

Overview

The Role of Animal Research in Pandemic Responses

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The significant advances made by the global scientific community during the COVID-19 pandemic, exemplified by the development of multiple SARS-CoV-2 vaccines in less than 1 y, were made possible in part because of animal research. Historically, animals have been used to study the characterization, treatment, and prevention of most of the major infectious disease outbreaks that humans have faced. From the advent of modern ‘germ theory’ prior to the 1918 Spanish Flu pandemic through the more recent Ebola and Zika virus outbreaks, research that uses animals has revealed or supported key discoveries in disease pathogenesis and therapy development, helping to save lives during crises. Here we summarize the role of animal research in past pandemic and epidemic response efforts, as well as current and future considerations for animal research in the context of infectious disease research.

Abbreviations: ACE2, angiotensin-converting enzyme 2; CEPI, Coalition for Epidemic Preparedness Innovations; RSV, respiratory syncytial virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TMPRSS2, transmembrane protease serine 2

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From the moment it began in late 2019, the COVID-19 pandemic has been met with remarkable scientific effort. In less than 1 y, substantial progress has been made in understanding the behavior of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, characterizing the damage it inflicts on the body, and developing safe and effective vaccines. Research in animals has provided many breakthroughs, as it has for most significant outbreaks in the past. Animals have been used to study infectious diseases long before disease-causing microorganisms were known to exist. Animal research in response to pandemics, past and present, provides a clear example of how such research can best serve the scientific community in the event of future outbreaks and other disease conditions. Response to a pandemic requires quick action to identify the emerging diseases, characterize transmission and pathogenesis, and develop preventative measures and therapies. Ideally, through the surveillance of environments and animal populations that may harbor pathogens with pandemic potential and through preclinical and basic science research in virology and vaccinology for diseases that are suspected to be potential threats, the pandemic response should begin before a disease gains the ability to spread easily through a population. In this article, we discuss the importance of animal research in all aspects of pandemic research response and the vital role it continues to play today.

Early Pandemic Research

The 1918 ‘Spanish flu’ influenza outbreak is one of the deadliest infectious disease events in modern history. Roughly a third

of the world’s population was infected with the 1918 H1N1 influenza A virus, and an estimated 50 million people died from the infection.^{4,11} Although Spanish flu was not the first pandemic that humans faced, its outbreak occurred when science and medicine had been revolutionized by new knowledge about the cause and treatment of disease. Public health officials at the time recommended wearing masks and practicing good hygiene to prevent the spread of illness.⁸⁸ These measures were instituted even though the etiologic agent of influenza had not been identified.^{4,88}

The prevailing theory of previous centuries was that ‘bad air’ from rotting food, waste, or other organic materials created a noxious ‘miasma’ that led to epidemics.³² The ‘germ theory of disease’—the now-accepted idea that microorganisms such as bacteria and viruses cause illness—superseded the ‘miasma theory’ in the late 19th century, precipitating a great search for disease-causing organisms.^{4,44} Leading this hunt was Robert Koch, a physician–scientist who developed a set of postulates that are still used to demonstrate the cause-and-effect relationship between infection with a specific agent and clinical disease. In the 1880s, Koch and fellow researcher Friedrich Loeffler used animal models to prove that bacteria caused tuberculosis, cholera, anthrax, and more.^{44,93} These models were fundamental to Koch’s postulates, allowing scientists to systematically link infection with a microbe to subsequent disease in the model organism (Figure 1).

The groundbreaking work done by Koch and colleagues led to a golden age of bacteriology.⁵ When the 1918 H1N1 outbreak emerged, scientists immediately began searching for the causative agent. Viruses were challenging to isolate, and little was known about them; therefore, bacteria were theorized to be the cause of influenza. At the time, many scientists mistakenly believed the bacteria *Haemophilus influenzae*—an opportunistic pathogen often found in flu-ravaged lungs—lay behind the

