required to protect whole populations and species scattered across large and mostly remote tropical forests will likely require a different approach, such as self-disseminating vaccines.

Summary
NHP models of Ebola virus disease have led to advancements in the field of vaccine development and therapeutic assessment. The Division of Clinical Research, NIAID is performing clinical trials in Western Africa on a potential vaccine. Partnership for Research on Ebola Vaccines in Liberia (PREVAIL) is a phase II/III randomized, double-blind, placebo-controlled study of the chimpanzee adenovirus 3 (ChAd3-EBO Z)-based vaccine and the vesicular stomatitis virus (VSVΔG-ZEBOV)-based vaccine. The vaccine was tested in macaques and provided protection against challenge with EBOV. A second trial, PREVAIL II: A Multicenter Randomized Safety and Efficacy Study of Putative Investigational Therapeutics in the Treatment of Patients with Known Ebola Infection is ongoing. The trial is a multi-country EVD treatment trial comparing ZMapp™, a cocktail of three monoclonal antibodies, to current optimized standard-of-care, including intravenous fluids, balancing electrolytes, maintaining oxygen status and blood pressure, and treatment of secondary infections. ZMapp was used to treat one of the patients evacuated from Liberia in 2014. Other potential vaccines and therapeutics are currently studied in laboratory animal models, and it is to be hoped that these agents will advance to clinical trials.

As noted previously, all experimental inoculation requires ABSL4 conditions and work practices. The importation of Reston virus to United States in the late 80s and 90s led to key additional guidance and oversight of primate importation and quarantine practices in this country. Nonhuman primates continue to be important to the study of Ebola in the biomedical research setting and in the wild. Animal models are a critical component of vaccine development and the laboratory animal community plays a key role in supporting this essential research. As presented here, the impact of Ebola research truly goes beyond human and domestic animal health to the overall health and long-term survival of wild nonhuman primate populations. These populations, along with research nonhuman primate models, will continue to help us further understand this virus and develop measures to prevent its spread and impact on key populations.

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