Chapter 16

Emerging Viral Diseases

Why We Need to Worry about Bats, Camels, and Airplanes

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One of the most dramatic aspects of virology is the emergence of new virus diseases, which often receives widespread attention from the scientific community and the lay public. Considering that the discipline of animal virology was established over 100 years ago, it may seem surprising that new virus diseases are still being discovered. How this happens is the subject of this chapter.

1. HOW DO NEW VIRAL DISEASES EMERGE?

There are many recent books and reviews (see Further Reading) that list the plethora of determinants that can lead to the emergence of infectious diseases (Table 1). In this chapter, we concentrate on those determinants that relate to viral pathogenesis and deal only briefly with the many societal and environmental factors that can be instrumental in disease emergence.

1.1 Discovery of the Etiology of an Existing Disease

In some instances, the “emergence” of a viral disease represents the first identification of the cause of a well-recognized disease. An example is La Crosse virus, a mosquito-transmitted bunyavirus that was first isolated from a fatal case of encephalitis in 1964. The isolation of the causal agent and the development of serological tests made it possible to distinguish La Crosse encephalitis from the rubric of “arbovirus encephalitis, etiology unknown.” Since that time, about 100 cases have been reported annually, without any significant increase since the 1970s. It appears that the emergence of this “new” disease reflected only the newfound ability to identify this etiologic entity, rather than any true change in its occurrence.

Hantavirus pulmonary syndrome is another example of the “emergence” of an existing but previously unrecognized disease. In 1993, in the four corners area of the southwestern United States, there occurred a small outbreak of cases of acute pulmonary illness with a high mortality. Epidemiologic and laboratory investigation rapidly identified the causal agent, a previously unknown hantavirus, now named Sin Nombre virus (SNV). SNV is an indigenous virus of deer mice (Peromyscus maniculatus) that are persistently infected and excrete the virus. Apparently deer mice produce virus-infected excreta and, when they infest human dwellings, aerosolized fomites can result in occasional human infections. The 1993 outbreak is thought to reflect...
a transient rise in deer mouse populations associated with an unusual crop of pine nuts, a major food source for these rodents. The recognition of SNV soon led to the discovery of other heretofore unrecognized hantaviruses in North, Central and South America, many of which also cause serious human disease.

### 1.2 Increase in Disease Caused by an Existing Virus

On occasion, a virus that is already widespread in a population can emerge as a cause of epidemic or endemic disease, due to an increase in the ratio of cases to infections. Such an increase can be caused by either an increase in host susceptibility or enhancement of the virulence of the virus. Although counterintuitive, there are some dramatic instances of such phenomena.

**Increase in host susceptibility.** Poliomyelitis first appeared as a cause of summer outbreaks of acute infantile paralysis in Sweden and the United States late in the nineteenth century (Figure 1). Isolated cases of infantile paralysis had been recorded in prior centuries, and an Egyptian tomb painting indicates that poliomyelitis probably occurred in early recorded history. Why then did poliomyelitis emerge abruptly as an epidemic disease? When personal hygiene and public health were primitive, poliovirus circulated as a readily transmitted enterovirus, and most infants were infected while they still carried maternal antibodies (up to 9–12 months of age). Under these circumstances, the virus produced immunizing infections of the enteric tract, but passively acquired circulating antibodies prevented invasion of

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**TABLE 1 Some of the Factors that Lead to Emergence or Reemergence of Viral Diseases**

<table>
<thead>
<tr>
<th>Factors Leading to Emergence</th>
<th>Determinant</th>
</tr>
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<tbody>
<tr>
<td>Economic and social</td>
<td>Population growth, density, distribution</td>
</tr>
<tr>
<td>development</td>
<td>Environmental changes such as deforestation, dam building, global warming</td>
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<td></td>
<td>Increased global travel</td>
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<td></td>
<td>Increased international commerce</td>
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<td></td>
<td>Agribusiness, food processing, distribution</td>
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<td>Poverty</td>
<td>Inadequate public health systems</td>
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<td>Open defecation</td>
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<td></td>
<td>Lack of safe water</td>
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<tr>
<td>Societal breakdown</td>
<td>Civil chaos</td>
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<td></td>
<td>War</td>
</tr>
<tr>
<td>Human factors</td>
<td>Sexual activity</td>
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<td></td>
<td>Substance abuse</td>
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<tr>
<td>Biological factors</td>
<td>Natural mutation</td>
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<tr>
<td></td>
<td>Antimicrobial resistance</td>
</tr>
<tr>
<td></td>
<td>Immunosuppression</td>
</tr>
</tbody>
</table>

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**FIGURE 1** The appearance of epidemic poliomyelitis in the United States, 1885–1916. The graph is based on reported cases (mainly paralytic) during an era when reporting was estimated at about 50%. Data from Lavinder CH, Freeman SW, Frost WH. Epidemiologic studies of poliomyelitis in New York City and the northeastern United States during the year 1916. Washington: United States Public Health Service (1918).
the spinal cord. With the improvement of personal hygiene in the late-nineteenth century, infections were delayed until 1–3 years of age, after the waning of maternal antibodies. Infections now occurred in susceptible children, resulting in outbreaks of infantile paralysis. This reconstruction is supported by seroepidemiological studies conducted in North Africa in the 1950s, when epidemic poliomyelitis first emerged in this region.

**Increase in viral virulence.** Viruses may undergo sudden increases in virulence resulting in emergence of dramatic outbreaks. An outbreak of lethal avian influenza in Pennsylvania in 1983 is one documented example. In eastern Pennsylvania, avian influenza appeared in chicken farms early in 1983, but the virus was relatively innocuous and most infections were mild. However, in the fall of that year a fatal influenza pandemic spread rapidly through the same farms. When viruses from the spring and fall were compared, it appeared that both isolates had almost identical genomes. The fall virus had acquired a single point mutation in the viral hemagglutinin that facilitated the cleavage of the hemagglutinin. The virus could now replicate outside the respiratory tract, markedly increasing its virulence (discussed in Chapter 7, Patterns of infection). This point mutation led to the emergence of an overwhelming epizootic, which was only controlled by a widespread slaughter program involving millions of birds. Similar outbreaks of avian influenza have occurred subsequently in other countries.