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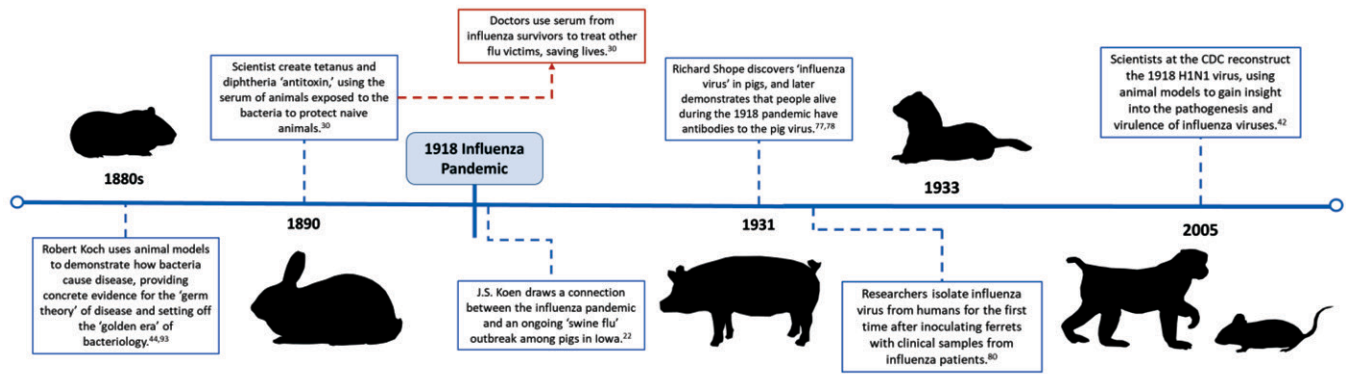


Figure 1. 1918 influenza pandemic: timeline of significant contributions from animal research.

disease, but they had trouble consistently isolating it from sick patients.^{4,26,84} The actual etiologic agent of influenza remained a mystery throughout the pandemic.

In 1918, the veterinarian JS Koen, who worked for the US Bureau of Animal Industry in Iowa, noted that pigs at the National Swine Show and Exposition in Cedar Rapids were experiencing an influenza-like illness similar to the one concurrently affecting humans (Figure 1). He called this new illness 'swine influenza' and, a year later, wrote:

"The similarity of the epidemic among people and the epizootic among pigs was so close...as to present a most striking coincidence, if not suggesting a close relation between the two conditions. It looked like 'flu,' it presented the identical symptoms of 'flu,' and until proved it was not 'flu,' I shall stand by that diagnosis."²²

This diagnosis was not popular among swine farmers, who feared that the negative connotation would make their product seem unhealthy, but years later Koen's observation garnered the attention of another scientist. In 1931, Richard Shope, a virologist working with the Rockefeller Institute, investigated Koen's 'swine flu' on farms in Iowa.²² Previous investigators had failed to isolate the specific agent behind the swine flu, as they had often inadvertently filtered out the small viral particles in their attempt to isolate bacterium. Shope repeated these experiments in pigs, taking filtrate from mucus collected from sick pigs and inoculating healthy swine. When the pigs became sick, Shope concluded that a 'filterable agent,' such as a virus, was the cause of swine influenza (Figure 1).⁷⁷ He also concluded that *H. influenzae*, which also had been isolated from pigs, worked with the virus to cause more severe disease—exactly what had been seen in humans during the 1918 pandemic. Shope went on to show that people old enough to have experienced the 1918 pandemic carried antibodies against the swine virus, drawing a concrete connection between the swine flu and the Spanish Influenza.⁷⁸

Inspired by Shope's work, in 1933, British physician and scientist Dr. Wilson Smith and colleagues obtained filtered throat washings from human influenza patients and exposed a variety of animals to the material.^{80,84} The investigators had little success infecting animals until they turned to ferrets, which were available at their institution because of ongoing canine distemper virus research.²⁵ Ferrets were the only animals that became sick and consequently they proved to be an excellent model for human disease in regard to both the clinical progression and transmission of influenza (Figure 1). In addition, the researchers used convalescent human sera protect ferrets against subsequent passage of the virus and showed that ferrets inoculated with Shope's swine influenza virus were immune to the human strain of the virus. When accidental ferret-to-human transmission

of influenza occurred after a sick animal sneezed near an investigator's face, Smith and colleagues further demonstrated that the virus grown in ferrets caused disease in humans and could subsequently be isolated again to infect other ferrets.⁸¹

With these early studies, the scientists isolated the influenza A virus from humans and developed an animal model—still critical to modern influenza research—with which laboratory research could be performed. Smith and colleagues noted that "direct experiments on man are fraught with difficulties" and discussed the potential value of parallel clinical and laboratory research during an ongoing epidemic.^{25,82} Dr Smith, along with scientists CH Andrewes and CH Stuart-Harris, went on to study influenza immunity and develop vaccines in ferrets and mice, followed by human volunteers, with the intention to deploy larger-scale clinical trials during the inevitable next epidemic.^{81,82} This foresight to initiate preclinical trials in advance of anticipated disease outbreaks is notable and reflects modern goals for animal research on pandemic diseases.

The breakthroughs achieved with animals before, during, and after the 1918 influenza pandemic exemplify the fundamental role animal research has played in humankind's fight against emerging infectious diseases. Scientists have expanded on this research throughout subsequent decades of epidemic response.

Identifying Etiology

Despite having modern techniques to help identify disease-causing organisms today, animals remain instrumental in understanding how pathogens infect, replicate, spread, and cause disease in living organisms. Categorizing the type of agent causing disease is an obvious critical first step in an outbreak response. As evidenced by the work of researchers on the bacteria *H. influenzae* during and after 1918 influenza pandemic, identifying the primary etiologic agent of a disease can be difficult when other factors such as secondary infections, coinfections, host immune response, and comorbidities complicate data interpretation.^{4,26,84} Animal model systems allow researchers to conduct highly controlled experiments with well-defined subjects in order to isolate and identify novel infectious pathogens in the face of an outbreak.

Koch's postulates served as guidelines for the identification of disease-causing agents for years after his original research was published.^{44,93} Shope found that although *H. influenzae* could be consistently isolated from pigs sick with swine influenza, the bacteria did not induce disease unless the animals were also inoculated with the influenza virus.⁷⁷ During an epidemic in Hong Kong, Alexandre Yersin, a Swiss-French bacteriologist and physician, discovered the etiologic agent of the bubonic

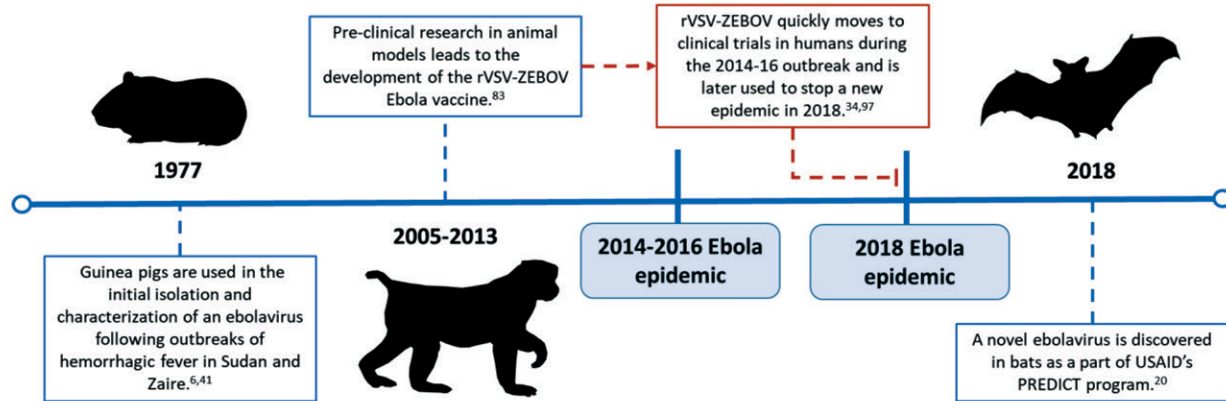


Figure 2. Ebola virus epidemics: timeline of significant contributions from animal research.

plague after isolating bacteria from the swollen lymph node of a patient, injecting it into mice and guinea pigs, and observing disease. His careful study of the bacillus found in the buboes of deceased plague patients and infected rodents, which developed similar clinical signs and dissemination of the bacteria, led to advances relevant to both transmission and treatment.⁸ Guinea pigs were also used to identify and characterize Ebola viruses after an outbreak of hemorrhagic fever among people in Sudan and Zaire (now the Democratic Republic of the Congo) in 1976 (Figure 2).^{6,41} Guided by epidemiologic and clinical data, the study teams working on the hemorrhagic fever outbreaks collected various pre- and postmortem samples and inoculated guinea pigs intraperitoneally with sample suspensions. The animals developed a 5-d course of febrile illness, and successive passage of infected guinea pig blood into additional guinea pigs produced a fatal febrile disease. The Ebola virus from these animals was isolated and cultured in Vero cells and was further characterized using immunofluorescent and electron microscopy.^{6,72} Similarly, West Nile virus was discovered in 1937 after mice were inoculated with serum isolated from a febrile patient in Uganda (Figure 3). Although the patient initially was assumed to have Yellow Fever, the mouse model and subsequent studies in NHP identified the etiologic agent as a novel virus with distinct pathogenicity and immunogenicity.⁷⁵

The discovery of HIV, the virus which causes AIDS, is a more recent example of how animals have been used to study connections between pathogens and human disease. HIV remains a global health threat: approximately 38 million people worldwide were reported to be infected in 2019.³⁵ AIDS was first observed in the United States in 1981, after outbreaks of typically rare opportunistic infections among otherwise healthy men. In 1983, French and American research groups independently uncovered a novel retrovirus in patients with AIDS.⁷⁶ Retroviruses had first been described by the physician–veterinarian team of Wilhelm Ellermann and Oluf Bang in 1908. They found that chickens developed a form of leukemia caused by viral infection.¹⁸ Many other animal viruses were subsequently discovered to be retroviruses, including the Visna–Maedi virus, which causes a progressive immunosuppressive disease in sheep.⁸⁷ In 1985, researchers Janice Clements and HIV codiscoverer Robert Gallo published research that compared HIV and Visna–Maedi virus.²⁹ This research helped to establish the identity of HIV as a lentivirus and provided initial clues regarding the mechanism of HIV infection. In addition, the sheep Visna–Maedi infection model was recognized as a model system for in vivo testing of antiHIV drugs.⁸⁷