### 1.3 Accumulation of Susceptible Hosts and Viral Reemergence

A virus that is endemic in a population may “fade out” and disappear, because the number of susceptibles has fallen below the critical level required for perpetuation in that population. If the population is somewhat isolated, the virus may remain absent for many years. During this interval, there will be an accumulation of birth cohorts of children who are susceptible. If the virus is then reintroduced, it can “reemerge” as an acute outbreak. In the years 1900–1950, Iceland had a population of about 200,000, which was too small to maintain measles virus, and measles periodically disappeared. When travelers to Iceland reintroduced the virus, measles reemerged in epidemic proportions.

### 1.4 Virus New to a Specific Population

On occasion, a virus can enter and spread in a region where it had never previously circulated, leading to the emergence of a disease new to that locale. A dramatic example is afforded by the emergence of West Nile virus (WNV) in the United States, beginning in 1999 (Figure 2). WNV, like most arboviruses, is usually confined to a finite geographic area, based on the range of its vertebrate reservoir hosts and permissive vectors. In an unusual event, WNV was imported into New York City, probably by the introduction of infected vector mosquitoes that were inadvertent passengers on a flight from the Middle East, where the virus is enzootic. This hypothesis was supported by the finding that the genomic sequence of the New York isolates was closely related to the sequence of contemporary isolates from Israel. Some American mosquito species were competent vectors for WNV, and certain avian species such as American crows were highly susceptible. As a result, West Nile encephalitis emerged as a significant disease new to the United States. Over a period of several years WNV spread across the continent, finally reaching the west coast and many areas in Latin America.

Chikungunya virus (CHIKV), another mosquito-borne arbovirus, has been endemic for many years in regions of Africa and Asia where it periodically caused outbreaks of a febrile illness associated with severe arthritis and arthralgia. In 2005–2006, CHIKV caused a large epidemic on the island of Reunion in the Indian Ocean, apparently associated with a mutation that allowed the virus to be more efficiently transmitted by vector mosquitoes. Chikungunya subsequently spread to India and elsewhere in Asia, followed by the Americas. As of November 2014, transmission of CHIKV had been documented in 40 countries or territories in the Caribbean, Central, South and North America, resulting in nearly one million suspected cases each year. It appears that CHIKV may become established in the New World, where virtually the entire human population currently lacks immunity.

### 2. ZOONOTIC INFECTIONS AS A SOURCE OF EMERGING VIRAL DISEASES

Zoonotic infections of animals that can be transmitted to humans are a major cause of emerging virus diseases of humans. These viruses are transmitted by direct contact, by virus-laden droplets or aerosols, or by insect vectors. All zoonotic viruses have one or more animal reservoir hosts, which play an important role in the epidemiological dynamics of human infections. Although many zoonotic viruses can be transmitted to humans on occasion, their relative ability to spread from human to human determines whether or not they emerge as significant new virus diseases of mankind (Table 2).

#### 2.1 Dead-end Hosts

Most zoonotic viruses that are transmitted to humans cannot be spread directly from person to person, so humans are considered to be “dead-end hosts.” One familiar example is rabies, which is enzootic in several animal hosts, such as dogs, skunks, foxes, raccoons, and bats. Humans are infected by bite of a rabid animal or by aerosol exposure.
Several zoonotic arenaviruses, such as Lassa, Machupo (Bolivian hemorrhagic fever), and Junin (Argentine hemorrhagic fever) viruses, are likely transmitted from the reservoir host (wild rodents) by inhalation of contaminated aerosols.

There are more than 500 viruses—belonging to several virus families—that are also classified as arboviruses (arthropod-borne viruses), based on a vertebrate–arthropod maintenance cycle in nature. Arboviruses replicate in both the vertebrate host and the arthropod vector (mosquitoes, ticks, sandflies, and others), and transmission occurs when the vector takes a blood meal. Typically, arboviruses have only a few vertebrate hosts and are transmitted by a narrow range of arthropods. Humans are not the reservoir vertebrate hosts of most arboviruses, but can be infected by many of these viruses, if they happen to be bitten by an infected vector. In most instances, arbovirus-infected humans are dead-end hosts for several reasons. Many arthropod vectors competent to transmit a zoonotic arbovirus prefer non-human hosts as a blood source, reducing the likelihood of transmission from human to vector. Also, infected humans are usually not sufficiently permissive to experience a high titer viremia, so they cannot serve as effective links in the transmission cycle. There are only a few exceptions: in urban settings, dengue, urban yellow fever, Oropouche, and Chikungunya viruses can be maintained by an arthropod vector–human cycle.

### 2.2 Limited Spread among Humans

As Table 2 shows, a few zoonotic viruses can be transmitted directly from human to human, at least for a few passages, and can emerge as the cause of outbreaks involving a few to several hundred cases. Since many viruses in this group cause a high mortality in humans, even a small outbreak constitutes a public health emergency. These viruses belong to many different virus families, and there is no obvious biological clue why they should be able to spread from human to human, in contrast to other closely related viruses. Typically, infections are mainly limited to caregivers or family members who have intimate contact with patients, often in a hospital setting. However, transmission is marginal, so that most outbreaks end after fewer than 5–10 serial transmissions, either spontaneously or due to infection-control practices.
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2.3 Crossing the Species Barrier

In the history of modern virology (the last 50 years) there are very few documented instances where zoonotic viruses have established themselves in the human population and emerged as new viral diseases of mankind (Table 2). Most viruses have evolved to optimize their ability to be perpetuated within one or a few host species, and this creates what is sometimes called the “species barrier.” In most instances, a virus must undergo some adaptive mutations to become established in a new species.