Animal research provides a foundation for current science on pathogenicity. In 2002, an outbreak of unidentified severe respiratory disease began in the Guangdong province of China. The illness was initially termed Severe Acute Respiratory Syndrome (SARS) because the microorganism causing the clinical signs was unknown.⁶⁸ After a promising viral candidate was identified by microbiologic studies,^{50,68,69} scientists at Erasmus University fulfilled Koch's postulates by verifying that the newly isolated coronavirus (SARS-CoV-1) caused disease in macaques and was likely the primary etiologic agent of SARS.²⁷ When the COVID-19 pandemic began in December 2019, previous research with animals and animal viruses permitted scientists to rapidly place the novel coronavirus within the *Coronaviridae* family tree, suggesting a probable zoonotic origin for SARS-CoV-2 and a likely mechanism of host-cell entry by the virus.¹⁰¹ Research with sophisticated animal models and modern genetic and molecular techniques will continue to provide key information about the relationship between pathogens and disease.

Describing Pathogenesis

Close scrutiny of COVID-19 over the past year has revealed a complex constellation of symptoms and conditions beyond what is typically associated with seasonal coronaviruses. SARS-CoV-2 has been implicated in neurologic, cardiovascular, and autoimmune pathology. The complex interplay between the novel coronavirus and various systems of the body is not yet well understood and raises many questions about disease pathogenesis. The advent of organoid and other complex tissue model systems has allowed the study of organ-specific problems,⁴⁵ but these systems do not model the entire body. Animal models of SARS-CoV-2 are important in answering these questions.

Animal research has contributed to our knowledge of disease pathogenesis, transmission, virulence factors, and immune response for most major infectious disease outbreaks. Research with rats conducted by Paul-Louis Simond in 1898 illustrated for the first time that flea-borne transmission was one of the main mechanisms behind the spread of bubonic plague.^{33,71} Although not well accepted at the time, Simond's experimental work with animal models of disease transmission eventually changed the way plague prevention and control was approached.²¹ Studies of influenza in ferrets, birds, and pigs have clarified viral tropism in the respiratory or gastrointestinal tracts and improved our understanding of the pathogenesis, transmission, and the epidemic potential of various flu strains.⁶⁶ Animal models have

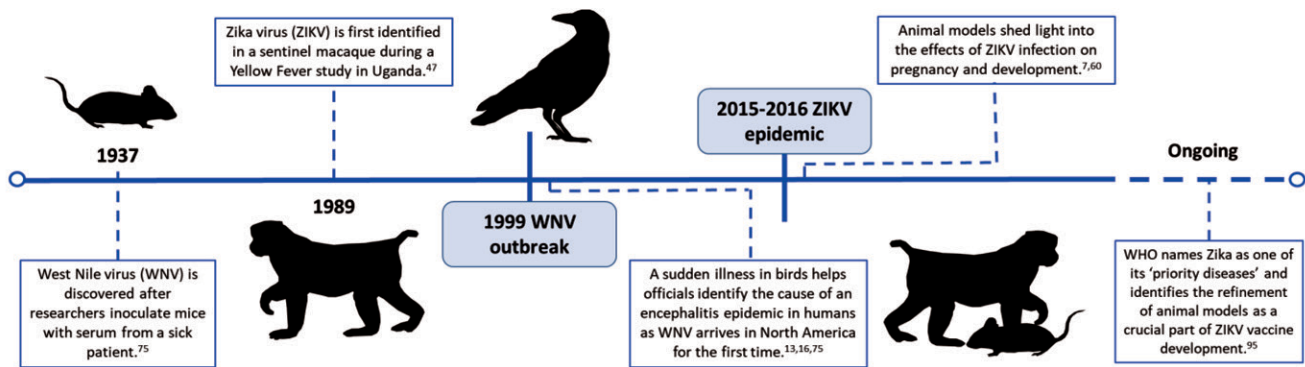


Figure 3. West Nile virus and Zika virus outbreaks: timeline of significant contributions from animal research.

been essential for investigating deadly human diseases such as Ebola and Nipah, which occur only sporadically and with high mortality in people and are therefore challenging to study.