**SARS coronavirus.** In November, 2002, an outbreak of severe acute respiratory disease (SARS) began in Guangdong Province, in southeast China near the Hong Kong border. In retrospect, the first cases were concentrated in food handlers, who then spread the virus to the general population in that region. Although not recognized immediately as a new disease, the outbreak continued to spread both locally and in other parts of China. In February, 2003, a physician who had been treating likely SARS patients, traveled to Hong Kong, where he transmitted SARS to a large number of contacts in a hotel. These persons, in turn, spread the infection to Singapore, Taiwan, Vietnam, and Canada, initiating a global pandemic that eventually involved almost 30 countries. From patient samples, several research groups isolated a novel coronavirus, which has been named the SARS coronavirus (SARS CoV). Clearly, this virus is new to the human population, and there is circumstantial evidence that it was contracted from exotic food animals that are raised for sale in restaurants in Guangdong Province. Recent studies suggest that horseshoe bats (genus *Rhinolophus*) may be the reservoir hosts and palm civets, consumed as food in China, may be the intermediary hosts for SARS CoV.

SARS CoV went through a large number of human passages (perhaps 25) before being contained by primary control measures, such as respiratory precautions, isolation, and quarantine. The virus has been eliminated from the human population, but the 2003 outbreak showed that it could be maintained by human-to-human transmission. From that perspective, it is potentially capable of becoming an indigenous virus of humans. Since many coronaviruses infect the respiratory system and are transmitted by the respiratory route, the SARS virus did not have to undergo any change in its pathogenesis. However, the virus did have to replicate efficiently in cells of the human respiratory tract and it is unknown whether this required some adaptive mutations from the virus that is enzootic in its reservoir hosts.

**Middle Eastern respiratory syndrome.** MERS is an acute respiratory disease of humans that was first recognized...
in 2012, when a novel coronavirus (MERS-CoV) was isolated from two fatal human cases in Saudi Arabia and Qatar. Since that time, more than 1400 clinical cases of MERS have been recognized, the great majority in Saudi Arabia but also in most of the other countries in the Arabian Peninsula and beyond (Figure 3). Hospitalized cases of MERS are characterized by an acute respiratory disease syndrome (ARDS) with a fatality rate over 25%. However, evolving evidence suggests that there may be many additional human infections with little or no associated illness.

What is the origin of this new disease? Based on fragmentary data now available, it appears that MERS-CoV is likely to be an enzootic virus of one or more species of bats. Camels are probably an intermediary host, and humans in close contact with camels can acquire infection. Also, MERS-CoV can spread from human to human, particularly to caretakers or others in close contact with acutely ill patients. However, there are many unknowns in this speculative reconstruction: How do camels become infected? Are camels the main source of human infections? What has caused this disease to emerge in 2012, or had it preexisted but was just recognized at that time? Is this an evolving outbreak, and if so, what is driving it?

Several relevant facts have been ascertained from basic studies. MERS-CoV replicates well in cell cultures obtained from many species, including bats and humans. The pantropic nature of this virus is explained in part by its cellular receptor, dipeptidyl peptidase 4 (DPP4), a widely distributed cell surface molecule. This would facilitate the transmission of this zoonotic infection from bats to camels to humans. Of interest, MERS-CoV infects type II alveolar pneumocytes while SARS CoV uses a different cell receptor (angiotensin-converting enzyme 2, ACE2) and infects type I alveolar pneumocytes. However, both viruses appear to cause a similar ARDS.

Type A influenza virus. Genetic evidence strongly implicates avian and porcine type A influenza viruses as the source of some past pandemics of human influenza. It appears that new epidemic strains are often derived as reassortants between the hemagglutinin (and the neuraminidase in some cases) of avian influenza viruses with other genes of existing human influenza viruses. The new surface proteins provide a novel antigenic signature to which many humans are immunologically naïve. The human influenza virus genes contribute to the ability of the reassortant virus to replicate efficiently in human cells. It is thought that reassortment may take place in pigs that are dually infected with avian and human viruses.

Currently, there is concern that a new pandemic strain of Type A influenza could emerge as a derivative of highly virulent avian H5N1 or H7N9 influenza viruses now causing epidemics in domestic chickens in southeast Asia. There have been several hundred documented human infections with each of these avian viruses in recent years—mainly among poultry workers—with significant mortality. However, few if any of these infections have spread from human to human, perhaps because the infecting avian virus has not undergone reassortment with a human influenza virus. A critical determinant is that avian influenza viruses preferentially attach to α2-3 sialic acid receptors while human viruses attach to α2-6 sialic acid receptors.

The 1918 pandemic of influenza is presumed to be an example of a zoonotic influenza virus that crossed the species barrier and became established in humans where it caused an excess mortality estimated at 20–40 million persons. Recent viral molecular archaeology has recovered the sequences of the 1918 H1N1 influenza virus from the tissues of patients who died during the epidemic.
All of the genes of the reconstructed virus are avian in origin, but it is unknown whether the virus underwent mutations that enhanced its ability to be transmitted within the human population. The reconstructed hemagglutinin of the 1918 virus has been inserted into recombinant influenza viruses, and—in mice—markedly increases the virulence of primary human isolates of influenza virus (Table 3), but the full virulence phenotype appears to require many of the avian influenza genes. Several physiological factors play a role in disease enhancement, including increased replication in pulmonary tissues and an enhanced ability to stimulate macrophages to secrete pro-inflammatory cytokines which, in turn, cause severe pneumonia.

**Human immunodeficiency virus.** HIV has emerged as the greatest pandemic in the recent history of medical science. Modern methods have made it possible to reconstruct its history in great detail (see Chapter 9, HIV/AIDS). A provisional reconstruction of the sequence of events is summarized in Figure 4. The emergence of HIV may be divided into two phases: What was the zoonotic source of HIV and when did it cross into humans? And when did HIV spread from the first human cases to become a global plague?