The 1918 H1N1 influenza pandemic was known from historical records to have an unusual characteristic: the virus was especially deadly among healthy adults from 15 to 34 y of age. This feature is in contrast to other seasonal or pandemic flu strains, which typically kill the very young or old.⁴² To understand the behavior of this virus, in 1997, a research team excavated a mass grave in Brevig Mission, Alaska, where nearly 80 of the village's inhabitants had been buried after dying from the 1918 influenza virus. Scientists were able to extract 1918 virus genetic material from the lungs of a deceased woman preserved in the permafrost and subsequently sequenced the hemagglutinin (HA) gene. This innovative work ultimately allowed a team at the Centers for Disease Control and Prevention to reconstruct a recombinant pandemic flu.⁴² A mouse model was used to characterize infection with 1918 H1N1, which confirmed that the influenza strain was incredibly adept at replicating in the lungs and over-activating the host immune response. The virus was highly lethal to the mice and potentially explained why healthy adults with robust immune systems fared worse during the pandemic.⁴³ Because the original 1918 strain no longer circulates in humans, much of what we know about its virulence is based on animal research. This research gave us insight into the complicated interplay between virus and immune system, including the role of specific genes and cytokines in triggering severe disease (Figure 1).^{17,43} Such knowledge, in turn, can help us identify danger signals in future flu strains.

Basic virology research in animals is also important during an outbreak. During the 2015–2016 Zika virus epidemic, animal models facilitated the progression from epidemiologic observations into viral pathogenesis research (Figure 3). Mouse and NHP models were used to correlate Zika-associated neurologic disease in humans with the neurotropic behavior of the virus in vivo.^{7,58,60} Both mice and NHP have similar placentation to humans, thus serving as useful models for intrauterine Zika virus infection and developmental studies.⁵⁸ These models offered crucial insight into infection during pregnancy and subsequent fetal CNS lesions.^{7,58,60,74} Mouse models of infection also established that the virus replicated in the testicles and female reproductive tract and therefore helped to explain reports of sexual transmission in humans.^{58,60,73} Ongoing Zika vaccine research uses animals to study acquired immunity and cross-protection in the context of distinct Zika virus lineages and other arboviruses, such as Dengue and Chikungunya (Figure 3).²³ Given that

overlap between these insect-borne diseases is common in the endemic range of Zika, animal studies are necessary to unravel the complexity of the immune response to Zika in association with prior or concurrent infections with other viruses. For example, a mouse model has been used to generate Zika vaccine candidates that protect against both antibody-dependent disease enhancement and disease exacerbation in vaccinated persons who produce deleterious antibodies that enhance subsequent natural infection.²³ Investigation of these problems in preclinical models is imperative before vaccine candidates are tested in virus-endemic regions.

COVID-19 pathobiology research has capitalized on animal studies performed with regard to the 2002 SARS outbreak. Like SARS-CoV-2, SARS-CoV-1 uses angiotensin-converting enzyme 2 (ACE2) as a receptor and means of entry into host cells.⁶⁸ Studies in rodents, combined with sequencing data from human tissues, elucidated the expression pattern of ACE2 through the body, explaining viral tropism and subsequent clinical disease.^{55,68} ACE2 knockout studies in mice proved that the protein was necessary for SARS-CoV-1 infection.⁵¹ This earlier work was important when SARS-CoV-2 was first described; researchers were quickly able to culture, isolate, and characterize the virus by using ACE2-expressing cells.¹⁰¹ The cell-surface transmembrane protease serine 2 (TMPRSS2) is another protein that facilitates the entry of SARS-CoV-1 and -2 into host cells.^{36,37} TMPRSS2-knockout mice infected with SARS-CoV-1 fail to develop pneumonia or weight loss, whereas wild-type mice develop significant clinical disease.⁴⁰ A TMPRSS2 inhibitor, camostat mesylate, has been shown to block SARS-CoV-1 infection in mice and is being investigated as a potential therapeutic agent for COVID-19.^{36,102}

Animal studies have also been used to investigate COVID-19 transmission and the behavior of viral variants. The scientific community has debated the contribution of airborne transmission (i.e., the dissemination of virus through small-particle aerosols suspended in the air) in COVID-19 spread.^{3,31} Studies in ferrets demonstrated that airborne exposure alone could result in transmission of SARS-CoV-2.^{46,52} Research such as that supports epidemiologic observations and facilitates controlled investigation into the mechanism of COVID-19 spread. Although next-generation genetic sequencing has given researchers the ability to closely monitor changes in circulating SARS-CoV-2 variants, the significance of a new mutation in the virus cannot necessarily be determined based on sequence alone. However, validated animal models can be used to test the effects of novel mutations on virulence or transmissibility, thus providing data

to inform public health actions. Animal models of SARS-CoV-2 infection were used in concert with population genetic modeling to study the D614G mutation of the SARS-CoV-2 spike protein, thereby offering initial evidence that the variant had greater transmissibility.^{38,54} Animal models are already being used to test mutations found in the novel, fast-spreading α variant and other variants.⁹ Similar studies will likely remain useful as the virus continues to circulate and mutate.