There have been several transmissions of simian immunodeficiency virus (SIV) to humans (Sharp and Hahn, 2011), and this account will focus on HIV-1 which appears to have been transmitted to humans in at least four separate instances, identified by individual HIV-1 lineages called groups (M, N, O, P). Of these, the most important was the M group of HIV-1, which has been responsible for the vast majority of human infections. Furthermore, HIV-1 is most closely related to SIVcpz, the SIV strain infecting two subpopulations of chimpanzees. Different segments of the SIVcpz genome, in turn, are closely related to genome segments of two SIVs of African monkeys, red-capped monkeys and *Cercopithecus* monkeys. It is hypothesized that chimpanzees, which regularly kill and eat monkeys, were infected during consumption of their prey; and that a recombination event produced SIVcpz, which was derived from parts of the genomes of the two acquired monkey viruses. It is speculated that a further transmission from chimpanzees may have occurred during the butchering of nonhuman primates, which occurs in rural Africa (Figure 5).

Amazingly, genomic similarities tentatively map the substrain of SIVcpz that is the ancestor of the M group of HIV-1, to chimpanzees in the southeastern corner of Cameroon, a small country in West Africa. Using a sequence analysis to compare diversity within current isolates, the common parent of the M group can be reconstructed. A molecular clock, derived from dated isolates, indicates that this virus was transmitted to humans during the period 1910–1930.

The period from 1930 to 1980 is a mystery (Pepin, 2011), but there are fragmentary data suggesting that the virus persisted as a rare and unrecognized infection in residents of jungle villages in West Africa during this time. It has also been proposed that the reuse of unsterilized needles—a frequent practice during the period of colonial rule—could have inadvertently helped to spread the virus. Starting about 1980, the virus began to spread more rapidly. It appears that accelerated spread began in the region centered on Kinshasa (previously Leopoldville) in the Democratic Republic of the Congo (previously the Belgian Congo, then Zaire) and Brazzaville, just across the Congo River in Congo. Transmission was exacerbated by the chaos in postcolonial Zaire.

During the period 1985–2004, HIV infection spread widely in Africa as shown in Figure 6. In the countries worst affected, the prevalence of infection among adults aged 15–49 years reached levels higher than 30%. The rapid spread was driven by many factors among which were: (1) a high frequency of concurrent sexual contacts in some segments of the population and the hidden nature of sexual networks; (2) the long asymptomatic incubation period during which infected individuals able to transmit the virus were sexually active; (3) the spread along commercial routes of travel within Africa; (4) the failure of health systems to

### TABLE 3 Genetic Determinants of the Virulence of the 1918 Type A Influenza Virus (Spanish Strain) Based on Intranasal Infection of Mice with Reassortant Viruses. The M88 Isolate is a Human Type A Influenza Virus Which is Relatively Avirulent in Mice, Typical of Human Isolates. The Hemagglutinin (H) of the 1918 “Spanish” Virus Confers Virulence Upon the M88 Isolate and the Neuraminidase (N) Does Not Appear to Enhance the Effect of the Hemagglutinin. MLD 50: Mouse 50% Lethal Dose Determined by Intranasal Infection with Serial Virus Dilutions

<table>
<thead>
<tr>
<th>Virus Strain</th>
<th>Origin of Viral Genes</th>
<th>Log 10 MLD 50</th>
<th>Log 10 Virus Titer in Lung (Day 3 after Infection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M88</td>
<td>H M88 N M88</td>
<td>&gt;6.2</td>
<td>2.9</td>
</tr>
<tr>
<td>M88/H99</td>
<td>Sp (1918) M88</td>
<td>4.4</td>
<td>5.1</td>
</tr>
<tr>
<td>M88/H99/N99</td>
<td>Sp (1918) Sp (1918) M88</td>
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<td>4.7</td>
</tr>
</tbody>
</table>

publicize the risks and the underutilization of condoms and other measures to reduce transmission; and (5) the slow introduction of antiviral treatment after it became available in the northern countries about 1996.

Concomitantly with the spread of HIV in Africa, the M group of HIV-1 evolved into nine different subtypes (A–D, F–H, J, K), based on sequence diversity. During the spread within Africa, there were population bottlenecks that resulted in the predominance of different M group subtypes in different regions. Subtype C is most frequent in southern Africa, and subtypes A and D are most frequent in eastern Africa. During the 1980s, HIV also spread globally, although prevalence rates did not reach the levels seen in some African countries. Subtype B is dominant in the western hemisphere and Europe, while subtype C is most frequent in India and some other Asian countries. This implies that each of these regional epidemics was initiated...
by a small number of founder strains of HIV-1, improbable though that may seem.

Thirty years after its appearance as a global disease, almost 40 million persons have died, and there are more than 30 million people living with HIV/AIDS. Although the global incidence of HIV has fallen slightly since 2010, there are still more than two million new infections each year (2014).

**Ebola hemorrhagic fever.** The Ebola pandemic of 2014–15 is the most recent emerging virus disease that has riveted the attention of the world. Where did it come from? Why did it cause a pandemic? Why did the international health community fail to control it? Where will it end? These questions are discussed below.

Ebola is a filovirus indigenous to Africa that is maintained in one or more reservoir species of wild animals, among which bats are a likely host. The transmission cycle is not well documented but it is thought that humans get infected, either by direct contact with bats, or while slaughtering infected wild animals who may act as intermediate hosts. Human-to-human spread can then occur.

The pathogenesis of Ebola virus infection may be briefly summarized. Presumably Ebola enters the human host via the mucous membranes or cuts in the skin. The virus infects mononuclear cells including macrophages and dendritic cells and traffics to lymph nodes, whence it spreads to target organs including the liver, spleen, and adrenal glands. It causes a high titer viremia and a dysregulation of the innate immune system. Clinically, Ebola patients undergo severe vomiting and diarrhea with massive fluid losses and become very dehydrated, with a mortality that varies from 25% to 75%. In fatal cases, the infection of the liver leads to disseminated intravascular coagulopathy, a shock syndrome, and multiorgan failure, although the sequential details are not well understood. Infection is transmitted between humans by contact with bodily fluids of patients, but not by aerosols. Therefore, the virus is spread most frequently to caregivers or others who are in intimate contact with patients and their fomites. Rituals associated with funerals and burial practices often serve to transmit the virus.