Developing Vaccines and Therapeutics

In addition to revealing the nature of the agent causing an outbreak, animal models have long been important in the development of treatments and vaccines. Antibody therapy—whether convalescent serum therapy or monoclonal antibody therapy—has been a mainstay of infectious disease treatment since the late 1800s.³⁰ The idea of ‘passive immunization’ began with the work of scientists Emil von Behring and Shibasaburo Kitasato, who showed that serum collected from rabbits that had been inoculated with tetanus or diphtheria toxin (i.e., convalescent serum) could prevent illness when administered to naive animals (Figure 1). After this breakthrough, standardized diphtheria and tetanus antitoxin for human use were produced in horses and cattle. Passive immunization via the administration of convalescent sera was also used during the 1918 influenza pandemic, thus reducing fatality rates in newly infected persons by nearly 50% (Figure 1).^{7,30,100} Convalescent serum and monoclonal antibody products have been used therapeutically in the treatment of several recently emergent infectious diseases, including SARS, Ebola, and COVID-19, and through genetic engineering, animals can be used to produce highly specific human monoclonal antibodies.^{24,61,63,85}

For cases in which human challenge studies would not be ethical or feasible, the FDA Animal Rule may be invoked to facilitate the approval of a medical product through the use of animal studies alone.⁸⁹ Such measures are important in preparing for events such as biologic terror attacks in which potential biologic weapons such as anthrax or smallpox cannot safely be given to volunteers and when natural disease occurs too sporadically to assess vaccines or therapeutics in the human population. To achieve approval through the Animal Rule, the effect of the product must be established in either multiple animal species or a single well-characterized animal model, and additional requirements surrounding pharmacokinetic and pharmacodynamic data must be met.⁸⁹ The FDA Animal Rule has been used to approve treatments for smallpox, inhaled anthrax, and others.^{90,91}

Animal use is also instrumental in the development of vaccines. The recipe for an effective, safe vaccine is not straightforward, given that pathogen biology, the innate and adaptive immune responses, and the recipient’s health and socioeconomic background can all affect vaccine performance. Although strategies surrounding vaccine design have changed over the years, animal models remain the optimal biologic systems for the investigation of complicated immunologic questions and therefore are a critical part of assessing efficacy and safety of vaccines before they enter human trials. For example, animal models of respiratory syncytial virus (RSV) infection have allowed researchers to determine the mechanisms behind the phenomenon of ‘disease enhancement’ in children vaccinated against RSV and subsequently exposed to the virus. These models are now used to predict the safety of new RSV vaccines under development.⁵³ Animal research has had an important role in the development of vaccines against many diseases, including measles, tuberculosis, polio, and influenza.²⁸

For many emerging and reemerging infectious diseases without known therapeutics, the use of vaccines is essential to interrupt disease transmission and prevent further morbidity and mortality during an epidemic. The response to the 2014–2016 Ebola outbreak in West Africa is an example of how prior animal research facilitated the rapid initiation of a lifesaving vaccine trial.⁸³ At the outset of the public health emergency, WHO experts—in collaboration with public health organizations, pharmaceutical companies, and regulatory agencies—rapidly developed a plan to review and accelerate the development of Ebola vaccine candidates based on efficacy and safety data from NHP research (Figure 2).³⁴ The rVSV-ZEBOV recombinant Ebola virus vaccine was thus able to move rapidly from preclinical research to clinical trials in humans. In 2018, this vaccine was able to effectively end the Ebola outbreak in the Équateur province of the Democratic Republic of the Congo while the outbreak was still in its early stages, and the rVSV-ZEBOV recombinant vaccine remains a vital tool in response to Ebola (Figure 2).^{94,97}

Vaccine technology using mRNA saw its clinical debut during the COVID-19 pandemic. From the publishing of the SARS-CoV-2 genome sequence in January 2020 to the first therapeutic doses given in December 2020, the Pfizer-BioNTech (BNT162b2) and Moderna (mRNA-1273) COVID-19 vaccines were developed in only 11 mo.^{2,39} Vaccine development timelines typically stretch into several years—on average 10 y—so the speed with which these mRNA vaccines were produced was unprecedented.⁸⁶ Numerous factors led to the rapid progress in vaccine creation,⁸⁶ but although the technology had not yet been used widely in a clinical setting, a long history of mRNA vaccine research in animals meant that a robust body of preclinical data was already available before the COVID-19 pandemic began.^{2,65}

Proof-of-concept research for mRNA therapeutics began in the early 1990s with studies in mice and rats. Animals given intramuscular injections of RNA were monitored for production of the associated protein. In vivo studies continued to be essential, because mRNA was quickly found to be challenging to work with: it is easily degraded by extracellular host enzymes, has high innate immunogenicity, and can be difficult to deliver to target cells.⁶⁵ Over the subsequent decades, studies in both large and small animals supported significant advances in modifying mRNA for stability and immunogenicity. Studies to develop novel vaccine delivery vehicles, such as lipid nanoparticles, were performed in animals to assess safety and efficacy.^{39,65} Building on this thorough preclinical evidence, vaccine candidate BNT162b moved into rodent and NHP studies just months after the initial publication of the SARS-CoV-2 genome sequence.⁹² Vaccine candidate mRNA-1273 moved into phase I clinical trials about 2 mo after sequence availability and into phase II and III trials 5 and 6 mo later, respectively.³⁹ Further research into mRNA vaccine technology—and other vaccination platforms—ideally should be performed for emerging disease candidates in advance of an outbreak.

In this vein, the success of the Ebola vaccine development program led WHO to create an *R&D Blueprint for Action to Prevent Epidemics*,^{95,96} described as “a global strategy and preparedness plan that allows the rapid activation of research and development activities during epidemics.” The R and D Blueprint outlines a list of priority pathogens (such as novel coronaviruses, Zika virus, and Nipah virus) that present global disease threats.^{95,96} The Oslo-based Coalition for Epidemic Preparedness Innovations (CEPI), a nonprofit organization that finances research projects, is working from this blueprint to develop vaccines against these priority pathogens before an outbreak or in rapid response to one.¹⁵ WHO and CEPI represent major

players in the international effort to reduce the time between the identification of an outbreak and approval of diagnostics, therapeutics, and vaccines. Both have emphasized the importance of preclinical studies in animals in laying the groundwork for clinical trials, as was done during the 2014–2016 Ebola epidemic and COVID-19 pandemic. In response to COVID-19, CEPI, the Geneva-based Gavi (the Vaccine Alliance), and WHO are coleading COVAX, with key delivery partner UNICEF, to ensure equitable access to COVID-19 vaccines and to end the acute phase of the pandemic by the close of 2021.¹⁵

Pandemic Preparedness

Scientists have taken a ‘One Health’ approach to pandemic research throughout history, even before the concept was given a name. When West Nile virus first arrived in the United States in 1999, doctors and scientists initially struggled to identify the agent at the root of the outbreak of encephalitis that erupted in New York City. Diligent investigation of a concurrent avian mortality event by veterinary pathologist Tracy McNamara led to the CDC’s isolation of West Nile virus (Figure 3).^{13,16,75} Reston ebolavirus was discovered after an outbreak of hemorrhagic fever in a colony of research macaques; blood samples from their human caretakers showed that they had been exposed to the virus, although none became ill.^{14,59} Alexandre Yersin demonstrated the origin of human plague epidemics for the first time when he noticed dead rats littering the streets of an affected city and subsequently isolated the same bacillus—now eponymously called *Yersinia pestis*—from both rats and humans.^{8,33} These examples underscore the importance of considering animal health in the context of emerging infectious diseases. Indeed, the spillover of SARV-CoV-2 into pets, zoo animals, and farmed mink highlights the pervasiveness of the human–animal interface.^{56,49,57,67}

An estimated 75% of emerging and reemerging infectious diseases are zoonotic.¹² Wild animals, livestock, and even pets can act as sentinels, alerting us to impending outbreaks or emerging pathogens. Zika virus, which rapidly spread to the Americas during the 2015–2016 epidemic, was first described in 1947 in a rhesus macaque in a forest of Uganda (Figure 3). Macaques were used as sentinels for the yellow fever virus by being placed in cages built into the tree canopy as part of a study to detect the virus circulating in the forest. When a macaque developed a fever, researchers took a sample of its blood and injected it into the brains of mice, which also fell ill. This newly identified virus was later named ‘Zika’ after the forest in which it was discovered.⁴⁷