Ebola virus was first isolated in 1976 in an outbreak near the Ebola River in the Democratic Republic of the Congo (then called Zaire) and almost concurrently in a second outbreak in southern Sudan. Viruses recovered from these two outbreaks were subsequently shown to be different and are now known as Ebola-Zaire and Ebola-Sudan. Since that time there have been more than 25 individual outbreaks of Ebola disease, mainly in central Africa. Past outbreaks have been controlled by use of protective garments by caregivers and quarantine of infected or potentially infected contacts. These controlled outbreaks have been limited to no more than ~5 serial human-to-human transmissions, and mainly ranged from about 25 to 300 cases.

In December, 2013, an Ebola-Zaire outbreak began in Guinea, West Africa. It appears that the initial case was in an infant who may have been infected by contact with bats. The outbreak then spread to two contiguous countries, Liberia and Sierra Leone. By the fall, 2014, the epidemic had become a catastrophe, and was raging out of control in several parts of these three countries. Although the data are incomplete, it is estimated that there have been at least 20,000 cases (with at least 50% mortality) through February, 2015 (Figure 7). The infection has spread to Nigeria, Senegal, Mali, the United States, and a few European countries, but these invasions have so far been controlled, with limited secondary cases. A global effort to control this pandemic was initiated, with participation from Doctors without Borders, the Red Cross, other non-government organizations, the World Health Organization, and the U.S. Centers for Disease Control and Prevention. As of March, 2015, it appeared that the epidemic was coming under control. Aggressive border screening, both on exit
from the affected countries and on arrival at destinations, has limited spread by air travelers, but the porous land borders remain areas of concern.

From an epidemiological viewpoint, why did this outbreak of Ebola explode into a massive pandemic, in contrast to the many prior outbreaks that were limited to no more than a few hundred cases at most? The outbreak began in Guinea and was mainly confined to that country for about 6 months before it spread to neighboring Liberia and Sierra Leone (Figure 8). During this first 6 months, incidence in Guinea varied from a few to about 50 cases a week, and cases were concentrated in rural areas. From a public health viewpoint, this represented a missed opportunity to contain the outbreak. Contributing to this omission were a combination of factors: a weak health system fragmented by social disruption, a failure of local health authorities to recognize or respond to the outbreak, the failure of international health organizations such as WHO to take aggressive action, and local societal norms that brought family and friends into close contact with Ebola victims, during their illness and at their funerals (Washington Post, October 5, 2014; Cohen, 2014). Once Ebola infections spread from rural villages to urban centers, the outbreak exploded.

Beginning in the fall of 2014, it was recognized that the pandemic was a global threat. In response, a number of countries and international organizations provided resources to Africa, including building facilities, sending equipment, and recruiting personnel to work in the pandemic areas. A major effort was made to get the local population to temporarily change some of their normal social responses to...
illness and death, to reduce the spread within the population. Combined with the efforts of the affected countries, these initiatives started to take hold about January, 2015. However, as of March, 2015, the epidemic was not yet terminated. In retrospect, the international community has acknowledged that it lacks a contingency plan to respond to global outbreaks wherever they may occur. Furthermore, because Ebola was a “neglected” disease, the tools to combat it had not been developed. The Ebola pandemic has spurred crash programs to develop a rapid diagnostic test, drugs and antibodies for treatment, and a vaccine for prevention (Product, 2015; Mire et al., 2015).

**Canine parvovirus.** CPV is another example of a disease that emerged due to the appearance of a virus new to its host species. In the late 1970s, a highly lethal pandemic disease appeared in the dog populations of the world. The etiologic agent was a parvovirus previously unknown in canines. The sequence of CPV is almost identical to that of feline panleukopenia virus (FPV), an established parvovirus of cats, which causes acutely fatal disease in kittens. CPV has a few point mutations that distinguish it from FPV, and these mutations permit CPV to bind to and infect canine cells, a property not possessed by FPV. It is presumed that these mutations permitted the emergence of a new virus disease of dogs.

### 2.4 The Species Barrier and Host Defenses

Many mammalian viruses have evolved with their hosts so that different members of a virus family are associated with each host species. Furthermore, under natural circumstances, each member of the virus family usually “respects” the species barrier and does not cross into other species, although it spreads readily between individual animals within its host species. Diverse mechanisms contribute to the species barrier, including host defenses and viral genes. For a zoonotic virus to establish itself in humans or another new species, it is probable that mutations are needed for full adaptation. This requirement is likely part of the explanation for the rarity of such events.

One of the best-studied examples is the transmission of SIVcpz, a virus of chimpanzees, to HIV, a human virus. Several recent studies have shown that APOBEC3 and tetherin are two very important gatekeepers for transmission of lentiviruses between different primate species (reviewed in Sharp and Hahn, 2011). APOBEC3 proteins represent a powerful first line of defense, believed to be responsible for preventing the transmission of SIVs from monkeys to chimpanzees and from monkeys to humans (Etienne et al., 2013). However, APOBEC3 proteins can be counteracted by the HIV/SIV viral infectivity factor (Vif), albeit generally in a species-specific fashion. Thus, mutations in Vif were required for monkey SIVs to be able to infect chimpanzees and then humans (Letko et al., 2013). Tetherin is a second line of defense, and only HIVs that have evolved an effective tetherin antagonism have spread widely in humans (Kluge et al., 2014). Different lentiviruses use different viral proteins to antagonize tetherin: HIV-1 group M uses the viral protein U (Vpu); HIV-2 group A uses the envelope glycoprotein (Env), and HIV-1 group O uses the negative regulatory factor (Nef). In contrast, HIV-1 groups N and P, whose spread in the human population has remained very limited, are unable to counteract tetherin efficiently.

Turning to another virus, recent studies using a mouse-adapted strain of Ebola virus to infect a panel of inbred mice provided by the Collaborative Cross (see Chapter 10, Animal models) found striking variation in the response to Ebola virus infection, from complete resistance to severe hemorrhagic fever with 100% mortality. This underlines the role of host genetic background as a determinant of susceptibility. Consistent with this are comments of clinicians that there are unpredictable differences in the outcome of individual Ebola cases. These studies suggest a dynamic relationship between host and pathogen that may determine when a virus can cross the species barrier and create a new virus disease of humankind.

### 3. WHY VIRAL DISEASES ARE EMERGING AT AN INCREASING FREQUENCY

Although difficult to document in a rigorous manner, it does appear that new virus diseases of humans (and perhaps of other species) are emerging at an increased tempo. There are a number of reasons for this trend (Table I).

#### 3.1 Human Ecology

The human population is growing inexorably, and is becoming urbanized even faster. As a result, there is an increasing number of large-crowded cities, which provide an optimal setting for the rapid spread of any newly emergent infectious agent.

In the nineteenth century, it was noteworthy that someone could circumnavigate the globe in 80 days, but now it can be done in less than 80 hours. However, the incubation period of viral infections (several days to several months) has stayed constant. Someone can be infected in one location and—within a single incubation period—arrive at any other site on earth. This enhances the opportunity for a new human virus to spread as a global infection before it has even been recognized, markedly increasing the opportunity for the emergence of a new disease. The same dynamics also apply to viral diseases of animals and plants, which has important economic and social consequences for humankind. The SARS pandemic of 2002–2003, described above, is an example of how rapidly and widely a new virus disease can emerge and spread globally. In this instance,
it is extraordinary that the disease was brought under control—and eradicated from the human population—by the simple methods of isolation, quarantine, and respiratory precautions. Although conceptually simple, a heroic effort was required for success. Another example is the 2009 emergence of a novel H1N1 strain of influenza A virus that spread rapidly around the world before a vaccine could be produced.

Remote areas of the world are now being colonized at a high frequency, driven by population pressures and economic motives, such as the reclamation of land for agriculture or other uses, and the harvest of valuable trees and exotic animals. The construction of new dams, roads, and other alterations of the natural environment create new ecological niches. It is possible that this is the origin of urban yellow fever. Yellow fever virus is an arbovirus maintained in a monkey–mosquito cycle in the jungles of South America and Africa. Humans who entered jungle areas became infected. When they returned to villages or urban centers, an urban cycle was initiated, where *Aedes aegypti* mosquitoes—that are well adapted to the urban environment and preferentially feed on humans—maintained the virus.

In the last 25 years, agriculture has undergone a dramatic evolution with the development of “agribusiness.” Food and food animals are now raised on an unprecedented scale and under very artificial conditions, where the proximity of many members of a single plant or animal species permits an infection to spread like wildfire. Furthermore, increasing numbers of plants, animals, and food products are rapidly transported over large distances; we enjoy fresh fruits and vegetables at any time, regardless of the season. International shipment of plants and animals can import viruses into new settings where they may lead to the emergence of unforeseen diseases. One example is the 2003 outbreak of monkeypox that caused about 80 human cases in the United States. This was traced to the importation from Africa of Gambian giant rats as exotic pets; several rodent species in west Africa appear to be reservoir hosts of this poxvirus. Monkeypox spread from these animals to pet prairie dogs and from prairie dogs to their owners. Because monkeypox causes infections in humans that resemble mild cases of smallpox, this outbreak was a major cause of public health concern.

### 3.2 Deliberate Introduction of a Virus New to a Specific Population

On occasion, a virus has been deliberately introduced into a susceptible population where it caused the emergence of a disease epidemic. For sport, rabbits were imported from Europe into Australia in the mid-nineteenth century. Because of the absence of any natural predator the rabbits multiplied to biblical numbers, and threatened natural grasslands and agricultural crops over extensive areas of southern Australia. To control this problem, myxomatosis virus was deliberately introduced in 1950 (Fenner, 1983). This poxvirus is transmitted mechanically by the bite of insects and is indigenous to wild rabbits in South America, in which it causes nonlethal skin tumors. However, myxomatosis virus causes an acutely lethal infection in European rabbits, and its introduction in Australia resulted in a pandemic in the rabbit population.

Following the introduction of myxomatosis virus in 1950, co-evolution of both virus and host were observed. The introduced strain was highly virulent and caused epizootics with very high mortality. However, with the passage of time field isolates exhibited reduced virulence, and there was a selection for rabbits that were genetically somewhat resistant to the virus. Strains of moderate virulence probably became dominant because strains of lowest virulence were less transmissible and strains of maximum virulence killed rabbits very quickly.

Concern has also been raised about disease emergence due to the deliberate introduction of viruses into either human or animal populations, as acts of bioterrorism.

### 3.3 Xenotransplantation

Because of the shortage of human organs for transplantation recipients, there is considerable research on the use of other species—particularly pigs—as organ donors. This has raised the question whether known or unknown latent or persistent viruses in donor organs might be transmitted to transplant recipients. Since transplant recipients are immnosuppressed to reduce graft rejection, they could be particularly susceptible to infection with viruses from the donor species. In the worst scenario, this could enable a foreign virus to cross the species barrier and become established as a new human virus that might spread from the graft recipient to other persons.

### 4. HOW ARE EMERGENT VIRUSES IDENTIFIED?

The impetus to identify a new pathogenic virus usually arises under one of two circumstances. First, a disease outbreak that cannot be attributed to a known pathogen may set off a race to identify a potentially new infectious agent. Identification of the causal agent will aid in the control of the disease and in prevention or preparedness for potential future epidemics. SARS coronavirus, West Nile virus, and Sin Nombre Virus are examples of emergent viruses that were identified in the wake of outbreaks, using both classical and modern methods. Alternatively, an important new virus may be discovered as a serendipitous by-product of research directed to a different goal, as was the case with hepatitis B virus (HBV). In this instance, a search for alloantigens uncovered a new serum protein that turned out to
be the surface antigen (HBsAg) of HBV, leading to the discovery of the virus.

When a disease outbreak cannot be attributed to a known pathogen, and where classical virus isolation, propagation, and identification fail, molecular virology is required. Hepatitis C virus (HCV), Sin Nombre and other hantaviruses, certain rotaviruses, and Kaposi’s sarcoma herpesvirus (HHV8), are examples of emergent viruses that were first discovered as a result of molecular technologies. Below, we briefly describe some methods of viral detection and identification. More detailed information and technical specifics can be found in several current texts.