Using animals as sentinels for potentially dangerous novel viruses is not a new idea, but is being increasingly recognized as important. In 2009, the United States Agency for International Development initiated the PREDICT program, a surveillance system designed to identify pathogens with zoonotic or pandemic potential before they caused outbreaks. The program, which ran through 2019, sent researchers to look for animal viruses in ‘hot spots’ of animal–human interaction; the program also provided disease outbreak prevention training and strengthened medical laboratories in developing countries.¹⁰ Through the investigation of areas with high biodiversity, dense human population, and environments where animals and humans have close contact, PREDICT identified nearly 1000 new viruses—including a new species of Ebola virus—from animal samples (Figure 2).^{10,20} Bat coronavirus RaTG13, the closest known relative to SARS-CoV-2, was identified through surveillance of wild bat populations near a mining town in Yunnan province, China. Researchers have discovered that wild

horseshoe bats have other circulating coronaviruses that are intrinsically capable of using the ACE2 receptor.^{48,101}

‘Virus hunting’ programs such as PREDICT are valuable for understanding what pathogens are circulating in potential animal or environmental reservoirs, but determining which of these pathogens are an actual threat is more challenging. The emergence of ‘ have identified crucial areas for acceleration in biomedical research and product development to prepare for Disease X, including animal model development, validation, and standardization. One group of authors recognized “insufficient available and standardized animal models” as a challenge facing the development of diagnostics, vaccines, and therapeutics during a Disease X epidemic and recommended expanding and curating databases of existing animal models to help researchers and scientific advisory boards to generate rigorous, reproducible research.⁷⁹ These principles were implemented during the current pandemic: the WHO R and D Blueprint team established an ad hoc COVID-19 modeling expert working group (WHO-COM), composed of more than 150 scientists from around the world, with the goal of advancing the development of COVID-19 medical countermeasures. WHO-COM’s aim is to provide a platform to share data, coordinate preclinical studies, foster collaboration, and promote the 3Rs principles to guide international efforts in modeling human disease.^{62,98} The group met in December 2020 to address the emerging SARS-CoV-2 variants, discussing plans for pathogenicity and transmission studies using COVID-19 animal models and the distribution of reagents and sera to group members to facilitate research on virus immune evasion.⁹⁹ Coordinated action such as this could be leveraged to standardize research on other infectious diseases in preparation for future epidemics and to support rigorous and ethical research during a public health crisis.

Animal research is necessary to elucidate the risks inherent at the human–animal interface and to predict and prevent future pandemics. For example, experimental SARS-CoV-2 infection studies not only helped identify animal models of disease but also revealed potential viral reservoir species. White-tailed deer (*Odocoileus virginianus*) were permissive to SARS-CoV-2 infection in the lab, shedding virus from their respiratory tissues and transmitting the virus to other deer.⁶⁴ In light of these data, researchers examined the blood of wild white-tail deer in the northeast United States and found that 40% of the animals had antibodies to SARS-CoV-2.⁵⁶ These findings are potentially troubling, given that wildlife reservoirs could promote the mutation and adaptation of viral strains and even reemergence of disease into new populations. Experimental transmission studies could also aid in the identification of species that may provide sources of future outbreaks, such as livestock or common commensal species that have frequent overlap with humans and human environments. Similar studies will be key to determining characteristics of pathogen or host species that permit movement of disease between animal and human populations, potentially offering critical information about pending outbreaks. These efforts, in concert with improvement and standardization of animal model design, can be a valuable component of pandemic countermeasures.

Conclusion

Comparative medicine has been an integral part of outbreak response research even before the first disease-causing organisms were identified. Over the course of history, people have worked with animals to increase our understanding of emerging infectious diseases, where they come from, and how to prevent them. Although scientific advances over the past century have

changed the way that we explore and combat disease, the field of infectious disease research has continuously highlighted the value of animal research inside the lab and out. Animal models allow for rapid, controlled investigation into disease processes, pathogen biology, and the development of therapeutics and vaccines, but these model systems must be used in ways that promote ethical, rigorous, and reproducible science. Rarely will one model perfectly capture all aspects of human disease, so the strengths and limitations for each model must be well defined. International organizations such as WHO and CEPI have led attempts to foster communication and collaboration in validating animal models during the COVID-19 pandemic. Researchers should strive to be thorough and detailed when reporting work with animals. The ARRIVE guidelines have been developed for the purpose of clarifying the reporting of animal studies.⁷⁰ Such guidelines can help to assure comprehensive descriptions of work with animals even in the fast-paced, global surge of published research that has become the norm during the current pandemic. As always, studies conducted in animals should follow the principles of the 3Rs—replacement, reduction, and refinement. In some circumstances, molecular diagnostics and genetic techniques may reduce or replace the need for in vivo animal work. Additional considerations regarding ethics and the design of animal studies of infectious disease have been covered elsewhere.^{1,19} When conducted appropriately, animal research can facilitate lifesaving science in the face of a global health crisis. Animal research will no doubt continue to inform our response to future significant infectious disease events, just as it has throughout history.

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