4.1 Classic Methods of Virus Discovery

The first question that confronts the investigator faced with a disease of unknown etiology is whether or not it has an infectious etiology? Evidence that suggests an infectious etiology is an acute onset and short duration, clinical similarity to known infectious diseases, a grouping of similar illnesses in time and place, and a history of transmission between individuals presenting with the same clinical picture. For chronic illnesses, the infectious etiology may be much less apparent and a subject for debate.

Faced with a disease that appears to be infectious, the next question is whether it is caused by a virus. A classical example that predates modern virology is the etiology of yellow fever. In a set of experiments that would now be prohibited as unethical, the Yellow Fever Commission, working with the US soldiers and other volunteers in Cuba in 1900, found that the blood of a patient with acute disease could transmit the infection to another person by intravenous injection. Furthermore, it was shown that the infectious agent could pass through a bacteria-retaining filter and therefore could be considered a “filterable virus.”

**Virus isolation in cell culture and animals.** The first step in identification of a putative virus is to establish a system in which the agent can be propagated. Before the days of cell culture, experimental animals were used for this purpose. Many viruses could be isolated by intracerebral injection of suckling mice, and some viruses that did not infect mice could be transmitted to other experimental animals. Human polioviruses—because of their cellular receptor requirements—were restricted to old world monkeys and great apes; the virus was first isolated in 1908 by intracerebral injection of monkeys and was maintained by monkey-to-monkey passage until 1949 when it was shown to replicate in primary cultures of human fibroblasts.

The modern era of virology (beginning about 1950) can be dated to the introduction of cultured cells as the standard method for the isolation, propagation, and quantification of viruses. There are now a vast range of cell culture lines that can be used for the isolation of viruses, and currently this is the first recourse in attempting to isolate a suspected novel virus. Some viruses will replicate in a wide variety of cells but others are more fastidious and it can be hard to predict which cells will support their replication.

It is also important to recognize that some viruses will replicate in cell culture without exhibiting a cytopathic effect. An important example is the identification of simian virus 40 (SV40). Poliovirus was usually grown in primary cell cultures obtained from the kidneys of rhesus monkeys, but SV40 had escaped detection because it replicated without causing a cytopathic effect. When poliovirus harvests were tested in similar cultures prepared from African green monkeys, a cytopathic effect (vacuolation) was observed, leading to the discovery of SV40 virus in 1960. Because inactivated poliovirus vaccine produced from 1955 to 1960 had been prepared from virus grown in rhesus monkey cultures, many lots were contaminated with this previously unknown virus, which inadvertently had been administered to humans. Since that time, viral stocks and cell cultures have been screened to exclude SV40 and other potential virus contaminants.

A number of methods are available to detect a noncytopathic virus that is growing in cell culture. These include visualization of the virus by electron microscopy, detection of viral antigens by immunological methods such as immunofluorescence or immunocytochemistry, the agglutination of erythrocytes of various animal species by virus bound on the cell surface (hemagglutination), the production of interferon or viral interference, and the detection of viral nucleic acids.

**Detection of nonreplicating viruses.** During the period from 1950 to 1980, there was a concerted effort to identify the causes of acute infections of infants and children. In seeking the etiology of diarrheal diseases of infants, it was hypothesized that—in addition to bacteria, which accounted for less than half of the cases—one or more viruses might be responsible for some cases of infantile diarrhea. Numerous unsuccessful attempts were made to grow viruses from stools of patients with acute diarrhea. It was conjectured that it might be possible to visualize a putative fastidious virus by electron microscopy of concentrated fecal specimens. When patients’ convalescent serum was added to filtered and concentrated stool specimens, aggregates of 70 nm virions were observed in stools from some infants with acute gastroenteritis. The ability of convalescent but not acute illness serum to mediate virion aggregation provided a temporal association of the immune response with an acute diarrheal illness. Within 5 years, rotavirus was recognized as the most common cause of diarrhea in infants and young children worldwide, accounting for approximately one-third of cases of severe diarrhea requiring hospitalization.

Once an emergent virus has been identified, it is necessary to classify it, in order to determine whether it is a known virus, a new member of a recognized virus group, or represents a novel virus taxon. This information
provides clues relevant to diagnosis, prognosis, therapy, and prevention.

In 1967, an outbreak of acute hemorrhagic fever occurred in laboratory workers in Marburg, Germany, who were harvesting kidneys from African green monkeys (Chlorocebus aethiops, formerly Cercopithecus aethiops). In addition, the disease spread to hospital contacts of the index cases, with a total of over 30 cases and 25% mortality. Clinical and epidemiological observations immediately suggested a transmissible agent, but attempts to culture bacteria were unsuccessful. However, the agent was readily passed to guinea pigs which died with an acute illness that resembled hemorrhagic fever. After considerable effort, the agent was adapted to tissue culture and shown to be an RNA virus. When concentrated tissue culture harvests were examined by electron microscopy, it was immediately recognized that this agent differed from known families of RNA viruses, since the virions consisted of very long cylindrical filaments about 70 nm in diameter. This was the discovery of Marburg virus, the first recognized member of the filoviruses, which now include Marburg and Ebola viruses.

### 4.2 The Henle–Koch Postulates

Isolation of a virus from patients suffering from an emergent disease provides an association, but not proof of a causal relationship. Formal demonstration that an isolated virus is the causal agent involves several criteria formulated over the past 100 years. These are often called the Henle–Koch postulates, after two nineteenth-century scientists who first attempted to enunciate the rules of evidence. Sidebar 1 summarizes these postulates, which have been modernized in view of current knowledge and experimental methods.

The classic version of the Henle–Koch postulates required that the causal agent be grown in culture. However, as discussed below, a number of viruses that cannot be grown in culture have been convincingly associated with a specific disease. Usually, this requires that many of the following criteria can be met: (1) Viral sequences can be found in the diseased tissue in many patients, and are absent in appropriate control subjects; (2) Comparison of acute and convalescent sera document induction of an immune response specific for the putative causal virus; (3) The disease occurs in persons who lack a preexisting immune response to the putative virus, but not in those who are immune; (4) The implicated virus or a homologous virus causes a similar disease in experimental animals; and (5) Epidemiological patterns of disease and infection are consistent with a causal relationship.

#### Sidebar 1 The Henle–Koch postulates (requirements to identify the causal agent of a specific disease)

The Henle–Koch postulates were formulated in 1840 by Henle, revised by Koch in 1890, and have undergone periodic updates to incorporate technical advances and the identification of fastidious agents with insidious disease pathogenesis.

Many of the following criteria should be met to establish a causal relationship between an infectious agent and a disease syndrome.

1. The putative causal agent should be isolated from patients with the disease; or the genome or other evidence of the causal agent should be found in patients’ tissues or excreta; and less frequently from appropriate comparison subjects. Temporally, the disease should follow exposure to the putative agent; if incubation periods can be documented they may exhibit a log-normal distribution.

2. If an immune response to the putative agent can be measured, this response should correlate in time with the occurrence of the disease. Subjects with evidence of immunity may be less susceptible than naïve individuals.

3. Experimental reproduction of the disease should occur in higher incidence in animals or humans appropriately exposed to the putative cause than in those not so exposed. Alternatively, a similar infectious agent may cause an analogous disease in experimental animals.

4. Elimination or modification of the putative cause should decrease the incidence of the disease. If immunization or therapy is available, they should decrease or eliminate the disease.

5. The data should fit an internally consistent pattern that supports a causal association.
and tissues and blood samples from these cases were tested extensively, but no virus was initially isolated in cell culture. However, when sera from recovered cases were tested, they were found to cross-react with a battery of antigens from known hantaviruses, providing the first lead (in June, 1993). DNA primers were then designed, based on conserved hantavirus sequences, and these were used in a PCR applied to DNA transcribed from RNA isolated from tissues of fatal cases. Sequence of the resulting amplicon suggested that it was a fragment of a putative new hantavirus (July, 1993), yielding a presumptive identification of the emerging virus within 2 months after the report of the outbreak. An intense effort by three research teams led to the successful isolation of several strains of SNV by November, 1993. SNV is a fastidious virus that replicates in Vero E6 cells but not in many other cell lines.

Kaposi’s sarcoma herpesvirus (HHV8). KS was described over 100 years ago as a relatively uncommon sarcoma of the skin in older men in eastern Europe and the Mediterranean region. In the 1980s, KS emerged at much higher frequency, as one of the diseases associated with AIDS. Furthermore, KS exhibited an enigmatic epidemiological pattern, since its incidence in gay men was more than 10-fold greater than in other AIDS patients, such as injecting drug users and blood recipients. These observations led to the hypothesis that KS was caused by a previously undetected infectious agent that was more prevalent among gay men than among other HIV risk groups. However, researchers were unable to isolate a virus from KS tissues.

Searching for footprints of such a putative agent, Chang and colleagues used the method of representational difference analysis to identify DNA sequences specific for KS tumor tissue. Several DNA fragments were identified, and found to be homologous with sequences in known human and primate herpesviruses. In turn, these sequences were used to design primers to obtain the complete genome of a previously undescribed herpesvirus, since named HHV8, human herpesvirus 8. To this date, HHV8 defies cultivation in tissue culture.

4.4 Computer Modeling of Emerging Infections

Is computer modeling a useful adjunct to the analysis or control of emerging viral diseases? The 2014–15 Ebola pandemic in West Africa offers an interesting case study. As the epidemic unfolded, several groups attempted to project how it would evolve (Meltzer et al., 2014; Butler, 2014). These projections had very wide confidence limits, and several of them had upper limits in the range of 100,000 or more cases. What the modelers could not foretell was that the infection did not spread across sub-Saharan Africa, and that several introductions (into countries such as Nigeria and Mali) were controlled by case isolation, contact tracing, and quarantine. However, modeling did contribute useful insights. Dobson (2014) suggested that rapid quarantine (within 5–7 days) of contacts of Ebola patients could be critical in epidemic control.

5. REPRISE

One of the most exciting current issues in virology is the emergence of new viral diseases of humans, animals, and plants. Even though the era of modern virology has been well established for more than 65 years, virus diseases continue to appear or reemerge. The Ebola pandemic of 2014–15 highlights the associated dangers and obstacles to control.

There are several explanations for emergence: (1) discovery of the cause of a recognized disease; (2) increase in disease due to changes in host susceptibility or in virus virulence; (3) reintroduction of a virus that has disappeared from a specific population; (4) crossing the barrier into a new species previously uninfected. Many zoonotic viruses that are maintained in a nonhuman species can infect humans, but most cause dead-end infections that are not transmitted between humans. A few zoonotic viruses can be transmitted between humans but most fade out after a few person-to-person transmissions. Rarely, as in the case of HIV, SARS coronavirus, and Ebola filovirus, a zoonotic virus becomes established in humans, causing a disease that is truly new to the human species.

There are many reasons for the apparent increase in the frequency of emergence of new virus diseases, most of which can be traced to human intervention in global ecosystems. Emergent viruses are identified using both classical methods of virology and newer genome-based technologies. Once a candidate virus has been identified, a causal relationship to a disease requires several lines of evidence that have been encoded in the Henle–Koch postulates, guidelines that are periodically updated as the science of virology evolves. Identifying, analyzing, and controlling emerging viruses involve many aspects of virological science. Virus–host interactions play a key role, to explain persistence in zoonotic reservoirs, transmission across the species barrier, and establishment in human hosts. Thus, the issues discussed in many other chapters contribute to our understanding of emerging viral diseases.

FURTHER READING

Reviews, Chapters, and Books


Howard CR, Fletcher NF. Emerging virus diseases: can we ever expect the unexpected? Emerging Microbes and Infection. 2012, 1, e46 doi. 10.1038/emr.2012.47.


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